COLLEGIUM INTERNATIONALE ALLERGOLOGICUM

CIA

Petersberg

13-18 SEPTEMBER 2014

GERMANY

30th SYMPOSIUM OF THE COLLEGIUM INTERNATIONALE ALLERGOLOGICUM

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Final Program

Allergies: Current challenges and solutions

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* "Fc-epsilon binding to FcεRI (in tan) from the mast cell perspective". The Fc-portion of IgE bends via conformational changes in order to bind, which improves mainly free entropy and confers a very slow rate of IgE dissociation from the receptor. Done with VMD[1] based on the 2Y7Q coordinates done by HOLDOM et al.

[1]Humphrey, W., Dalke, A. and Schulten, K., "VMD - Visual Molecular Dynamics", J. Molec. Graphics, 1996, vol. 14, pp. 33-38.

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Dear Colleagues,

It is our great pleasure to welcome you to the 30th Symposium of the Collegium Internationale Allergologicum entitled ***Allergies: Current challenges and solutions*** from **13-18 September 2014** in **Petersberg, Germany.**

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

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at the biennial symposia of the *Collegium* is not only important, but includes many late-breaking and in many cases unpublished developments.

We believe you will find that the venue selected by the Council of the *Collegium* for its 30th meeting will accommodate scientists and accompanying persons in the tradition and style that have characterized past meetings of the *Collegium*.

Petersberg is a prominent hill with a height of 331 meters (1,086 ft) in the Siebengebirge mountain range near Bonn, Germany. It overlooks the cities of Königswinter on the right bank of the Rhine River, and Bonn on the opposite side. The Siebengebirge ("seven hills" in German) is a German upland range to the East of the Rhine, southeast of Bonn, consisting of more than 40 hills. It is of volcanic origin and much of the territory covered by the Siebengebirge belongs to the Naturpark Siebengebirge, which is under environmental protection. It is a popular tourist destination for hiking, because of its natural beauty.

On behalf of the *Collegium* Council Members, and the group of local organizers who have worked so effectively to prepare for this meeting, thank you for attending the 30th Symposium in Petersberg. From the beginning, the *Collegium* was intended to be an exclusive group of friends pursuing excellence in research in a spirit of open intellectual exchange at a meeting held in interesting and stimulating locations. We will again strive to fulfill this vision over the next week in Petersberg.

Yours sincerely,

Stephen J. Galli
President

Marcus Maurer
Symposium Organizer

Heidrun Behrendt
Symposium Organizer

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General Information

The 30th Symposium of the *Collegium* will be held in Petersberg, Germany. Petersberg overlooks the cities of Königswinter on the right bank of the Rhine River, and Bonn on the opposite side. The Siebengebirge ("seven hills" in German) is a German upland range to the East of the Rhine, southeast of Bonn, consisting of more than 40 hills. It is a popular tourist destination for hiking, because of its natural beauty.

Airport Transfers

Transfers to and from the Cologne/Bonn airports on Saturday, 13 September and Thursday, 18 September are included in the registration fee.

Coffee Breaks

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

Currency

The currency used in Germany is the Euro. There are ATMs, or Bancomats, widely available for cash withdrawal. Credit cards are also accepted at most hotels, restaurants, and shops.

Electricity

The electrical current in Germany is 220 volts. The European Plug with round prongs is acceptable.

Evaluations

Evaluation forms are included in the registration packet. Please fill out your form and return it to the Registration Desk in the Steigenberger Hotel.

Hospitality Desk

The Hospitality Desk is located at the registration desk in the Steigenberger Grand Hotel. Delegates and accompanying persons will be able to sign up for excursions and ask for advice regarding activities around Petersberg.

Hours:

Saturday, 13 September	14:00 – 20:00
Sunday, 14 September	7:00 – 13:30
Monday, 15 September	7:30 – 14:15
Wednesday, 17 September	7:30 – 13:30
Thursday, 18 September	7:30 – 13:00

Language

The official language of the 30th Symposium is English.

"Life in Science" Breakfast Discussions

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

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

Lunches

Buffet-style lunches will be served in the Steigenberger Hotel in Südflügel during the following times:

Sunday, 14 September	13:00 – 14:30
Monday, 15 September	13:30 – 15:00
Wednesday, 17 September	13:30 – 15:00

Lunch is included in the registration fee for delegates, accompanying persons and children. A lunch will be available for delegates on the boat ride on Tuesday, 16 September.

Oral Abstract Sessions

Oral Abstract Sessions will take place on 14, 15, 16, 17, and 18 September. Oral Abstract Sessions on 14, 15, 17, and 18 will take place at the Steigenberger Hotel and oral abstracts on 16 September will take place at the Maritim Hotel.

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

Poster Sessions

Poster Sessions will take place from 14:30 – 17:00 on 14 September, 17:00 – 19:30 on 15 September and from 15:30 – 18:00 on 17 September at the Maritim Hotel. An assortment of wine, cheese and other refreshments will be served. Poster presenters will stand next to their posters during the assigned sessions and be available for questions and discussion.

Proceedings

Papers from the 30th Symposium will be published by Pacini Editore.

Online submission is now open at www.ciaweb.org.

Deadline for Submission: 27 March 2015

General Information

Registration

The Registration Desk is located in the Steigenberger Hotel on 13, 14, 15, 17, and 18 September, and in the Maritim Hotel on 14, 15, 16, and 17 September (as listed).

Hours:

Saturday, 13 September	14:00 – 20:00
Sunday, 14 September	7:00 – 13:30
	14:30 – 17:00 (Maritim Hotel)
Monday, 15 September	7:30 – 14:15
	15:45 – 19:00 (Maritim Hotel)
Tuesday, 16 September	7:30 – 10:30 (Maritim Hotel)
Wednesday, 17 September	7:30 – 13:30
	15:30 – 17:30 (Maritim Hotel)
Thursday, 18 September	7:30 – 13:00

Registration Fees

The registration fee for delegates includes:

Airport Transfer on Arrival on 13 September
- From Cologne/Bonn Airport to the hotels

Airport Transfer on Departure on 18 September
- From the hotels to the Cologne/Bonn airport

Oral Abstract Sessions

Poster Sessions

Coffee Breaks

Lunches

Social Events
- Welcome Reception, Boat Ride, Informal Dinner, Gala Dinner
- Hotel Shuttle Transportation

The registration fee for accompanying persons and children includes:
- Airport Transfer on Arrival 13 September
- From Cologne/Bonn Airport to the hotels

Airport Transfer on Departure 18 September
- From the hotels to the Cologne/Bonn airport

Lunches

Social Events
- Welcome Reception, Boat Ride, Informal Dinner, Gala Dinner

Hotel Shuttle Transportation

Speaker Preview Room

The Speaker Preview Room is located in the Foyer Bankettsaal at the Steigenberger Hotel. Speakers will be able to check and upload their presentations before the Oral Abstract Sessions.

Hours:

Sunday, 14 September	7:00 – 13:30
Monday, 15 September	7:30 – 14:15
Wednesday, 17 September	7:30 – 13:30
Thursday, 18 September	7:30 – 13:00

Time Zone

Germany is on Central European Time Zone, which is two hours ahead of Greenwich Mean Time (GMT) during the summer.

Tipping

Tipping is generally done at the table with the waiter or waitress who served you. Depending on the service you received, you should tip the normal 10 percent or so. Of course, if the service was poor, you don't have to tip at all. It is wise to always check about credit card payments before you order. For a taxi, tipping isn't necessary, but most round-up the fare to the nearest Euro.

Venue

The oral abstract sessions of the 30th Symposium will take place at the Steigenberger Hotel on 14, 15, 17, and 18 September. The poster sessions on 14, 15, and 17 September, the Relaxing Lecture on 15 September, and the Oral Abstract Session on 16 September will take place at the Maritim Hotel.

Social Events

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

Welcome Reception

Saturday, 13 September 2014, 18:30 – 21:30

The Welcome Reception will be held on the terrace of the Steigenberger Hotel overlooking the Rhine Valley with refreshments and an assortment of hors d'oeuvres.

Lunch and Boat Ride

Tuesday, 16 September 2014, 11:00 – 19:00

Following in the tradition of past Collegium meetings, a boat ride will take place on the fourth day of the meeting. Participants will enjoy a leisurely cruise with fantastic views of the Rhine. The Boat will dock in the town of Leutesdorf and attendees will enjoy a wine tasting at Winery Mohr. The Winery was founded in 1875 and has been operated by the family for four generations. Participants will return via boat and will be dropped off at the Informal Dinner.

Informal Dinner

Tuesday, 16 September 2014, 19:00 – 22:00

After the boat ride, the Informal Dinner will take place at the Rheinhotel Dreesen located along the Rhine River. The evening will include musical entertainment.

Gala Dinner

Wednesday, 17 September 2014, 19:00 – 23:00

An elegant dinner will be held on the last evening of the Symposium at The Steigenberger Hotel in the Rotunde room. Live classical music will be provided at the opening of the evening.

Optional Excursions

All tours will depart from the lobby of the Maritim Hotel, at the times listed below.

Tour 1

Organ Builder Klais

Sunday, 14 September 2014, 9:30 – 12:30

Orgelbau Klais Bonn is a German organ builder firm that designs, builds and restores pipe organs. The company was founded in 1882 by Johannes Klais, Sr. and is now run by his great-grandson Philipp Klais. Based in Bonn with approximately 65 employees, Orgelbau Klais Bonn has completed many large-scale building and restoration projects around the globe in more than a century of organ building. This includes the main organ in the famous Cologne Cathedral and the largest organ in China at the National Centre for the Performing Arts in Beijing. You will have an exclusive guided tour through the organ builder's impressive workshop by Philipp Klais himself. This tour is perfect for getting first-hand information about a rare and globally admired art and handicraft before you have the opportunity to enjoy a fabulous organ concert in the Cologne Cathedral in the evening (see tour 2 or 3).

Price: €35 per person

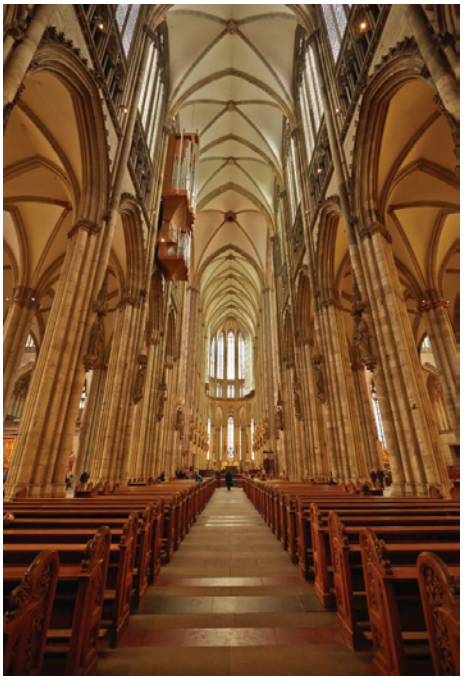
Tour fee includes transportation to/ from Orgelbau Klais Bonn and an English speaking tour of the organ builder's workshop.

Tour 2

Cologne Cathedral

Sunday, 14 September 2014, 14:00 – 19:00

The Cathedral of St. Peter and St. Mary in Cologne is the second highest Catholic Church in the world at more than 157 meters high. It is probably the most important church of Germany, surely one of the most famous examples of Gothic architecture, and the main inspiration for creators of Gothic Revival buildings. The Cologne Cathedral officially became a World Heritage Site in 1996 and is Germany's most visited landmark, attracting approximately 6 million visitors each year. You will have an English speaking guided tour of the inner Cathedral and the treasure chamber as well as the chance to climb the 533 stairs to the top of the Cathedral's tower with a spectacular view over the city. If you book the basic package, we will organize your return to Königswinter by bus afterwards. If you opt for the Combination Package (strongly recommended!) you will have an extended leisure time to discover Cologne. Create some special memories by strolling through the old parts of the city, having dinner at one of the many traditional restaurants or tasting a real "Kölsch" beer on the Domplatte (not included in the price). Afterwards, a real and rare highlight awaits you: Enjoy the exclusive Organ Concert at the Cathedral after opening hours. You will be seated in the illuminated nave and have an English speaking introduction to the church and its instruments by the Cathedral's organist, Professor Bönig. After the Organ Concert on the Klais organ, you will get the chance to explore some parts of the Cathedral that even members of its congregation rarely see.



© Köln Tourismus GmbH – Axel Schulten

Tour 2 (Basic): **Cologne Cathedral Only**

Price: €50 per person
14:00 – 19:00
Tour fee includes transportation to/from Cologne Cathedral, English speaking tour of Cathedral and the treasure chamber, entrance fees for Cathedral and top of the tower.

Tour 2 (Combination): **Cologne Cathedral and Organ Concert**

Price: €85 per person
14:00 – 24:00
Combine your Cologne Cathedral tour with the Organ Concert in the evening (find more information below, see Organ Concert at Cologne Cathedral). Buy the combined ticket and save €20 (in comparison to the regular Organ Concert price)! Tour fee includes transportation to/from Cologne Cathedral, English speaking tour of Cathedral and the treasure chamber, entrance fees for Cathedral and top of the tower, extended leisure time in the evening, and an English speaking tour and Organ Concert at Cologne Cathedral.

Tour 3

Organ Concert at Cologne Cathedral

Sunday, 14 September 2014, 18:00 - 24:00

The famous and impressive Cologne Cathedral, a World Heritage Site and Germany's most visited landmark, is the spectacular location of an exclusive Organ Concert on Sunday night after opening hours. You will be seated in the illuminated nave and have an English speaking introduction to the church and its instruments by the Cathedral's organist Professor Bönig. After the Organ Concert on the Klais organ, you will get the chance to explore some parts of the Cathedral that even members of its congregation rarely see. The tour will depart right after the poster session so that you will have time to stroll through the old parts of the city, have dinner at one of the many traditional restaurants or taste a real "Kölsch" beer on the Domplatte (not included in the price).

Price: €55 per person

Tour fee includes transportation to/from Cologne Cathedral, English speaking tour and Organ Concert at Cologne Cathedral.

Optional Excursions

Tour 4

Beethoven House and Bonn

Monday, 15 September 2014, 9:45 - 15:30

Bonn is not only famous for being West Germany's capital during the Cold War, but also for its rich history of classical music. The well-known German composer and pianist Ludwig van Beethoven played a crucial role in the transition between the Classical and Romantic eras in Western Art Music. Beethoven was born in Bonn in 1770. He spent the first 22 years of his life in Bonn learning the arts of composing and piano before he moved to Vienna where he became one of the most famous and influential of all composers. You will visit the Beethoven House, the historic building where Ludwig van Beethoven was born that now hosts a museum with interesting displays from Beethoven's life. Afterwards, you will have some leisure time to walk through the old part of Bonn, unwrap your lunch package in front of the venerable Town Hall of Bonn or have lunch at one of the traditional restaurants in the adjacent old town center (not included in the price).

Price: €45 per person

Tour fee includes transportation to/from Bonn, English speaking tour of Beethoven House, one lunch package, entrance fee for Beethoven House.

Tour 5

Drachenfels and Drachenburg Castle

Monday, 15 September 2014, 9:30 - 13:30

Mount Drachenfels is the landmark of the romantic mountain range Siebengebirge east and south of Königswinter. With its height of 321 meters and striking silhouette, mount Drachenfels has been attracting people from all parts of the world for more than 100 years. The main attractions of the Drachenfels are the oldest German cog railway leading the way up to the top of the hill as well as the "Neuschwanstein on the Rhine," the romantic castle Drachenburg (literally: Dragon Castle). The tour starts with meeting the English speaking guide and a ride on the miniature train Lokomobil from the Maritim Hotel to the cog railway station. After the ten-minute train journey up-hill, the guide will offer you a peek into the interesting history of the cog railway, the Drachenfels-Bahn, while you enjoy a fantastic panoramic view of the Siebengebirge and the Rhine. After a short walk, you will get a tour of the chateau Drachenburg full of funny, romantic and quaint anecdotes from its turbulent history. Then, after some leisure time in the castle or the adjacent forest, the cog railway will take you back to Königswinter.

Price: €45 per person

Tour fee includes train rides with traditional Drachenfels-Bahn and Lokomobil, English speaking guide and tour of Drachenburg Castle, entrance fee for Drachenburg Castle.

Optional Excursions

Tour 6

Castles Augustusburg and Jagdschloss Falkenlust/Max Ernst Museum

Wednesday, 17 September 2014, 9:30 – 16:00

The palace Augustusburg in Bonn-Brühl is said to be the most meaningful Baroque palace of the Rhineland. No wonder that in 1984 the Augustusburg, the little pleasure palace Jadgschloss Falkenlust and the large-scale park were declared an official World Heritage Site. The palaces that were built during the 18th century as a summer and hunting residence were commonly used for recreation such as falconry or great gala dinners by the Cologne electors. You will be guided by an English speaking tour through the historical building complex of the Augustusburg. After the tour, the participants are invited to take a walk through the beautiful and spacious garden of the park. Lunch can either be enjoyed in the park (lunch packages) or in an Italian restaurant close by (not included in the price). In the afternoon, the other part of the well maintained baroque palace, the Jagdschloss Falkenlust, will be toured individually with audio guides.

Alternately, you have the chance to visit the Max Ernst Museum right next to the palace Augustusburg and the park.

Price per person: €45

Tour fee includes transportation to/from Augustusburg/ Jagdschloss Falkenlust, English speaking tour of Augustusburg, individual audio-guided tour of Jagdschloss Falkenlust, one lunch package, entrance fees for Augustusburg and Jagdschloss Falkenlust.

Tour 7

Wine Valley Ahrtal and Government Bunker

Wednesday, 17 September 2014, 10:00 - 17:30

The Ahr river twists and turns its way through a rocky landscape where lush vineyards cling to the bare stone. The valley Ahrtal or "the wildest daughter of the Rhine" as it was called by the Romanticists is widely known for its excellent red wines. It is home of many historic and nature attractions and the tour will cover a few of the most interesting sights. Most importantly, the tour includes a visit to the Government Bunker (Regierungsbunker) which was a top secret and massive underground complex built and designed to house the German government, parliament and all federal personnel needed to keep the government working in the event of war or severe crisis. The Bunker was built between 1960 and 1972 inside two abandoned railway tunnels and kept in a working condition for about 30 years, including a fully equipped hospital or the former "Mobile Office" of the German Federal President. Accompanied by an English speaking guide, you will start the tour by passing by several castles on the way from Königswinter to the Ahrtal. In Ahrweiler, you will be taken on a tour through of the Government Bunker and then move on to the vineyard and former monastery Kloster Marienthal where a traditional lunch of tarte flambée ("Flammkuchen") awaits you as well as a short walk around the vineyard. In the afternoon, you will visit the town Bad-Neuenahr/ Ahrweiler with its medieval centre. After a tour of Ahrweiler and some leisure time for coffee or souvenir shopping, you will return to Königswinter in the early evening.

Price: €70 per person

Tour fee includes transportation to/from Ahrtal/Government Bunker, English speaking guide for the whole day, guided tour of Government Bunker, lunch and short tour at Monastery/Vineyard Marienthal (excluding drinks), guided tour of Ahrweiler, entrance fee for Government Bunker.

Alain L. de Weck Travel Grant Recipients

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

Oliver Burton, United States

Yu-Chang Bryan Wu, United Kingdom

Dawn Newcomb, United States

Nicole Leib, Germany

Claudia Hui, Canada

Stefanie Eyerich, Germany

Beatriz Cabanillas, Germany

Roopesh Singh Gangwar, Israel

Liliana Cifuentes, Germany

Sylwia Smolinska, Poland

Massimo Caruso, Italy

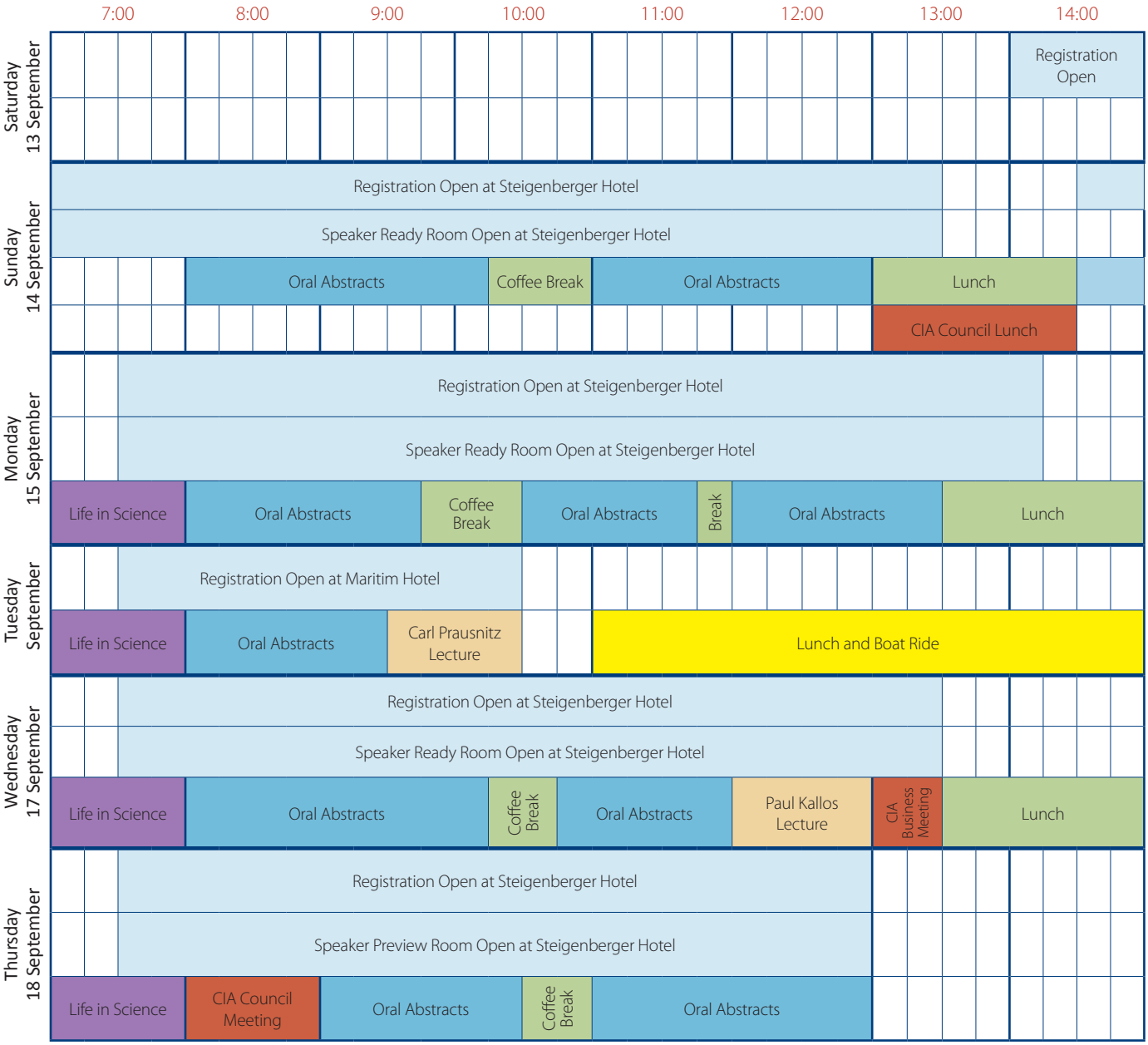
Bettina Margrethe Jensen, Denmark

Travel Grant Recipients will be awarded with a certificate during the Gala Dinner on 17 September 2014.

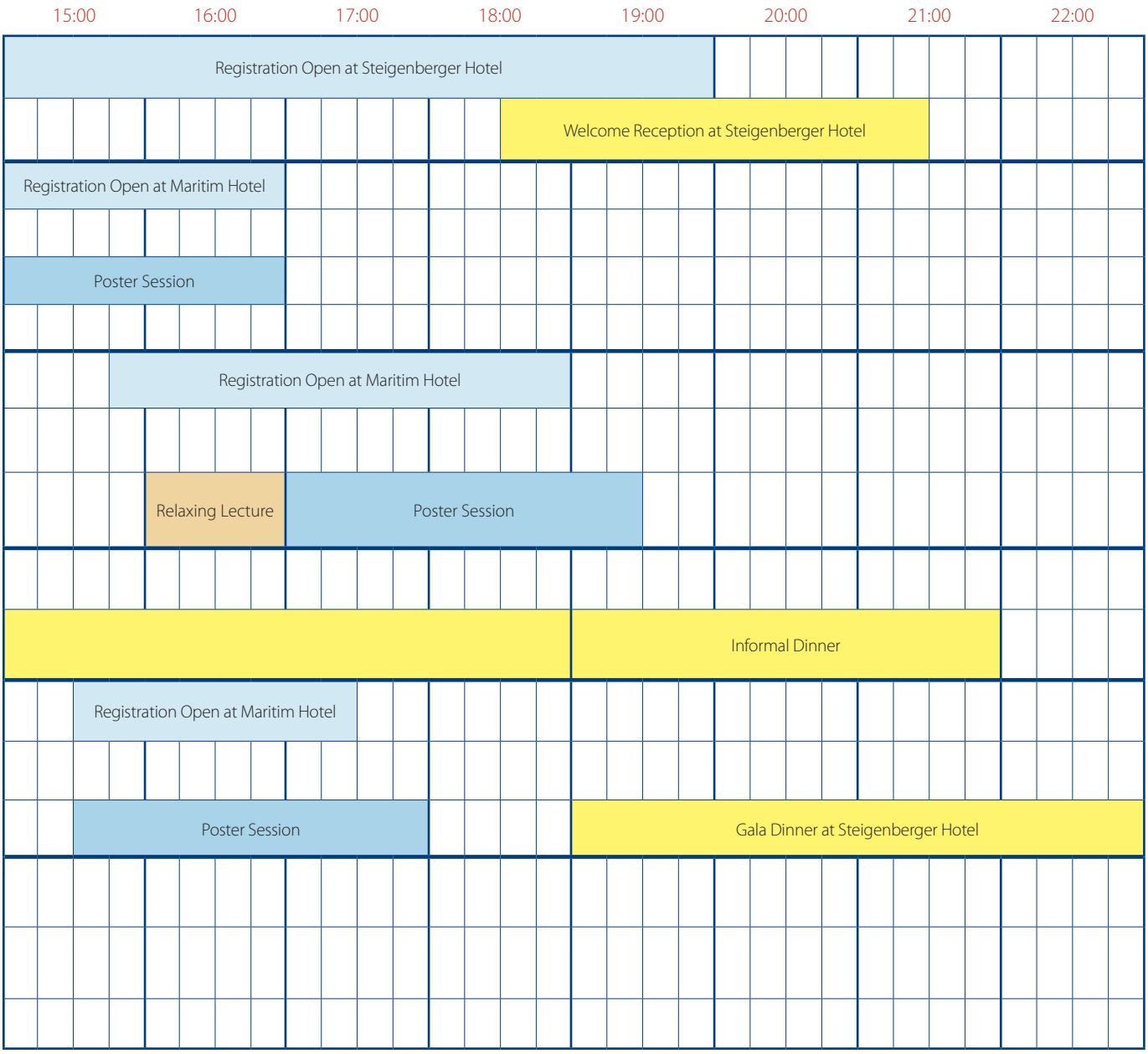
The *Collegium* would like to thank the members who contributed to the Alain L. de Weck Travel Grant Fund (included in the membership renewal form) and the following company:

INDOOR biotechnologies

2014 Program-at-a-Glance



2014 Program-at-a-Glance



*Schedule subject to change.

Map of Hotels

Steigenberger Grandhotel Petersberg:
Petersberg, 53639 Königswinter/Bonn, Germany

Maritim Hotel:
Rheinallee 3, 53639 Königswinter, Germany

Reinhotel Loreley:
Rheinallee 12, 53639 Königswinter, Germany

Schedule of Events

Saturday, 13 September 2014

14:00 – 20:00 Registration Opens . . . . . Steigenberger Hotel Foyer

18:30 – 21:30 Welcome Reception . . . . . Steigenberger Hotel, Südflügel
Stephen J. Galli, United States
Marcus Maurer, Germany
Thomas Bieber, Germany

Sunday, 14 September 2014

7:00 – 13:30 Registration Open . . . . . Steigenberger Hotel Foyer

7:00 – 13:30 Speaker Preview Room Open . . . . . Steigenberger Hotel, Foyer Bankettsaal

7:00 – 8:00 Authors Set Posters . . . . . Maritim Hotel

8:00 – 10:15 Oral Abstract Session 1 . . . . . Steigenberger Hotel, Bankettsaal
Genetic and Environmental Factors in Allergy
Chairpersons: Claudia Traidl-Hoffman, Germany
Marianne van Hage, Sweden

8:00 1 *Neonatal DNA methylation signatures in immunoregulatory pathways predict asthma during childhood*
Donata Vercelli, United States

8:17 2 *Allergic sensitization and current allergen exposure: Big data from the U.S. National Health and Nutrition Examination Survey (NHANES)*
Martin D. Chapman, United States

8:34 3 *Micro-RNA-mediated transcriptional regulation of the high-affinity receptor for IgE on human Langerhans cells*
Nicole Leib, Germany

8:51 4 *Differential roles of BCL-XL and MCL-1 in the development of mast cells*
Karin Hartmann, Germany

Dendritic Cells and Mast Cells
Chairpersons: Gianni Marone, Italy
Gunnar Nilsson, Sweden

9:08 5 *The human lung in 3-D: The use of micro-CT*
Jane Anne Warner, United Kingdom

9:25 6 *Rab5 is a novel regulator of mast cell secretory granule biogenesis*
Ronit Sagi-Eisenberg, Israel

9:42 7 *Virus-infected human mast cells enhance Natural Killer cell functions*
Jean S. Marshall, Canada

9:59 8 *Mitochondrial STAT3 plays a major role in IgE-Ag mediated mast cell exocytosis*
Ehud Razin, Israel

10:15 – 11:00 Coffee Break . . . . . Steigenberger Hotel, Südflügel

11:00 – 13:00 Oral Abstract Session 2 . . . . . Steigenberger Hotel, Bankettsaal
Mast Cells, Basophils and Eosinophils
Chairpersons: Hirohito Kita, United States
Jean Marshall, Canada

11:00 9 *Critical role for mast cell Stat5 activity in atopic dermatitis*
Toshiaki Kawakami, United States

11:17 10 *Evolution of mast cell and basophil tryptases from transmembrane proteases*
George H. Caughey, United States

Sunday, 14 September 2014 (continued)

11:34 11 *A universal antidote? Human tryptase degrades and detoxifies snake venom*
Martin Metz, Germany

11:51 12 *Siglec-7 is an inhibitory receptor on human mast cells and basophils*
Francesca Levi-Schaffer, Israel

12:08 13 *NADPH oxidase-independent formation of extracellular DNA traps by basophils*
Hans-Uwe Simon, Switzerland

12:25 14 *Thymic stromal lymphopoietin promotes human eosinophil-basophil lineage commitment: a new vista on 'in situ hemopoiesis'*
Claudia Hui, Canada

12:42 15 *Airway eosinophilopoietic events in severe prednisone-dependent asthma*
Parameswaran Nair, Canada

13:00 – 14:30 Lunch . . . . . Steigenberger Hotel, Südflügel

13:00 – 14:30 CIA Council Lunch . . . . . Steigenberger Hotel, Salon Drachenfels

14:30 – 17:00 Registration Open . . . . . Maritim Hotel Foyer

14:30 – 17:00 Poster Session 1 . . . . . Maritim Hotel
Genetic and Environmental Factors in Allergy, Dendritic Cells, Mast Cells, Monocytes and Granulocytes, and Lymphocytes and Mediators of Immunoregulation
Chairpersons: Stephen J. Galli, United States
Deborah A. Meyers, United States
Rosetta Pedotti, Italy

Genetic and Environmental Factors in Allergic Disorders . . . . . Maritim Hotel, Rheinblick

16 *Nitration of the birch pollen allergen Bet v 1.0101 leads to immunological and structural features that may link pollution to enhanced allergy*
Albert Duschl, Austria

17 *Environment-environment interactions impacting on human health: Influence of biogenic and anthropogenic factors on birch pollen's allergenicity*
Claudia Traidl-Hoffmann, Germany

19 *Genetic variants influencing serum total IgE levels and the specific IgE response to parasites and allergens*
Nathalie Acevedo, Sweden

20 *Profiling of the rapid IgE sensitization in Netherton syndrome*
Annamari Ranki, Finland

21 *A variant of Interleukine-13 (IL13) gene is associated with food allergy in the Japanese population*
Tomomitsu Hirota, Japan

22 *The association of 36 susceptibility loci of psoriasis with atopic dermatitis and psoriasis in the Japanese population*
Mayumi Tamari, Japan

23 *Genetic and environmental factors of allergy in Lithuanian birth cohort*
Ruta Dubakiene, Lithuania

24 *A prospective nation-wide study on allergen sensitization patterns in Mexican rhinitics*
Désirée Larenas Linnemann, Mexico

Dendritic Cells, Mast Cells, Monocytes and Granulocytes . . . . . Maritim Hotel, Lowenburg

25 *Targeted delivery of signal transduction inhibitors to human allergic effector cells using highly specific nanoconjugates*
Bernhard Gibbs, United Kingdom

27 *Effects of roasting on the uptake of peanut allergen Ara h 3 by monocyte-derived dendritic cells and on the induction of basophils degranulation*
Beatriz Cabanillas, Germany

Sunday, 14 September 2014 (continued)

28 *Direct immune modulatory effect of non-digestible oligosaccharides mimicking the functionality of human breast milk oligosaccharides on human monocyte derived dendritic cells*
Leon Knippels, Netherlands

29 *In vitro generation of oral mucosal Langerhans cell-like cells from cord blood CD34+ stem cells*
Jean-Pierre Allam, Germany

30 *Clinical efficacy of allergen specific immunotherapy correlates with changes of proinflammatory (DC2)/regulatory DC ratios in peripheral blood*
Philippe Moingeon, France

31 *Regulation of CD48 in allergic inflammation*
Roopesh Singh Gangwar, Israel

32 *The stealthy fusion nano-machine*
Ilan Hammel, Israel

33 *Mast cells control skin wound infection with Pseudomonas aeruginosa*
Marcus Maurer, Germany

34 *IL-8 derived from mast cells induces epithelial-to-mesenchymal transition and stem cell features in human thyroid cancer cells*
Nella Prevete, Italy

35 *Mast cell-derived IL-1β contributes to uric acid crystal-induced acute arthritis in mice*
Laurent L. Reber, United States

37 *Cannabinoids modulate immune cell-assisted angiogenesis and lymphangiogenesis*
Rosaria I. Staiano, Italy

38 *Differences in eosinophilic and mast cell dependent mediator patterns in chronic rhinosinusitis with and without nasal polyps*
Torsten Zuberbier, Germany

39 *Yield and quality of basophil RNA are highly dependent on the RNA extraction technique*
Bettina M. Jensen, Denmark

40 *Human basophil-monocyte interaction mediated by IL-3*
Francesco Borriello, Italy

41 *Basophil-derived IL-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation*
Masato Kubo, Japan

42 *Group V secreted phospholipase A_2 mediates the production of angiogenic and anti-angiogenic factors from human neutrophils*
Stefania Loffredo, Italy

Lymphocytes and Mediators of Immunoregulation . . . . . Maritim Hotel, Ölberg

43 *The fate of IL-4-switched B cells: dynamics of IgE and IgG4 in the hyper-IgG4 syndrome*
Rob C. Aalberse, Netherlands

44 *Interleukin-21 induced IgE-synthesis in memory B cells: A role for T follicular helper cells in Seasonal Allergic Rhinitis*
Gilda Varricchi, United Kingdom

45 *HLA-class II peptide tetramers vs. allergen-induced proliferation for identification of allergen-specific CD4 T cells in the model of mugwort pollen allergy*
Beatrice Jahn-Schmid, Austria

46 *Increased expression of periostin and IL-33 in chronic rhinosinusitis with nasal polyps*
Ruby Pawankar, Japan

47 *Effects of corticosteroid on cytokine-induced periostin production by primary human lung and dermal tissue cells*
Tetsuo Shoda, Japan

48 *Platelets constitutively express interleukin-33 protein*
Tomohiro Takeda, Japan

49 *Cytokine secretion profiles in the tears of patients with chronic allergic conjunctivitis*
Naoko Okada, Japan

Sunday, 14 September 2014 (continued)

50 *In vitro effects of Interleukin-17 on cultured human bronchial epithelial cells*
Akio Matsuda, Japan

52 *In depth comparison of Monophophoryl Lipid A (MPLA) and Lipopolysaccharide (LPS): immune activation, adjuvant capacity and application as part of a MPLA:allergen fusion protein*
Stefan Schülke, Germany

Monday, 15 September 2014

7:00 – 8:00 "Life in Science" Breakfast Discussion . . . . . Steigenberger Hotel, Winter Garden
Donata Vercelli, United States

7:30 – 14:15 Registration Open . . . . . Steigenberger Hotel Foyer

7:30 – 14:15 Speaker Preview Room Open . . . . . Steigenberger Hotel, Foyer Bankettsaal

8:00 – 9:45 Oral Abstract Session 3 . . . . . Steigenberger Hotel, Bankettsaal
Lymphocytes and Mediators of Immunoregulation
Chairpersons: Hans-Uwe Simon, Switzerland
Donata Vercelli, United States

8:00 53 *The persistence of asthma requires the establishment of multiple interconnected feedback circuits involving airway epithelial cells and type 2 innate lymphoid cells but not adaptive immune cells*
Rafeul Alam, United States

8:17 54 *PGI_2 signaling inhibits IL-5 and IL-13 protein expression by group 2 innate lymphoid cell in response to inhaled aeroallergen*
R. Stokes Peebles, United States

8:34 55 *Symbiotic microbiota regulates type 2 immunity through RORgt+ Tregs*
Caspar Ohnmacht, Germany

8:51 56 *Let-7f miRNA differentially regulates IL-17A protein expression in Th17 cells from women compared to men*
Dawn C. Newcomb, United States

9:08 57 *TSLP induces corticosteroid-resistance in the IL-33/natural helper cell pathway*
Koichiro Asano, Japan

9:25 58 *Immune suppression in severe atopic dermatitis mediated by myeloid-derived suppressor cells*
Tilo Biedermann, Germany

9:42 59 *Human novel effector and regulatory subsets of memory B cells*
Mubeccel Akdis, Switzerland

9:45 – 10:30 Coffee Break . . . . . Steigenberger Hotel, Südflügel

10:30 – 13:30 Oral Abstract Session 4 . . . . . Steigenberger Hotel, Bankettsaal
Lymphocytes and Mediators of Immunoregulation
Chairpersons: Heimo Breiteneder, Austria
Hannah Gould, United Kingdom

10:30 60 *Generation and characterization of allergen-specific B cell clones from tolerant and allergic individuals*
Willem Van de Veen, Switzerland

10:47 61 *Follicular helper T cells mediate IgE antibody production in response to airborne allergens in mice*
Hirohito Kita, United States

11:04 62 *Influence of natural pollen exposure on local and peripheral IgE repertoires in allergic rhinitis revealed by next-generation sequencing*
Yu-Chang B. Wu, United Kingdom

11:21 63 *MicroRNA-155 deficiency affects allergen-induced CD4+ T cell plasticity*
Madeleine Rådinger, Sweden

Monday, 15 September 2014 (continued)

11:38 – 11:55 Break . . . . . Foyer Bankettsaal

Allergen Specific Immunotherapy
Chairpersons: Mübeccel Akdis, Switzerland
Stephen Durham, United Kingdom

11:55 64 *Diversity of allergen-specific IgG4 responses during birch pollen immunotherapy*
Barbara Bohle, Austria

12:12 65 *Seasonal increases in peripheral innate lymphoid type 2 cells (ILC2s) are inhibited by grass pollen immunotherapy*
Mohamed H. Shamji, United Kingdom

12:29 66 *Facilitated induction of Tregs by anti-IL-4: A RDBPC immunotherapy under the umbrella of anti-IL-4*
Carsten B. Schmidt-Weber, United Kingdom

12:46 67 *Safety and tolerability of multiple food oral immunotherapy with and without Omalizumab*
Kari C. Nadeau, United States

13:03 68 *Vaccination with SG100 attenuates aeroallergen-induced early and late phase asthmatic responses in house dust mite sensitive non-human primates*
Geert C. Mudde, Austria

13:20 69 *A recombinant mutant of the major fish allergen parvalbumin shows reduced allergenic activity in skin tested fish allergic children and reduces allergic symptoms in a mouse model of fish allergy by blocking antibodies*
Birgit Linhart, Austria

13:30 – 15:00 Lunch . . . . . Steigenberger Hotel, Südflügel

15:45 – 19:00 Registration Open . . . . . Maritim Hotel Foyer

16:00 – 17:00 Relaxing Lecture . . . . . Maritim Hotel, Maritim Hall
The secret of sacred lotus: Learning from biological surfaces

Chairperson: Thomas Bieber, Germany

Prof. Dr. Wilhelm Barthlott
University of Bonn

Wilhelm Barthlott is a German botanist and bionics expert. He studied biology at the University of Heidelberg, Germany. Barthlott's areas of specialization are systematics and biodiversity research, with the main focus devoted to tropical ecosystems and the global distribution of biodiversity. He is one of the pioneers in the field of biological and technical interfaces. Based on his systematic research on scanning electron microscopy of plant surfaces, he developed self-cleaning (lotus effect) technical surfaces and, in recent years, surfaces which permanently retain air under water. This led to a paradigm shift in particular areas of materials science and facilitated the development of superhydrophobic biomimetic surfaces. This technology has successfully been launched on the market under the trademark Lotus-Effect®.

Barthlott has been honored with various awards and prizes, e.g. the German Environment Prize (Deutscher Umweltpreis). He is a member of the Academy of Sciences and Literature of Mainz, the North Rhine-Westphalia Academy of Sciences and Arts, the German Academy of Sciences Leopoldina, and is a Foreign Member of the Linnean Society of London.

Monday, 15 September 2014 (continued)

17:00 – 19:30 **Poster Session 2 . . . . . Maritim Hotel**

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

Chairpersons: Kurt Blaser, Switzerland
Mariana C. Castells, United States
Thomas A. E. Platts-Mills, United States

Conventional and Novel Biomarkers of Asthma And COPD . . . . . Maritim Hotel, Petersberg

70 *Clinical review of 18 patients with late-onset anaphylaxis after ingestion of Bacillus subtilis-fermanted sybeans (natto)*
Zenro Ikezawa, Japan

71 *The usefulness of thymus and activation-regulated chemokine (TARC) in early diagnosis of typical DIHS, in which reactivation of HHV-6 is observed*
Zenro Ikezawa, Japan

Allergens and Diagnosis of Allergy . . . . . Maritim Hotel, Petersberg

72 *A quantitative multiplex immunoassay for food allergen proteins*
James P. Hindley, United Kingdom

73 *Allergen food matrix interaction – impact on allergenicity*
Karin Hoffmann-Sommergruber, Austria

74 *Detection of food allergen-specific immunoglobulin free light chains in serum of food-intolerant patients*
Frank A. Redegeld, Netherlands

75 *Periostin as a novel clinical biomarker of disease severity and chronicity in patients with atopic dermatitis*
Yukie Yamaguchi, Japan

76 *Systems biology to understand the reaction of the allergenic pollen from common ragweed (Ambrosia artemisiifolia) to air pollutions and climate change*
Ulrike Frank, Germany

77 *Natural variability of allergen release from plants (olive, birch, and grass pollen) and animals (cats)*
Jeroen Buters, Germany

78 *Low molecular weight factors from pollen mediate aggravation of the allergic immune response to pollen allergen in humans*
Stefanie Gilles-Stein, Germany

79 *House dust mite allergens manipulate barrier integrity and immune balance*
Erika Jensen-Jarolim, Austria

80 *Bet v 1, a lipocalin-like allergen, whose function depends on iron*
Franziska Roth-Walter, Austria

81 *Structure-function analysis of the highly immunogenic protein tropomyosin – a major cross-reactive pan-allergen in allergic sensitization*
Andreas L. Lopata, Australia

82 *Identification of the muscle protein, myosin light chain 1, as an important chicken meat allergen*
Ines Swoboda, Austria

83 *Analysis of Can f 5 content in urine and fur samples from male and female dogs*
Jonas Lidholm, Sweden

84 *In vitro immunomonitoring of Hymenoptera venom-allergic patients*
Liliana Cifuentes, Germany

85 *IgE and IgG4 profiles to a panel of recombinant CCD free honeybee venom allergens in honeybee venom allergic patients*
Edzard Spillner, Denmark

86 *Investigating the delayed adverse reactions to non-steroidal anti-inflammatory drugs by an ex-vivo stimulation test: A new safety approach to support the drug allergy diagnosis*
Massimo Caruso, Italy

Monday, 15 September 2014 (continued)

87 *Basophil activation and skin tests with cetuximab in patients sensitized to Galactose-alpha-1,3-Galactose*
Andreas J. Bircher, Switzerland

88 *Allergy to formaldehyde: basophil histamine-release test with a modification is useful for diagnosis*
Masao Yamaguchi, Japan

89 *Basophil activation test as a biomarker in allergic patients to platins undergoing rapid desensitization*
Mariana C. Castells, United States

90 *IgE-mediated sensitization against storage pests: relevance of cross-reactivity and improvement of diagnostics*
Monika E. Raulf-Heimsoth, Germany

Allergen Specific Immunotherapy . . . . . Maritim Hotel, Siebengebirge

91 *Immune mechanisms of induction and long-term maintenance of allergen tolerance in patients allergic to hymenoptera venom*
Wolfgang Pfüetzner, Germany

92 *Grass pollen immunotherapy: suppression of nasal symptom scores after nasal allergen challenge correlates with nasal fluid IL-9 concentrations*
Stephen R. Durham, United Kingdom

93 *Frequency of CD39+ cells in the upper airways – a marker of tolerance in hayfever?*
Adam Chaker, Germany

94 *Nasal inhibitory IgG4 antibodies: potential biomarkers for monitoring grass pollen immunotherapy*
Stephen R. Durham, United Kingdom

95 *Birch pollen immunotherapy in mice: inhibition of Th2 inflammation is not sufficient to decrease airway hyper-reactivity*
Hanneke PM Van Der Kleij, Netherlands

96 *Observations on skin test reactivity and allergen-specific IgE in ragweed-allergic patients*
Peter S. Creticos, United States

97 *Neuraminidase-coated, allergen-loaded microparticles are a safe and efficient novel treatment option for food allergy*
Eva Untersmayr-Elsenhuber, Austria

98 *Peanut allergy can be cured by oral immunotherapy*
Anne D. Moneret-Vautrin, France

99 *Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3)*
Kari C. Nadeau, United States

100 *Nanoparticles as new adjuvant for peanut oral immunotherapy*
Marta Ferrer Puga, Spain

101 *Oral immunotherapy for cow's milk and egg allergy with heat modified milk and egg derivatives*
Antonella M. Muraro, Italy

102 *Peptide immunotherapy is not associated with deletion of allergen-specific T cells, or modulation of chemokine receptor expression*
Mark Larche, Canada

103 *Development of a new SQ HDM SLIT-tablet for house dust mite allergy immunotherapy*
Jorgen Larsen, Denmark

104 *AAAAI membership experience with allergen immunotherapy safety in patients with special medical conditions varies according to practice characteristics*
Désirée E. Larenas Linnemann, Mexico

Tuesday, 16 September 2014

7:00 – 8:00 "Life in Science" Breakfast Discussion . . . . . Maritim Hotel, Room Gourmet
Bruce S. Bochner, United States

7:30 – 10:30 Registration Open . . . . . Maritim Hotel Foyer

7:30 – 10:30 Speaker Preview Room Open . . . . . Maritim Hotel

8:00 – 9:30 Oral Abstract Session 5 . . . . . Maritim Hotel, Maritim Hall
Allergens, Antigen Presentation and Diagnosis of Allergy
Chairpersons: Barbara Bohle, Austria
Peter Weller, United States

8:00 105 *Natural clinical tolerance to peanut in African patients is caused by poor allergenic activity of peanut IgE*
Marianne van Hage, Sweden

8:17 106 *Bet v 1 binds lipids from birch and grass pollen but not from peanuts*
Heimo Breiteneder, Austria

8:34 107 *Serum periostin is a biomarker reflecting tissue remodeling in bronchial asthma*
Kenji Izuhara, Japan

8:51 108 *Live imaging of the skin immune responses: Identification of inducible skin associated lymphoid tissue (iSALT)*
Kenji Kabashima, Japan

9:30 – 10:30 Carl Prausnitz Lecture . . . . . Maritim Hotel, Maritim Hall
Contributions of mast cells and IgE to innate and acquired resistance to venoms: Is this the "good side" of allergy?

Chairperson: Gianni Marone, Itally

Stephen J. Galli, MD

Stephen J. Galli, MD, is chair of the Department of Pathology, the Mary Hewitt Loveless, MD Professor, and a professor of pathology and of microbiology and immunology at Stanford.

Steve investigates the development and function of mast cells and basophils and has developed new animal models for analyzing the roles of these cells in health and disease. This work has elucidated diverse functions of mast cells in models of allergic diseases and other disorders, and also has shown that mast cells can contribute to innate and, along with IgE antibodies, adaptive immune responses that increase host resistance to animal venoms.

Steve was president of the *American Society for Investigative Pathology* (2005-2006) and has been elected to the *Pluto Club (Association of University Pathologists*), the *Collegium Internationale Allergologicum* (serving as president from 2010-2014), the *American Society for Clinical Investigation*, the *Association of American Physicians*, and *the Institute of Medicine of the U.S. National Academies*, and is a foreign member of the *Accademia Nazionale dei Lincei* in Rome.

11:00 – 19:00 Lunch and Boat Ride . . . . . Departs from Maritim Hotel

19:00 – 22:00 Informal Dinner . . . . . Rheinhotel Dreesen

Wednesday, 17 September 2014

7:00 – 8:00 "Life in Science" Breakfast Discussion . . . . . Steigenberger Hotel, Winter Garden
Mübeccel Akdis and Cezmi Akdis, Switzerland

7:30 – 13:30 Registration Open . . . . . Steigenberger Hotel Foyer

7:30 – 13:30 Speaker Preview Room Open . . . . . Steigenberger Hotel, Foyer Bankettsaal

8:00 – 10:15 Oral Abstract Session 6 . . . . . Steigenberger Hotel, Bankettsaal
Treatment of Immune Disorders
Chairpersons: Heidrun Behrendt, Germany
Marcus Maurer, Germany

8:00 109 *Targeting IgE production using antibodies against the M1 prime segment of human membrane IgE*
Lawren Wu, United States

8:17 110 *Critical role for IL-27 in induction of Foxp3- regulatory T cells by retinoic acid-differentiated dendritic cells, and their role in reversal of anaphylaxis sensitivity*
John Robert Gordon, Canada

8:34 111 *Development of oral plasma kallikrein inhibitors for prophylactic treatment of hereditary angioedema*
Yarlagadda Babu, United States

8:51 112 *IL-31 regulates skin barrier and antimicrobial function through IL-1 signaling*
Jens M. Baron, Germany

Anaphylaxis, COPD, Allergic Colitis, and Eczema
Chairpersons: Bruce S. Bochner, United States
Ruby Pawankar, Japan

9:08 113 *Delayed anaphylaxis to red meat and the nature of the IgE response to galactose alpha-1, 3-galactose*
Thomas A. E. Platts-Mills, United States

9:25 114 *Structure-function relationships that govern FceRI signaling by allergens*
Bridget Wilson, United States

9:42 115 *Importance of mast cell Prss31/transmembrane tryptase/tryptase gamma in lung function and experimental chronic obstructive pulmonary disease and colitis*
Richard Stevens, United States

9:59 116 *Inhibition of allergen-induced colitis by activated regulatory T cells in a humanized mouse model*
Joachim Saloga, Germany

10:15 – 10:45 Coffee Break . . . . . Steigenberger Hotel, Südflügel

10:45 – 12:10 Oral Abstract Session 7 . . . . . Steigenberger Hotel, Bankettsaal
Pathophysiology of Allergic Disorders and Inflammation, Regulation of Humoral and Cellular Immune Responses, Tolerance, and Effects of Virus Infections
Chairpersons: Kenji Izuhara, Japan
A. Barry Kay, United Kingdom

10:45 117 *Benign versus pathologic aeroallergen-specific Th2-immunity: maturation of disease-associated immunophenotypes involves parallel changes in both humoral and cellular response profiles*
Patrick G. Holt, Australia

11:02 118 *Targeted inhibition of IgE:FcεRI signals during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells*
Oliver T. Burton, United States

11:19 119 *Dissecting cellular targets of IL-10 in allergen specific tolerance induction*
Thilo Jakob, Germany

11:36 120 *Bacterial modulation of epithelial innate immune responses to human rhinovirus infection*
David Proud, Canada

11:53 121 *A critical role for IgE in pulmonary vascular leak during an anti-viral immune response*
Mitchell H. Grayson, United States

Wednesday, 17 September 2014 (continued)

12:10 – 13:10 **Paul Kallós Lecture** . . . . . **Steigenberger Hotel, Bankettsaal**
Genetic analysis of immune responses

Chairperson: Stephen J. Galli, United States

Bruce Beutler, MD
Regental Professor and Director of the Center for Genetics of Host Defense
University of Texas Southwestern Medical Center

Bruce Beutler is a Regental Professor and Director of the Center for the Genetics of Host Defense at UT Southwestern Medical Center in Dallas, Texas. He received his medical training at the University of Chicago, graduating in 1981. As a postdoctoral fellow at The Rockefeller University (1983-1986), he isolated mouse tumor necrosis factor (TNF) and discovered its importance as a mediator of inflammation. Subsequently, at UT Southwestern, he analyzed mammalian responses to bacterial lipopolysaccharide. This work culminated in the identification of Toll-like receptors as key sensors of the innate immune system, used to detect infection. In further studies, Beutler employed a forward genetic strategy to elucidate many aspects of mammalian immunity.

He has received numerous awards for his work including the Balzan Prize (2007), the Albany Medical Center Prize (2009), the Shaw Prize (2011), and election to both the US National Academy of Sciences and the Institute of Medicine (2008). In 2011, he shared the Nobel Prize in Physiology or Medicine for "discoveries concerning the activation of innate immunity."

13:10 – 13:40 CIA Business Meeting . . . . . Steigenberger Hotel, Bankettsaal

13:40 – 15:00 Lunch . . . . . Steigenberger Hotel, Südflügel

15:30 – 17:30 Registration Open . . . . . Maritim Hotel Foyer

15:30 – 18:00 **Poster Session 3** . . . . . **Maritim Hotel, Drachenfels**
Treatment of Immune Disorders, Pathophysiology of Allergic Disorders and Inflammation, Urticaria and Angioedema, Clinical Aspects of Allergic Disorders
Chairpersons: Susan M. MacDonald, United States
Yoseph Mekori, Israel
Sally Wenzel, United States

Treatment of Immune Disorders

51 *Escherichia coli heat-labile detoxified enterotoxin modulates dendritic cell function and attenuates allergic airway inflammation*
Jiu-Yao Wang, Taiwan

122 *Rapid antigen desensitization through the IgE receptor on wild type and humanized FcεRIα murine bone marrow-derived mast cells*
Mariana C. Castells, United States

123 *Lactobacillus strains differentially activate immunomodulatory and neuromodulatory pathways to attenuate symptoms of food allergy*
Paul Forsythe, Canada

124 *Vitamin D inhibits human innate inflammatory cytokine responses following bacterial (TLR4) but not TLR7/8 or respiratory virus stimulation*
Kent T. HayGlass, Canada

125 *Involvement of the bioactive peptide prokineticin 2 in autoimmune demyelinating disease of the central nervous system*
Rosetta Pedotti, Italy

126 *The N-formyl peptide receptors (FPRs) expression and functions in systemic sclerosis*
Francesca Wanda Rossi, Italy

127 *Rule of different memory cells in diagnosis of common variable immunodeficiency and specific antibody deficiency*
Amer M. Khojah, United States

Wednesday, 17 September 2014 (continued)

Pathophysiology of Allergic Disorders and Inflammation

128 *The signal molecule, Regulator of Calcineurin 1 (RCAN1), is a potential regulator of sensitivity to anaphylaxis in humans*
Lars K. Poulsen, Denmark

129 *Association of serum zinc concentration with allergic disorders in Korean National Health and Nutrition Examination Survey*
Ho Joo Yoon, Korea

130 *Human Rhinovirus-induced airway smooth muscle chemotaxis: a potential mechanism for airway remodeling in asthma*
Richard Leigh, Canada

131 *Diverse allergens require MD2-mediated neutrophil recruitment to mount allergic airway inflammation*
Sanjiv Sur, United States

132 *T cell induced-bronchoconstriction in vitro and in vivo*
Akio Mori, Japan

133 *Reduced anti-viral innate immunity in severe asthma is associated with neutrophilic inflammation and high dose inhaled steroids*
John W. Upham, Australia

134 *Regulation of proteinase activated receptor-2 (par-2) on airway epithelium*
Harissios Vliagoftis, Canada

135 *Interleukin 33 exacerbates allergic airway inflammation and bronchoconstriction via modulation of mast cell responses*
Gunnar Nilsson, Sweden

136 *A role for mast cell tryptase in histone modification*
Gunnar Pejler, Sweden

137 *The formyl peptide receptor 1 exerts a tumor suppressor role in human gastric cancer by suppressing angiogenesis*
Nella Prevete, Italy

138 *Evolution of antibody responses to allergenic molecules in childhood and implications for immunological intervention*
Paolo M. Matricardi, Germany

139 *In vivo effects of silica crystals on airway inflammation in mice*
Hirotoshi Unno, Japan

140 *Effects of a new potent PARP-1/PARP-2 inhibitor in an in vivo murine model of bleomycin-induced lung fibrosis*
Emanuela Masini, Italy

141 *Interleukin-32 attenuates collagen production in fibroblasts via modulation of focal adhesion kinase signaling*
Hee-Bom Moon, Korea

142 *"Auto-anti-IgE": naturally occurring IgG anti-IgE antibodies may inhibit allergen-induced basophil activation*
Christopher J. Corrigan, United Kingdom

143 *Of mice and not men: Vitamin D3 differentially induces TSLP in mouse and human skin*
Edward F. Knol, Netherlands

144 *What can we learn from East-West German-reunification about causes of the allergy epidemic?*
Johannes Ring, Germany

145 *Histamine 4 receptor stimulation modulates the differentiation process and chemokine release in human macrophages*
Alexander Kapp, Germany

146 *Histamine and histamine receptor expression in mucosal inflammatory diseases*
Sylwia Smolinska, Poland

147 *The effect of oral challenge with wheat protein in mice systemically or percutaneously sensitized with hydrolyzed wheat protein*
Hiroyuki Tanaka, Japan

Wednesday, 17 September 2014 (continued)

148 *Advertisements impact the physiological efficacy of a branded drug*
Robert M. Naclerio, United States

Urticaria and angioedema

149 *Hyperexpression of Mas-related gene X2 (MrgX2) in skin mast cells of severe chronic urticaria patients; MrgX2 is a . . receptor for basic eosinophil granule proteins*
Yoshimichi Okayama, Japan

150 *Enhanced expression of vasoactive mediators (CGRP, VEGF and IL-25) and increased vascularity in weals from skin biopsies in chronic spontaneous urticaria*
A. Barry Kay, United Kingdom

151 *Expression of IL-33 in lesional skin in chronic spontaneous urticaria*
A. Barry Kay, United Kingdom

152 *Aspirin-exacerbated cutaneous disease: A subphenotype of chronic urticaria*
Mario Sanchez-Borges, Venezuela

153 *Management of hypersensitivity reactions from intravenous iron compounds*
Andreas J. Bircher, Switzerland

Clinical Aspects of Allergic Disorders

154 *Long-term safety of replication-defective smallpox vaccine (MVA-BN) in atopic eczema and allergic rhinitis*
Ulf G. Darsow, Germany

155 *A randomized, controlled intervention trial of early emollient use in prevention of atopic dermatitis and allergic sensitization during infancy*
Yukihiro Ohya, Japan

156 *Classification of elderly asthma phenotypes and its implementation*
You-Young Kim, Korea

157 *Late-onset asthma in the elderly: roles of upper airway diseases and staphylococcal enterotoxin sensitization*
Sang Heon Cho, Korea

158 *Serial oral provocation tests in ASA-Intolerance Syndrome (M. Samter) demonstrate a high variance of results*
Ludger Klimek, Germany

160 *Asthma susceptibility genes: Which genetic variants are important?*
Eugene R. Bleecker, United States

161 *Association of rare variants in β2-Adrenergic receptor pathway genes with asthma severity in African Americans*
Deborah A. Meyers, United States

19:00 – 23:00 Gala Dinner . . . . . Steigenberger Hotel, Rotunde Room
19:00 Reception
19:30 Concert
20:15 Dinner/Awards

Thursday, 18 September 2014

7:00 – 8:00 "Life in Science" Breakfast Discussion . . . . . Steigenberger Hotel, Winter Garden
Stephen J. Galli, United States

7:30 – 13:00 Registration Open . . . . . Steigenberger Hotel Foyer

7:30 – 13:00 Speaker Preview Room Open . . . . . Steigenberger Hotel, Foyer Bankettsaal

7:30 – 8:30 Poster Dismantle . . . . . Maritim Hotel

8:00 – 9:00 CIA Council Meeting . . . . . Steigenberger Hotel, Salon Drachenfels

9:00 – 10:30 **Oral Abstract Session 8 . . . . . Steigenberger Hotel, Bankettsaal**
Pathophysiology of Allergic Disorders and Inflammation

Epithelial and Airway Function and Pathology in Asthma
Chairpersons: Francesca Levi-Schaffer, Israel
Johannes Ring, Germany

9:00 162 *Epigenetic mechanisms for the regulation of barrier integrity and bronchial epithelial tight junctions in asthma*
Cezmi Akdis, Switzerland

9:17 163 *Epigenetic regulation as an important mechanism in the origin and early development of allergy and asthma*
Harald E. Renz, Germany

9:34 164 *Loss of normal peripheral airway bronchial epithelial gene profile in severe asthma*
Peter H. Howarth, United Kingdom

9:51 165 *The airway mucin Muc5b is an endogenous ligand for Siglec-F on mouse eosinophils and reduces eosinophil survival in vitro and in vivo*
Bruce S. Bochner, United States

10:08 166 *Type 1 and Type 2 immunity in severe asthma: Convergence at the airway epithelium*
Sally E. Wenzel, United States

10:30 – 11:00 Coffee Break . . . . . Steigenberger Hotel, Südflügel

11:00 – 13:00 Oral Abstract Session 9 . . . . . Steigenberger Hotel, Bankettsaal
Pathophysiology of Allergic Disorders and Inflammation

Skin Barrier Function, Eczema, Contact Dermatitis and Auto-IgE
Chairpersons: Thomas Bieber, Germany
Natalija Novak, Germany

11:00 167 *RabGEF1, a negative regulator of thymic stromal lymphopoietin production and skin inflammation*
Mindy Tsai, United States

11:17 168 *Distinct mechanisms for allergic sensitization to protease antigen between skin and airway*
Toshiro Takai, Japan

11:34 169 *The BET family of epigenetic readers contributes to chronic airways inflammation*
Cara Williams, United States

11:51 170 *The heterogeneous molecular signature of eczema subtypes*
Stefanie Eyerich, Germany

12:08 171 *Th17 cells and tissue remodeling in atopic and contact dermatitis*
Dagmar Simon, Switzerland

12:25 172 *Autoimmunity ("autoallergy") – a driving mechanism in the perpetuation of atopic dermatitis?*
Thomas Werfel, Germany

12:42 173 *The story goes on: Auto-IgE represents a natural response that is not necessarily associated with pathology in childhood*
Jan Gutermuth, Belgium

1

Neonatal DNA methylation signatures in immunoregulatory pathways predict asthma during childhood

Avery DeVries[1*], Gabriela Wlasiuk[1*], Susan J. Miller[1], Anthony Bosco[2], Debra A. Stern[1], Jessie Nicodemus-Johnson[3], Anya C. Jones[2], Janet Rothers[1], I. Carla Lohman[1], Anne L. Wright[1], Marilyn Halonen[1], and Donata Vercelli[1]

[1]University of Arizona, Tucson, AZ, USA; [2]University of Western Australia, Perth, Australia; [3]University of Chicago, Chicago, IL, USA
*These authors contributed equally to this work

Background: Asthma, the most prevalent chronic disease of childhood, is thought to begin during the pre-school years even when chronic symptoms appear in early adulthood. However, firm diagnostic criteria to pinpoint the true beginning of the asthma trajectory are still lacking. Immune alterations accompany and often precede the diagnosis of childhood asthma, but the underlying pathways remain unknown. Because environmental and developmental factors are essential for asthma pathogenesis, epigenetic mechanisms are plausible contributors to disease risk. Indeed, epigenetic mechanisms provide the plasticity required for biological responses to environmental cues and the timed unfolding of developmental processes.

To define when the trajectory to asthma begins and identify the underlying pathways, we performed a genome-wide search for DNA methylation signatures predictive of childhood asthma in cord blood mononuclear cells from children enrolled in the Infant Immune Study, an unselected birth cohort in which asthma and immunity were closely monitored from birth to age 9. We reasoned that the detection at birth of differentially methylated regions (DMRs) associated with development of asthma in childhood would both support a perinatal origin of the disease and highlight epigenetic mechanisms potentially involved in asthma inception.

Methods: Genome-wide DNA methylation was assessed using 2.1M Human Promoter Deluxe microarrays (Roche-NimbleGen).

Results: The immune methylome differed between neonates who did and neonates who did not develop asthma by age 9. *Asthma-associated DNA methylation signatures were detected in 589 regions* significantly enriched for intergenic locations and DNase I hypersensitive sites. Analysis of a molecular interaction network of genes containing asthma-associated DMRs identified TGFB1, an innate cytokine critical for immunoregulation, as a major hub. DNA methylation levels in DMRs that mapped to the TGFB1 pathway were significantly associated with the capacity to produce TGF-b1 in neonates who did not become asthmatic but not in neonates diagnosed with asthma by age 9.

Conclusions: In children who will be diagnosed with asthma during childhood, a rewired methylome is in place already at birth and may promote a trajectory to asthma by altering responsiveness to innate immunoregulatory signals. This is the first study that identified epigenetic predictors of childhood asthma in the neonatal methylome.

2

Allergic sensitization and current allergen exposure: Big data from the U.S. National Health and Nutrition Examination Survey (NHANES)

Martin Chapman, Herman Mitchell, Agustin Calatroni, Renee Jaramillo, Eva King, Paivi Salo and Darryl Zeldin

Background: The relationship between allergic sensitization and current allergen exposure is a major public health issue which has not previously been investigated in a large study of the general population. The NHANES 2005-2006 was the first, large (N=10,348) population-based study to provide nationally representative data on both allergic sensitization and indoor allergen exposures in the U.S. population. Here, we present data on current allergen exposures among NHANES participants and factors that influence allergen levels in the home.

Methods: A reservoir dust sample (bedding and bedroom floor) was obtained from 6957 NHANES subjects and analysed for eight allergens (Der p 1, Der f 1, Fel d 1, Can f 1, Bla g 2, Rat n 1, Mus m 1 and Alt a 1 using multiplex array technology (MARIA). This generated 58,500 allergen test results and 5,868 bench QC data points. Exposure data were analysed according to prevalence, subject and housing characteristics and whether allergic sensitization was predictive of allergen exposure.

Results: While over 2/3 of participants had detectable allergen at levels considered above conventional asthma morbidity 'threshold' values, an unexpected finding was that in 32% of participants' homes, allergen levels in the home did not exceed morbidity thresholds. Cat and dog allergens were the most common allergens above the morbidity threshold (29-35%), while exposure to cockroach, mouse and rat allergens was least common (7%, 1.5% and 0.7%, respectively). Exposure to cat and dog was significantly associated with non-Hispanic whites ($p<0.01$) and with older single family housing. Allergen specific IgE was not a strong predictor of allergen exposure. In subjects with allergen specific IgE >3.5kU/L (CAP class3), exposure levels greater than threshold were found in 44.2% of dog allergic subjects, 37.8% cat, 7.3% mouse, 35.5% *D. farinae* and 20.6% *D. pteronyssinus*.

Conclusions: The results show a high prevalence of environmental allergen exposures in U.S. homes, though to a large extent the nature of these exposures was not strongly predictable from housing characteristics or from allergen specific IgE. Current asthma guidelines and practice parameters use allergen specific IgE as a surrogate for assessing likely allergen exposure. The NHANES data suggests that the level of allergen specific IgE is not a good predictor of allergen exposure in the home. The data also suggests that allergen exposure should be taken into account in studies of immunotherapy using environmental allergens.

3

Micro-RNA-mediated transcriptional regulation of the high-affinity receptor for IgE on human Langerhans cells

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Background: Epidermal Langerhans cells (LC) and other dendritic cells (DC) from patients with atopic dermatitis (AD) express the high-affinity receptor for IgE (FceRI). We showed previously that Toll-like receptor 2 (TLR2) stimulation can down-regulate FceRI on transcriptional level on human LC via transcription factor (TF) PU.1. TLR4 signaling leads to an up-regulation of Micro-RNA (miR)-155 and down-regulation of PU.1 in B cells and DC. We speculated that miR-155 is involved in TLR2-mediated down-regulation of FceRI. 1,25-dihydroxyvitamin D3 (calcitriol) is an immune regulator and can inhibit DC maturation. Aryl hydrocarbon receptor (AHR), an environmentally responsive TF, is thought to be a crucial factor that regulates immune responses. We analyzed maturation and FceRI expression after AHR engagement with its agonist 3-methyl cholanthrene (3-MC) and calcitriol treatment in CD34LC.

Methods: Human LC were generated *in vitro* from cord blood-derived CD34+ hematopoietic stem cells (CD34LC) which express TLR2 and FceRI. TLR2-, calcitriol- or 3-MC-mediated impact on LC maturation, expression of FceRI, its known TF and miR was analyzed on the protein (flow cytometry, western blotting) and RNA level (real-time PCR). Lipofection of CD34LC with pre-miR has been performed and changes in receptor and its TF expression were analyzed as described.

Results: In human CD34LC, stimulation of TLR2 resulted in down-regulation of FceRI surface expression at transcriptional level in a PU.1-dependent manner. MiR-155 was up-regulated upon TLR2 engagement. Over-expression of miR-155 resulted in down-regulation of PU.1 and subsequently FceRI, independent from TLR2 engagement. Calcitriol down-regulated FceRI in a maturation-independent manner. PU.1 and miR-155 were little affected, indicating a different mechanism than TLR2-induced FceRI regulation. Additionally, we found that 3-MC down-regulated FceRI in the absence of LC maturation, too.

Conclusions: Taken together, we show that in human LC TLR2-mediated regulation of FceRI expression via TF PU.1 involves miR-155 and FceRI expression can be regulated by the mean of pre-miR-155. Calcitriol and 3-MC down-regulate FceRI expression independent from CD34LC maturation. The underlying molecular mechanism remain to be elucidated but differ from those of TLR2 signaling. Understanding the regulation of FceRI expression on LC in the skin could open new therapeutic avenues in AD.

4

The human lung in 3-D: The use of micro-CT

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Background and Method: Microfocus X-ray micro-computed tomography(μ-CT) offers complete, high resolution, non-destructive 3D imaging of the structural features in a given volume of tissue. We have demonstrated that we can reconstruct the network of small airways(<2mm diameter) and vessels in samples of formalin fixed, paraffin embedded human lung tissue using μ-CT with a resolution of 3-10 microns. Using this technique we examined the relationship between small airways and vasculature in peripheral lung tissue of patients with normal airways function and diseases such as COPD, asthma and IPF and correlated our findings with traditional histological analysis.

Results: Healthy lung revealed a wealth of structural detail and the main architectural features could be identified by simple thresholding and semi-automatic segmentation. The small airways could be followed down to the alveoli budding from the respiratory bronchioles. The complex branching nature of the small airways was clearly discernible in 3D and closely paralleled by the accompanying vessels. In contrast, emphysematous lesions were evident in a patient with moderate COPD with obliteration of the small airways and airway wall thickening. The increased secretions in the COPD tissue meant it was not possible to set an appropriate threshold value and the airways and vessels were segmented out using a manual approach. Blockages in the small airways were common in the COPD tissue but rare in the healthy lung. Sectioning of the tissue blocks revealed an excellent correlation between features identified on micro-CT and traditional histological analysis. We used immunohistochemistry to verify the small airways and vessels and extended these studies to stain lymphatic vessels and mast cells in the tissue. Correlating these images with the segmented images from the μ-CT allows us to produce a 3D image dataset of the peripheral airways showing the distribution of the mast cells in relation to the small airways, the vasculature and the lymphatic vessels.

Conclusion: In summary, we have shown that it is possible to non-destructively obtain a 3D representation of the small airways, vasculature and lymphatics in the peripheral lung of healthy patients and those with different lung diseases and confirm our findings using traditional histology.

5

Differential roles of BCL-XL and MCL-1 in the development of mast cells

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BCL-XL and MCL-1 are anti-apoptotic members of the BCL-2 protein family, which play essential roles during embryogenesis and are indispensable for survival of many cell types. Recent studies suggest that BCL-XL and MCL-1 play a role in the development and homeostasis of mast cells.

To explore the roles of BCL-XL and MCL-1 in mast cells, we crossed *Bcl-xfl/fl* and *Mcl-1fl/fl* mice (in which the *Bcl-x* or *Mcl-1* genes are flanked with loxP sites and therefore can be deleted by a CRE recombinase) to the *Mcpt5Cre* strain, which expresses CRE selectively in connective tissue type mast cells. *Mcpt5Cre/Bcl-xfl/fl* mice had a relatively minor reduction of mast cell counts in the tongue, heart and peritoneal cavity (40-55% reduction). Consequently, there was no impairment of IgE-mediated passive systemic anaphylaxis in these animals. *In vitro* studies confirmed that BCL-XL is not essential for the development or sustained survival of mast cells. Conversely, *Mcpt5Cre/Mcl-1fl/fl* mice showed complete ablation of mast cells in various tissues, such as the dermis of back skin, mesentery, tongue, heart as well as submucosa and muscularis of the glandular stomach (<1% mast cells compared to control mice). We also observed significantly reduced numbers of mast cells in the peritoneal cavity and ears of *Mcpt5Cre/Mcl-1fl/fl* mice. In contrast, and as expected from the expression of this *Cre* transgene, the numbers of T cells, B cells, dendritic cells, macrophages and granulocytes were normal in these animals. Thus, *Mcpt5Cre/Mcl-1fl/fl* mice show specific depletion of connective tissue type mast cells. Accordingly, *Mcpt5Cre/Mcl-1fl/fl* mice were completely protected from IgE-mediated passive systemic anaphylaxis.

In conclusion, our studies define for the first time differential roles of BCL-XL and MCL-1 in the development and sustained survival of mast cells. The *Mcpt5Cre/Mcl-1fl/fl* strain represents a new mast cell-deficient mouse model, which will be useful to specifically analyze functions of connective tissue type mast cells in immune responses and pathologic processes.

6

Rab5 is a novel regulator of mast cell secretory granule biogenesis

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Background: Secretion of inflammatory mediators prestored in mast cells secretory granules (SGs) enhances immune responses such as in allergy and host defenses. Yet, the mechanisms underlying the biogenesis of the SGs remain largely unresolved. Rab GTPases are master regulators of vesicular trafficking that mediate vesicle transport, tethering and fusion within the secretory and endocytic pathways. Therefore, unveiling the Rab networks associated with a cellular process provides invaluable tools for decoding the intermediate pathways and mechanisms involved in such process regulation or execution. Along this line of thoughts, we recently developed a high-resolution imaging-based methodology that allowed screening Rab GTPases for their phenotypic and functional features. This screen identified 30 Rabs as general regulators of exocytosis in the widely studied mast cell line, RBL-2H3 [Azouz et al., J. Immunol. 189, 2169-2180, (2012)]. Intriguingly, our screen implicated Rab5, an established regulator of endocytosis and endosomal fusion, as potential regulator of the SG size.

Method: To explore the mechanism by which Rab5 controls the SG size, we combined high-resolution live cell confocal imaging, electron microscopy and quantitative morphometric analyses.

Results: We show that Rab5 promotes SG homotypic fusion in a VAMP8-dependent fashion. We also show that Rab5 controls the SG cargo composition by mediating SG fusion with early endosomes. Finally, we show that Rab5 mediated SG fusion precedes their maturation process as reflected by the replacement of Rab5 by Rab27B.

Conclusions: Collectively, our results assign Rab5 a key role in controlling the amount, composition and rate of secretion of the SGs content and maintaining the communication between new and pre-existing SGs.

7

Virus-infected human mast cells enhance Natural Killer cell functions

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Background: Mast cells are most frequently studied in the context of allergic disease, however, they also have important roles as sentinel cells in host defence against infection. Recently, human mast cells have been shown to produce chemokines that selectively recruit human natural killer (NK) cells in models of viral infection. We therefore examined whether virus infected mast cells could further activate NK cells to enhance anti-viral immunity.

Methods: The ability of virus infected human mast cells to enhance the function of human NK cells was examined using a model of viral infection, which induces a highly effective immune response. Human cord blood derived mast cells and human peripheral blood derived NK cells were used for these studies. mRNA expression of NK cell proteins was examined by qPCR while CD69 and IFN expression were determined by flow cytometry. Production of cytokines by mast cells was determined by ELISA and/or luminex analysis of supernatants following infection with reovirus type 3 Dearing (reovirus), UV inactivated virus or control (mock).

Results: Human cord blood derived mast cells infected with reovirus produced several potential NK activating mediators including type 1 and type 3 IFNs (IL-28a, IL-28b and IL-29) and IL-10. The products of virus infected human mast cells significantly induced enhanced expression of perforin ($p<0.01$) and TIA-1 ($p<0.05$) by NK cells. They also significantly enhanced NK cell cytotoxic activity in a killing assay using NK target cells ($p<0.05$). In separate studies, mast cell products induced expression of the activation marker CD69 ($p<0.001$) and increased IFNγ production in the context of exogenous IL-18 ($p<0.01$). Blockade of type 1 IFN receptors revealed that the majority of the impact of supernatants from virally infected mast cells on NK cell function, including IFNγ production, was mediated by type 1 IFNs.

Conclusions: These data demonstrate a novel co-operative function of human mast cells and NK cells in response to viral infection. This includes mast cell-mediated enhancement of both NK cytotoxic functions and their ability to produce IFNγ. These findings are important for understanding the role of mast cells during viral infection as well as the mechanism of action of reovirus-mediated cancer therapies.

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Mitochondrial STAT3 plays a major role in IgE-Ag mediated mast cell exocytosis

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It has been recently shown in a variety of cell types that signal transducer and activator of transcription 3 (STAT3) has a noncanonical role in mitochondria whereby it regulates oxidative phosphorylation (OXPHOS) by modulating mitochondrial electron transport chain activity. Studies in a human mast cell line showed that degranulation and TNFα secretion require mitochondrial translocation into the exocytosis sites. Moreover, adequate ATP levels in mast cells have been shown to be essential for degranulation and cytokine secretion. However, the role played by mitochondrial STAT3 during mast cell degranulation and its regulation remains elusive. Here we show that mitochondrial STAT3 is essential for immunologically mediated degranulation of human and mouse mast cells and in RBL cells. In immunologically activated RBL cells, mitochondrial STAT3 was phosphorylated on serine 727 in an ERK1/2 dependent manner which was followed by induction of OXPHOS activity. Furthermore, the endogenous inhibitor of STAT3, PIAS3, was found to inhibit OXPHOS activity in the mitochondria, resulting in inhibition of mast cell degranulation. Mice injected with Stattic, a STAT3 inhibitor, had a significant decrease in histamine secretion.

These results provide the first evidence of a regulatory role for mitochondrial STAT3 in mast cell functions and therefore, mitochondrial STAT3 could serve as a new target for the manipulation of allergic diseases.

References: Erlich, T. H. et al, J Allergy Clin Immunol, 2014, in press.

9

Critical role for mast-cell Stat5 activity in atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease. Although the etiology of AD is not completely

understood, numerous studies suggest that immune dysregulation and impaired skin barrier function underlie the disease.

Methods: In order to analyze the immune cell contributions to AD pathogenesis, we used both spontaneously developed and allergen-induced models of AD.

Results: Here we show that phospholipase C-b3 (PLC-b3)-deficient mice spontaneously develop AD-like skin lesions and more severe allergen-induced dermatitis than wild-type mice. Mast cells were required for both AD models and remarkably increased in the skin of *Plcb3-/-* mice due to the increased Stat5 and reduced SHP-1 activities. Our previous study showed that PLC-b3 interacts with SHP-1 and Stat5 to prevent overactivation of Stat5 by dephosphorylation of Stat5-Tyr694 by SHP-1. Ryan et al. showed that Stat5 is essential for mast cell development and survival. Consistent with these previous observations, mast cell-specific deletion of *Stat5* gene ameliorated allergen-induced dermatitis, whereas that of *Shp1* gene exacerbated it. We found that PLC-b3 negatively regulates the expression of periostin in fibroblasts and TSLP in keratinocytes, two proteins critically involved in AD pathogenesis. The receptor for TSLP was essential for dermatitis development in *Plcb3-/-* mice. Furthermore, polymorphisms in *PLCB3*, *SHP1, STAT5A* and *STAT5B* genes were associated with human AD, and increased mast cells with high levels of phospho-STAT5 were found in lesional skin of >50% of AD patients.

Conclusions: Therefore, STAT5-regulatory mechanisms in mast cells are important for AD pathogenesis and could be therapeutic targets.

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Evolution of mast cell and basophil tryptases from transmembrane proteases

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Background: Evidence converging from a variety of pharmacological and genetic studies in mice, other mammals, and humans suggests roles for mast cell and basophil tryptases in allergic inflammation, infection, arthritis, immune tolerance, inflammatory bowel disease, tissue fibrosis and other pathologies. Ongoing major pharmaceutical efforts are devoted to developing selective tryptase inhibitors for anti-asthmatic and other immunosuppressive applications. Tryptases in blood have become clinically useful as markers of mast cell activation, mastocytosis, and anaphylaxis risk. However, the origins of mast cell tryptases, which are products of at least four gene loci in humans, are obscure in comparison to other biologically important proteases.

Methods: We used data-mining, deep sequencing, and phylogenetic approaches to explore the genetic origins, drivers, and biological significance of mast cell and basophil tryptase variation.

Results: Tryptases were not detected in non-mammalian vertebrates, but a great variety occur in mammals, many of which, including mice, express one or more tryptases not expressed in humans, and vice versa. Even among humans there is major variation, with individuals inheriting as many as four or as few as two active beta tryptases. Mice, dogs and pigs express a tryptase, mastin, which is especially abundant in basophils, but a pseudogene in humans, who—perhaps consequently—express very little basophil tryptase. Delta tryptase, which is primate-specific, is truncated and catalytically impaired in humans, but is full-length and active in other primates, like macaques. Human and mouse gamma tryptases have appeared to be anomalous, being transmembrane proteins with C-terminal peptide anchors, and are present in some mammalian genomes but absent in others in clade-specific patterns.

Conclusions: Here we present phylogenetic evidence that all tryptases, including soluble alpha, beta, delta, and basophil mastins, and membrane-anchored gamma, evolved in mammals from ancestral, tryptic, type I transmembrane proteases related to marapsins and prostasins, which, unlike tryptases, have readily identifiable counterparts in reptiles and amphibians and are expressed primarily in epithelial cells, where they regulate ion flux and barrier integrity. Gene duplications, gene conversions, pseudogenizations, and complete gene loss have dramatically transformed tryptases during mammalian evolution. Transmembrane gamma tryptase appears to be a “living fossil” and “missing link” to ancestral tryptases. We propose that liberation from membrane attachments allowed soluble tryptases to proliferate and evolve new functions, now including innate and adaptive host defense.

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A universal antidote? Human tryptase degrades and detoxifies snake venom

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Background: Snake envenoming is a severe health problem that is responsible for as many as 125,000 deaths per year. Current antivenom therapy has many drawbacks ranging from poor availability and serious immune side effects to limitations inherent in species-specific treatment. In mice, mast cells have been shown to detoxify snake and other animal venoms, we therefore investigated the potential of human mast cells and mast cell proteases to be used as a universal antidote for the treatment of snakebite in humans.

Methods: Activation of human mast cells by snake venoms has been tested in LAD-2 and primary human skin mast cells. In a newly developed zebrafish model, we monitored the efficacy of mast cells and different concentrations of various

mast cell-derived proteases in deactivating venoms from six phylogenetically distinct snakes. To characterize the mode of deactivation of the toxins, we analyzed protein degradation in all venoms by mass spectrometry. Finally, we produced recombinant human tryptase and employed this enzyme in the zebrafish model.

Results: All snake venoms tested activated human skin mast cells and were detoxified by products released from human mast cells, purified human mast cell tryptase, and recombinant human tryptase β. For example, while all venoms pretreated with vehicle induced 100% mortality within 2-5 hours, tryptase-pretreatment of venom from three of six snakes resulted in complete protection (100% survival, $p<0.0001$) and in partial protection in two (89% and 27% survival, $p<0.0001$).

Conclusions: The data presented here clearly indicate that a wide range of snake venoms can get detoxified by human tryptase. These findings make recombinant human tryptase β a promising candidate for use as a universal antidote to venomous snakebites. Future research will have to address the efficacy and safety of recombinant tryptase in humans and the full spectrum of venoms detoxified by tryptase.

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Siglec-7 is an inhibitory receptor on human mast cells and basophils

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Background: Targeting mast cells (MC) and basophils (Baso), key effectors of allergy, via activation of inhibitory receptors (IRs) can be a strategy in downregulating this disease. Siglec-7 (p75/AIRM1, CD328) is a sialic acid binding Ig-like IR that transduces inhibitory signals by association of its ITIM with the phosphatase SHP-1.

Method: We analysed expression, function and signal transduction of Sigliec-7 on human MC and basophils. Human cord blood derived MC (CBMC), HMC-1 and LAD-2 and peripheral blood Baso were investigated for Siglec-7 expression by FC, immunofluorescence microscopy and IP/SDS-PAGE/WB with mouse anti-human Siglec-7 mAbs. Inhibition of MC (CBMC and LAD-2 presensitized with IgE Abs) and Baso activation was carried out by pre-incubating cells with anti-Siglec-7 Abs and afterwards by co-cross-linking IgE and Siglec-7 with goat anti-mouse F(ab)2 . Inhibition of activation was monitored by assessing release of β-hexosominidase, tryptase, PGD2 and GM-CSF by MC and histamine by Baso. SHP-1 involvement was studied in orthovanadate activated CBMC by IP.

Results: Siglec-7 was expressed on the surface of all studied MC (on CBMC already at 4 weeks of culture) and on Baso. Siglec-7 in CBMC and Baso had a MW of 75KDa. Co-aggregation of Siglec-7 with IgE, not just aggregation of Siglec-7 to itself, on the surface of CBMC significantly inhibited in a dose-dependent fashion release of the preformed mediators tryptase and β-hexosaminidase (3.5 folds), of PGD2 (3.8 fold) and of GMCSF (1.4 folds) (n=3, $p\leq0.03$). Anti-Siglec-7 co-cross linking with IgE also significantly downregulated β-hexosaminidase release from LAD-2 cells (3.5 folds) and histamine from Baso, although weakly (1.3 folds, n=5, $p\leq0.04$). Siglec-7 co-precipitated with p-SHP-1 in activated CBMC indicating a role for this phosphatase in the inhibitory signal transduction of Siglec-7 in MC.

Conclusions: This is the first report of Siglec-7 expression and function on human MC and Baso. Since Siglec-7 is expressed on MC, Baso and Eos (Eos still under investigation) its specific targeting in allergic diseases might be a powerful pharmacological tool to limit allergy by directly inhibiting allergic effector cell responses.

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NADPH oxidase-independent formation of extracellular DNA traps by basophils

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Basophils are primarily associated with a pro-inflammatory and immunoregulatory role in allergic diseases and parasitic infections. Recent studies have shown that basophils can also bind various bacteria both in the presence and the absence of opsonizing antibodies. In this report, we show that both human and mouse basophils are able to produce mitochondrial reactive oxygen species and to form extracellular DNA traps upon IL-3 priming and subsequent activation of the complement factor 5 or high-affinity IgE receptor. Such basophil extracellular traps (BETs) contain mitochondrial, but not nuclear DNA, as well as the granule proteins basogranulin and mouse mast cell protease 8. BET formation occurs despite the absence of any functional NADPH oxidase in basophils. BETs can be found in both human and mouse inflamed tissues, suggesting that they also play a role under *in vivo* inflammatory conditions. Taken together, these findings suggest that basophils exert direct innate immune effector functions in the extracellular space.

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Thymic stromal lymphopoietin promotes human eosinophil-basophil lineage commitment: a new vista on 'in situ hemopoiesis'

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Rationale: An important immunopathological hallmark of allergic disease is tissue eosinophilic and basophilic inflammation, a phenomenon which originates from hemopoietic progenitors (HP). The fate of HP is determined by local inflammatory cytokines that permit "in situ hemopoiesis", which lead to the accumulation of eosinophils and basophils (Eo/B). We have previously shown that phenotypic and functional HP-related Eo/B lineage commitment

alterations in neonates are also evident in adult HP. Given that recent evidence supports a critical immunomodulatory role for thymic stromal lymphopoietin (TSLP) in allergic inflammation, as well as TSLP effects on CD34+ progenitor cytokine and chemokine secretion1, we investigated the role of TSLP in mediating eosinophilo- and basophilopoiesis, the mechanisms involved, and the association of these processes with atopic sensitisation.

Methods: Human peripheral blood (PB) HP were stimulated with hemopoietic cytokines and/or TSLP and assessed for Eo/B colony forming units (CFU) by methylcellulose cultures and cytokine/chemokine secretion by Luminex. Neutralizing antibodies and pharmacological inhibitors were used to elucidate signalling molecule(s) responsible for regulating TSLP-mediated Eo/B differentiation. Lastly, the relevance of atopy was addressed by assessing variations in TSLP-induced Eo/B CFU from 20 adult PB samples.

Results: TSLP significantly increased the formation of IL-3-responsive Eo/B CFU. TSLP-stimulated HP actively secreted an array of cytokines/chemokines, key among which was TNFα, with consequent autocrine regulation on Eo/B colony formation ex vivo. Furthermore, we demonstrate preferential dependence of p38MAPK signalling in TSLP-mediated Eo/B colony formation and TNFα secretion in PB HP. Lastly, enhanced IL-3-responsive Eo/B CFU and cytokine/chemokine secretion was observed from atopic HP post TSLP-stimulation.

Conclusions: This is the first study to demonstrate p38MAPK-dependent autocrine signalling by TNFα in TSLP-mediated human PB Eo/B differentiation ex vivo. Enhanced TSLP-mediated hemopoiesis in relation to clinical atopy is a novel mechanism underlying eosinophil and basophil accumulation at sites of inflammation in atopic individuals. Recently, a single nucleotide polymorphism (SNP) rs1837253 in the TSLP gene was identified as highly associated with asthma and airway hyper-responsiveness2. We are currently investigating whether the relationship of atopy to this variant SNP in the TSLP gene is linked to alterations in TSLP secretion which carry functional consequences for Eo/B differentiation.

1. Allakhverdi Z et al. J Allergy Clin Immunol. 2009;123:472-8.
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Airway eosinophilopoietic events in severe prednisone-dependent asthma

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Rationale: Eosinophil progenitor cells have been demonstrated in the airways and in blood of patients with mild atopic asthma. However, there is no information on the numbers, clonogenic potential, or clinical relevance of these lineage-committed progenitors in patients with severe prednisone-dependent eosinophilic asthma.

Methods: We enumerated, in patients with severe asthma (n=21), the numbers of circulating and sputum eosinophils, hemopoeitic progenitors ($CD45^+CD34^+$) and the clonal outgrowth of these cells in response to IL-5, eosinophil progenitors ($CD45^+CD34^+CD125^+$) and their intracellular expression levels of IL-5 and IL-13, and innate lymphoid type 2 cells (ILC2: $lin^-CD45^+127^+ST2^+IL5^+IL\text{-}13^+$). These outcome measures were compared to patients with mild asthma (n=19), non-asthmatic COPD (n=8) and normal controls (n=8). In a subset of patients with severe asthma, we repeated these measurements after treatment with a humanized anti-IL5 monoclonal antibody.

Results: Compared to patients with mild asthma, COPD and normal controls, patients with severe eosinophilic asthma have, as expected, higher numbers of mature eosinophils in blood and sputum. The numbers of hemopoeitic and eosinophil progenitors in blood were not significantly different from those with mild asthma. However in colony assays, circulating hemopoietic progenitors from severe eosinophilic asthma were exquisitely more sensitive to low concentrations of IL-5 than milder asthmatic patients. In addition, the numbers of eosinophil-lineage committed progenitors in sputum were 10-fold higher than in all other subject groups including patients with mild asthma. A small but substantial proportion of hemopoietic progenitors and ILC2 cells in sputum expressed IL-5 and IL-13. These numbers were relatively unaffected in those patients with severe asthma who continue to have sputum eosinophilia after anti-IL5 therapy despite normalizing blood eosinophilia.

Conclusion: Locally derived eosinophilopoeitic Th2 cytokines may promote local eosinophil maturation and differentiation. ILC2 cells may contribute to this process. In addition, the increased clonogenic potential of hemopoietic progenitors in severe eosinophilic asthma may favour this process. Taken together with our previous observation of the inability of a potent oral CCR3 inhibitor to decrease sputum numbers of eosinophils or eosinophil progenitors, our findings suggest a significant role of *in-situ* eosinophiopoietic events in promoting the development of airway eosinophilia in severe prednisone-dependent eosinophilic asthma.

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Nitration of the birch pollen allergen Bet v 1.0101 leads to immunological and structural features that may link pollution to enhanced allergy

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Background: Air pollution has been considered as one of the factors leading to increased allergy prevalence in industrialized countries. One possible mechanism is nitration of allergens, which can occur exogenously (in polluted urban air), but also endogenously (in inflamed lung tissue). In air, "summer smog" conditions give rise to NO2 and O3 in concentrations that are sufficient to nitrate tyrosine residues in proteins. It has been previously shown that nitrated birch pollen allergen Bet v 1.0101 induces increased allergy in BALB/c mice (Gruijthuijsen et al., IAAI 141:265-275 (2006)) and that human dendritic cells present higher amounts and more different epitopes from nitrated Bet v 1.0101 in the MHC II complex (Karle et al., PLoS ONE 7(2) e31483 (2012)). We have investigated the impact of nitration on Bet v 1.0101 to determine whether nitration might be a factor affecting its immunogenicity and/or allergenicity.

Methods: Nitrated allergen was obtained using tetranitromethane and was characterized by high-performance liquid chromatography mass spectrometry (HPLC-MS), Western blot, size exclusion chromatography and dynamic light scattering. The structure of the nitrated allergen was analyzed by X-ray crystallography. Monocyte derived dendritic cells were differentiated in vitro into MoDCs and primary dendritic cells were isolated from human blood using MACS. Cytokine secretion upon stimulation was followed by ELISA. Allergen-specific T-cells were tested for proliferation activity.

Results: We found that specific T-cell lines of birch pollen-allergic patients proliferate stronger upon stimulation with nitrated compared to unmodified Bet v 1.0101. This response requires the presence of the intact protein, since nitrated immunodominant epitopes of the allergen had no comparable effect. A structural consequence of nitration is oligomerization, which may increase the immune response towards the allergen, i.e. its immunogenicity. In addition, analysis of the crystal structure of nitrated Bet v 1.0101 showed that amino acid residue Y83, located in the hydrophobic cavity, is nitrated to 100%, which might influence the ligand binding capacity of the protein. Thus, nitration might interfere with the ability of hydrophobic ligands to bind to the hydrophobic cavity of Bet v 1. Both monocyte-derived dendritic cells (DCs) and primary DCs showed a decrease in release of TH1-priming cytokines, thus favoring a TH2 response.

Conclusions: Taken together, we conclude that nitration enhances the immunogenicity of Bet v 1.0101 by favoring oligomerization. In addition, the cytokine production by dendritic cells is affected to shift the response towards TH2. Modified binding of hydrophobic ligands may be involved in this response, but other mechanisms may be involved within the dendritic cells as well. Overall, nitration of Bet v 1.0101 has several consequences for the protein and the immune response against it, which leads to an increase both in immunogenicity and in allergenicity.

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Environment-environment interactions impacting on human health: Influence of biogenic and anthropogenic factors on birch pollen's allergenicity

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Evidence is compelling for a positive correlation between urbanisation and increment of allergic sensitisation and diseases. The reason for this association is not clear to date. Some data point to a pro-allergic effect of anthropogenic factors on susceptible individuals. Studies analysing the impact of environmental – biogenic and anthropogenic – factors on the allergenicity of allergen carriers such as pollen grains are scarce. Within a transdisciplinary research team (REKLIM Initiative) we evaluate the effect of natural (e.g. climate) and anthropogenic (e.g. traffic pollutants) factors on birch pollen in a holistic approach. Herein, allergenicity of pollen is placed in context to allergic sensitisation and symptoms of subjects (KORA study) living in the surrounding of the respective tree. Birch trees from urban and rural sites in the surrounding of KORA-individuals in Augsburg, Germany, were selected and sites were characterized for NO_2 and O_3 by passive samplers (birch trees n=40). Temperature, NOx, PM2.5, PM10 and black smoke exposure of the trees was determined by temperature- and pollution models. Pollen was categorized according to maturation state; allergenicity was assessed by means of allergen content and adjuvant factor release – both on descriptive and functional level. Our data in two different cities (Munich, Augsburg) and consecutive years

(2012, 2013) reveal ozone as a prominent environmental factor influencing the allergenicity of birch pollen. Enhanced allergenicity, as assessed in skin prick tests, was mirrored by enhanced allergen content. Beyond that, ozone induced changes in lipid composition and chemotactic and immune modulatory potential of the pollen. Higher ozone-exposed pollen were characterized by less immune modulatory but higher immune stimulatory potential. The results of this study add significantly to our understanding how urbanisation and climate change influence the allergenicity of birch pollen and how this impacts on allergic sensitisations and symptom severity.

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Genetic variants influencing serum total IgE levels and the specific IgE response to parasites and allergens

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Background: Quantitative traits such as total and specific IgE levels, phenotypes associated with helminth infections and asthma may be regulated by the burden of common and rare genetic variants. Our previous findings suggest that specific antibody levels to the nematode Ascaris are under genetic control of chromosome 13q33. The aim of this study was to explore other chromosomal regions and select candidate markers for genotyping two populations with different genetic backgrounds: Colombian (CGA Study) and Swedish (MALF Study).

Methods: Coding and non-coding regions of 14 genes,*CHI3L1, CHIA, IL10, FCER1A, RAD50/IL13, IL4, IL5, TSLP, IL33, STAT6* and the Ascaris-infection susceptibility locus in 13q33 *(LIG4, ABHD13, TNFSF13B, IRS2)* were sequenced using targeted enrichment (SureSelect, Agilent) and massive parallel sequencing (Illumina HiSeq2000) in 48 subjects (24 asthmatics/24 controls) from Colombia at the extreme distributions (< 25th and >75th percentiles) of total and specific IgE/IgG levels to Ascaris. Variant discovery and genotyping were done using GATK; burden analyses using Ingenuity Variant Analysis.

Results: There were 21 variants in high IgE responders to Ascaris (at least 4 subjects) that were not observed in those < 25th percentile. They were distributed in *STAT6, CHIA, CHI3L1, IL13, LIG4* and *ABHD13*. Subjects with high specific IgE levels to Ascaris had a significant enrichment of 6 variants in the promoter and 5'UTR of *STAT6* (burden p value = 0.009) and 7 coding variants in *CHIA* (p = 0.04). Enrichment of *STAT6* variants was also found in subjects with high total IgE (p = 0.04) and high IgG levels to Ascaris (p = 0.005). Two IgE-related *STAT6* variants were also overrepresented in asthmatics but the overlap between IgE and asthma enriched variants was low. Asthmatic patients had a significant enrichment of variants in the *TSLP* gene (p = 0.02).

Conclusions: Deep sequencing revealed a panel of variants related to total IgE and IgE/IgG response to Ascaris in Colombian individuals. Non-coding variants in *STAT6* as well as coding variants with predicted deleterious effects on chitinase genes were enriched in subjects with high IgE responsiveness. These associations are being evaluated in the context of Ascaris infection and atopy in two independent populations.

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Profiling of the rapid IgE sensitization in Netherton syndrome

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Background: Netherton syndrome (NS) is a rare genodermatosis with severe erythroderma, brittle hair, and a profound atopic diathesis. NS is caused by *SPINK5* mutations which lead to a severe epidermal barrier defect. No microarray IgE profiling of the extensive IgE sensitization in relation to the clinical atopic manifestations of NS patients has been performed earlier.

Methods: Ten NS patients, aged 1 to 51 years and with confirmed *SPINK5* mutations, were included in this study. IgE sensitization profiles to specific allergens were studied with the IgE ImmunoCAP method and additionally, with the IgE ImmunoCAP ISAC® microarray in seven patients.

Results: We found an extensive IgE sensitization profile to 11-37% allergen components. Importantly, the IgE sensitization developed rapidly during the first 2-3 years of life and was directed to common food, animal protein and pollen components, but also to uncommon allergen components in Northern Europe e.g. lipidtransferproteins (LTPs). Two patients were beneficially desensitized to wheat and milk. As clinical symptoms, 90% of patients developed erythrodermic eczema within three days of birth, asthma developed in 30%, angioedema and anaphylactic reactions to food allergens occurred in 30%, and urticaria in 40%. Serum IgE levels were elevated up to 17 433 kU/l and eosinophilia were found in all but the youngest patient. Eosinophilic esophagitis was found in one patient and early gastrointestinal inflammation in four patients.

Conclusion: The multiple atopic manifestations in Finnish NS patients initiated from birth. The ISAC microarray enabled the most precise IgE sensitization profiling and revealed that the broad IgE sensitization develops very rapidly during the first years of life, including stable allergens inducing severe allergic reactions. Desensitization is a treatment option.

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A variant of Interleukine-13 (IL13) gene is associated with food allergy in the Japanese population

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Background: The prevalence of food allergy (FA) has increased over the past two decades, particularly in the developed countries. FA is an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food. Recent GWASs have shown that IL13 gene locus on chromosome 5q31 is associated with bronchial asthma and atopic dermatitis; however influences of genetic variations in the IL13 gene on susceptibility to FA are unclear.

Objective: To investigate the association between variants of IL13 gene and FA and FA related phenotypes in the Japanese population.

Methods: We re-sequenced the IL13 gene region by PCR-directed sequencing. Four tag SNPs were selected using the Tagger algorithm and genotyped by Invader methods. We performed association studies of FA using two independent Japanese populations (1st population, 603 cases and 938 controls; 2nd population 282 cases and 1004 controls). Among the 1st FA population, a total of 182 subjects have bronchial asthma.

Results: We found a total of 17 variants including a non-synonymous substitution (rs20541; Arg144Gln). We observed a significant association at rs1295686 (meta-analysis, combined P = 1.7×10^{-7}; OR, 1.4; 95% CI, 1.2-1.6). In further analyses of patient subgroups, we observed a strong association between rs1295686 and FA with bronchial asthma (P = 8.1×10^{-6}; OR, 1.7; 95% CI, 1.3-2.1).

Conclusion: rs1295686 of IL13 gene is significantly associated with FA in the Japanese population. Although further genetic and functional analyses are needed, our findings could help elucidate common genetic factors for bronchial asthma, atopic dermatitis, and FA.

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The association of 36 susceptibility loci of psoriasis with atopic dermatitis and psoriasis in the Japanese population

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Background: Atopic dermatitis (AD) and psoriasis vulgaris (PsV) are chronic inflammatory skin diseases caused by a combination of genetic and environmental factors, and recent genetic studies have reported overlapping susceptibility loci between the diseases. Genome-wide association studies (GWASs) and meta-analysis of GWASs of PsV have identified a total of 36 susceptibility loci of PsV.

Objective: To explore the genetic components involved in AD and PsV in the Japanese population.

Method: We recruited a total of 259 patients with PsV, 991 patients with AD and 935 healthy individuals. We genotyped a total of 41 SNPs in the 36 previously reported PsV risk loci and conducted an association study. Since the MHC class I region is associated with both of the diseases, we conduct genotyping of HLA alleles in patients with AD and PsV.

Results: We found significant associations with PsV in four SNPs after Bonferroni correction, *TNIP* (rs2233278, $P = 3.5\times10^{-8}$), *IL12B* (rs4379175, $P = 1.2\times10^{-6}$), the MHC class I region (rs4406273, $P = 5.9\times10^{-7}$) and *TRAF3IP2* (rs33980500, $P = 9.4\times10^{-6}$). The direction of associations of susceptibility to PsV was similar to that in the recent study. Although we could not find a significant association between the PsV susceptibility SNPs and AD, marginal associations were observed for *IL13* (rs1295685, $P = 3.5\times10^{-3}$) in the opposite direction and *ZMIZ1* (rs1250546, $P = 1.7\times10^{-3}$) in the same direction.

Conclusions: Our data strongly support the important genetic influences of the polymorphisms in the *TNIP, IL12B,* the MHC class I region and *TRAF3IP2* on the susceptibility to PsV in the Japanese population. In this study, the sample size was relatively small. In addition, we conducted the validation study using only reported SNPs in the previous GWASs of PsV, and associations of the other SNPs within the susceptibility loci remain unclear. Further studies of these loci are necessary for better understanding of the genetic etiology and the pathophysiology of the chronic inflammatory skin diseases.

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Genetic and environmental factors of allergy in Lithuanian birth cohort

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The aim of the was to evaluate study genetic and environmental factors based on birth cohort data. The collecting blood samples and clinical investigation was done in Lithuanian birth cohort participants (n=1320, mean age 7.11 ± 1.23). The selected group from the cohort of bronchial asthma was studied. We found that risk of the the late onset of asthma was increasing, when IgE antobodies against d1 (main mite D.pteronyssinus) allergen), d202 (mite D.pteronyssinus) ir f1 (egg) allergens and were detected in sera and when ECP (R^2=0,28; p=0,02), was found. The risk for development of asthma was increased if, besides them, IgG4 antibodies against d1and f1 allergens and IgA antibodies against f1 allergen (R^2=0,38; p=0,027) were found. The risk for the later development of asthma was also increasing, when, at the age of 2 years, child have been was ill with bronchitis (R=0,58; p<0,05).

But the risk for asthma is decreasing, when sensibilization to d1 alergenui (R=-0,55; p<0,05) was increasing. Risk for the development of asthma was not associated with hRSV, hMPV, hPIV 1 ir 3 ir HBoV induced respiratory infections (p>0,05) and ECP amount in the blood sera (p>0,05). Control and study groups differed statistically significant for hPIV 1-4 infekcions (p=0,03).

New system for PGR for FCER1A gene VNP rs2251746 genotype detection was created and optimised. It was establish, that in the control and allergy group system FCER1A gene VNP rs2251746 C/T ir C/C genotipe were more frequent in the study group comparing with the control (p=0,046). In the study and control group it was found that, CD14 gene VNP rs2569190 A/A genotype is statistically significant in the control group comparing to study group (p<0,05).

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A prospective nation-wide study on allergen sensitization patterns in Mexican rhinitics

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Purpose of the study: Allergic rhinitis (AR) is a disease with changeable expression depending on climate and allergen exposure. These variables differ between Europe and Latin America. We investigate the characteristics of allergic sensitization in skin prick testing of AR patients in Mexico.

Methods: Patients with confirmed rhinitis symptoms, 2-65 years of age (either sex), and a positive screening allergen skin test (SPT), seen by allergists in 25 centers spread over the six climatologic zones in Mexico were studied. The subjects filled in a validated questionnaire on AR and underwent SPT with a blinded, standardized panel of 18 allergens using a uniform method. We report here on the skin prick test results using descriptional statistics.

Results: A total of 529 skin prick tests were analyzed. The nationwide results showed (% positivity of all tests done per allergen): HDM (house dust mite = Dermatophagoides pteronyssinus and farinae mix) 56, Blomia 25, Cat 22, Cockroach 21; Ash 24, Oak 23, Mesquite 22 and Cypress 11 tree pollen; Bermuda grass 26, Kentucky Bluegrass 21 and Timothy 20 pollen. Nationwide weed pollen sensitization (e.g. ragweed 13%) is less important as is sensitization to fungi (aspergillus 6, alternaria 7%). We also tested 2 pan allergens: peach 8.5 and profilin 10%. Ash and oak sensitivity is less frequent in the dry North, where sensitivity to Mesquite -which grow better in dry zones- predominates among trees. Bermuda grass, as a tropical grass, causes most frequently grass pollen sensitivity in Mexico. Ragweed pollen sensitivity is mostly found in the dry (North) and semi-dry (Queretaro) zones (18-26%), but is not of importance in the area around Mexicocity or in the humid South-East (6-9%). Remarkable findings were: fungal sensitization was 4 times higher in the dry North. Other differences between the zones were low HDM 18.5% and high ragweed 26% in the very dry North. Very high positivity for tree 63%, grass 55% and weed 37% pollen in the central zones (semi-dry, temperate). AR patients from the very humid, tropical South-East had almost all SPT(+) to mites 87%, but very low SPT(+) to pollens nor cat (7%).

Conclusion: Allergic sensitization patterns in Mexico vary from that reported in US and Europe. Striking regional differences exist in allergic sensitization of patients with AR, within one country in which several different climatologic zones can be detected, as in Mexico.

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Targeted delivery of signal transduction inhibitors to human allergic effector cells using highly specific nanoconjugates

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Background: The pro-allergic responses of basophils and mast cells can be substantially reduced using various signal transduction inhibitors (e.g. calcineurin and Syk inhibitors). However, these agents are rarely used therapeutically, especially systemically, due to ubiquitous expressions of signalling proteins which results in adverse drug reactions and side-effects. Our aims were to discover whether the above problem could be solved by specifically targeting allergic effector cells with signal transduction inhibitors using gold nanoparticles (AuNPs) as a delivery platform.

Method: AuNPs were conjugated to antibodies against CD203c, which is specifically expressed on human basophils and mast cells, by amine-coupling to acidic groups of reduced glutathione (GSH). GSH was also used as a spacer for immobilizing the calcineurin inhibitor ascomycin on the gold surface.

Results: AuNPs alone did not affect basophil histamine release or viability in purified human basophils. However, AuNPs conjugated with anti-CD203c specifically bound to basophils and, after conjugation with ascomycin, they strikingly inhibited IgE-dependent basophil histamine release by more than 70% at effective concentrations of 5 nM ascomycin (an increase in potency by more than 20-fold compared to ascomycin alone). In contrast, nanoconjugates containing antibodies not expressed on the surface of basophils (e.g. anti-Tim-3, anti-HIF-1alpha) had no effect on basophil function. Similar results were obtained using mast cells and unpurified basophils present in mixed leukocyte preparations, suggesting specific targeting of these cells.

Conclusions: The ability to successfully target allergic effector cells using gold nanoconjugates indicates that this technology could be useful in anti-allergic therapy for the specific delivery of highly effective signalling inhibitors or toxins without side-effects.

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Effects of roasting on the uptake of peanut allergen Ara h 3 by monocyte-derived dendritic cells and on the induction of basophils degranulation

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Rationale: Peanut allergy is one of the most severe food allergies. Heat processing such as roasting has been implicated in the increase of allergenicity of peanuts due to reactions between reducing sugars and proteins which can create neoallergens. However this increase is not fully understood. The potential role of modifications generated after roasting in the uptake of peanut allergens by dendritic cells has not been addressed yet.

Aim: We sought to analyze differences in the uptake of the peanut allergen Ara h 3 by immature monocyte-derived dendritic cells (MDDC) in raw and roasted form. We also sought to study the ability of raw and roasted Ara h 3 to cross-link IgE on effector cells.

Methods: Ara h 3 was purified from raw and roasted peanuts and labelled with Alexa fluor 488 dye. Labelled allergens (10 and 50 µg/ml) were added to MDDC obtained from five donors on day six of culture and internalization was analyzed after 10, 30 and 120 minutes by flow cytometry. In parallel mannan, which blocks the mannose receptor, was added 30 minutes before adding labelled allergens. Furthermore, the ability of raw and roasted Ara h 3 to cross-link IgE on effector cells was evaluated using a rat basophil leukaemia (RBL) cell line transfected with human FcεRI. Cells were passively sensitized by incubation with 8 individual sera from patients with peanut allergy and exposed to 30 µg/ml of raw or roasted Ara h 3. The release of β-hexosaminidase was measured.

Results: We found that the uptake of raw and roasted Ara h 3 by MDDC was increased after application of higher allergen concentration and duration of time. The internalization of roasted Ara h 3 was significantly enhanced in comparison with raw Ara h 3 after 30 minutes using a concentration of 50 µg/ml ($p < 0.05$) and after 120 minutes at 10 µg/ml ($p < 0.05$) and 50 µg/ml ($p < 0.01$). The uptake of roasted Ara h 3 but not raw Ara h 3 was significantly reduced by mannan ($p < 0.05$). Our results showed an increase of β-hexosaminidase release after incubation with roasted Ara h 3 compared to raw Ara h 3 in RBL mediator release assay.

Conclusions: Roasting increases the uptake of Ara h 3 by MDDC and mannose receptor could be involved in this increase. Roasted Ara h 3 induces also higher basophil degranulation.

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Direct immune modulatory effect of non-digestible oligosaccharides mimicking the functionality of human breast milk oligosaccharides on human monocyte derived dendritic cells

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Background: The combination of lactic acid bacteria (LAB) with oligosaccharides is considered to selectively manipulate the composition of the microbiota of the host. This is of special interest regarding the growing application of LAB in combination with specific mixtures of non-digestible oligosaccharides mimicking oligosaccharides present in human milk in prevention and treatment of allergic diseases. Aim of the study was to investigate the impact of different bacterial strains in combination with oligosaccharides on cytokine release by human monocyte-derived dendritic cells (MoDC).

Method: Immature MoDC prepared from peripheral blood monocytes of healthy non-atopic volunteers were stimulated with a *Lactobacillus* and *Bifidobacterium* strain in different concentrations in the presence of different combinations of GOS (galacto oligosaccharides), FOS (fructose oligosaccharides) and AOS (pectin derived acidic oligosaccharides). IL-12p70 and IL-10 release was analyzed after 24h in cell-free supernatants.

Results: Incubation of MoDC with the *Lactobacillus* and *Bifidobacterium* strains in combination with neutral or acidic oligosaccharides revealed that GOS/FOS and GOS/FOS/AOS had a significant additive effect on bacteria-induced IL-10 release by human MoDC, while the ability of these oligosaccharides to increase IL-12p70 production was less pronounced. Enhanced release of IL-10 by bacteria-treated human MoDC in the presence of GOS/FOS and GOS/FOS/AOS suggests specific immune regulatory capacities *in vitro*.

Conclusions: Thus, the tested LAB in combination with oligosaccharides might be considered as potential health promoting providing new strategies for the prevention or therapeutic treatment of diseases including immune regulatory disorders, such as skin diseases, allergy or infection and open new aspects for the application as allergy preventing ingredients in food.

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In vitro generation of Oral mucosal Langerhans cell-like cells from cord blood CD34+ stem cells

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Background: Sublingual immunotherapy (SLIT) has been shown to be efficient in the treatment of allergic rhinitis. Data from classical subcutaneous immunotherapy (SIT) suggest tolerance induction to be a key immunological mechanism. However, only little is known about immunological mechanisms underlying SLIT. It is more than likely that dendritic cells such as oral mucosal Langerhans cells (oLC) play a central role. Nevertheless, functional data are difficult to acquire due to limited tissue obtained from oral mucosal biopsies. Thus this study intended to develop an *in vitro* model for oLC using cord blood $CD34^+$ stem cell-derived dendritic cells (CD34dDC).

Methods: Cultured CD34DC from atopic and non-atopic donors were phenotyped by flow cytometry.

Results: Thereby we could show that specific concentrations of TGF-b1 in combination with GM-CSF, TNF-α and SCF induces $CD1a^+$ CD34dDC displaying a phenotype similar to oLC expressing the high affinity receptor for IgE (Fc□RI), Langerin, TLR4/CD14, CD11b and co-inhibitory B7-H molecules. In line with data from *ex-vivo* oLC, Fc□RI expression was significantly higher on *in vitro* generated oLC from atopic donors. Moreover, *in vitro* generated oLC were able to stimulate IL-10 producing T cells spontaneously and after allergen uptake.

Conclusion: This novel *in vitro* model for oLC described here enables studies on functional mechanisms involving oLC leading to allergen-specific tolerance induction in SLIT.

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Clinical efficacy of allergen specific immunotherapy correlates with changes of proinflammatory (DC2)/regulatory DC ratios in peripheral blood

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Background: Whereas allergen immunotherapy (AIT) is known to reorient allergen-specific immune responses from a Th2 to a Th1/TReg profile, it remains difficult to correlate clinical benefit with changes in the polarization of allergen-specific CD4 T cells in peripheral blood, due to a lack of well standardized assays. Dendritic cells (DCs) orient adaptive immune responses by supporting the differentiation of effector Th1, Th2, Th17 or regulatory $CD4^+$ T cells. In this context, we investigated molecular changes associated with polarized DCs in the blood of grass pollen allergic patients to predict the orientation of allergen-specific T cell responses during sublingual immunotherapy.

Methods: We compared the proteomes and transcriptomes of immature monocyte-derived DCs as well as DC1, DC2 and DC Reg to identify specific markers for each of these DC subsets. These markers were assessed by quantitative PCR in the patients' blood before, during and after immunotherapy.

Results: The induction of regulatory DC markers (*i.e.* C1Q and Stabilin-1) in peripheral blood mononuclear cells of grass pollen allergic patients was found to correlate with clinical efficacy of AIT. We further identified specific markers for pro-allergic DCs (DC2) capable to support the differentiation of $CD4^+$ Th2 cells. As a first step, we selected a cocktail of cytokines inducing the differentiation of immature monocyte-derived DCs (MoDCs) toward a DC2 phenotype, i.e. leading to a Th2 polarization of allogeneic naive $CD4^+$ T cells in co-culture experiments. DC2 secrete IL-6, IL-8 and IL-13 cytokines, but not IFN-γ, IL-12p70 nor TNF-α. Comparative studies of immature DCs, DC2 and DC Regs using cDNA microarrays together with quantitative proteomics (label-free mass spectrometry), identified 121 and 32 genes/proteins up- and down-regulated, respectively, in DC2. We subsequently assessed the relevance of these markers by PCR in a cohort of 82 grass pollen allergic patients receiving daily sublingual immunotherapy for 4 months and regularly exposed in a challenge chamber. We observed specific alterations in both DC2 and DCReg markers which correlated with clinical improvement during immunotherapy.

Conclusions: Our results support the hypothesis that markers documenting the DC2/DC Reg balance in peripheral blood can be used to monitor AIT efficacy.

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Regulation of CD48 in allergic inflammation

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Background: Mast cells and eosinophils, the main effector cells of allergic inflammation, express high levels of CD48, a glycosylphosphatidylinositol (GPI)-anchored cell-surface protein (mCD48) existing also in a soluble form (sCD48). Its high affinity receptor, 2B4 is also expressed by eosinophils. CD48 has an important role in allergy. For example, its neutralization down-regulates the lung-associated inflammation in a murine asthma model. Its cross-linking on human eosinophils activates them. Moreover, *Staphylococcus aureus* and its toxin (SEB) activate mast cells and eosinophils through CD48. Although many GPI-molecules exist as both soluble and membrane-bound, the mechanism/s of cleavage is not yet well established. Therefore we wondered how sCD48 is released from the eosinophils and what its functional role is.

Methods: Eosinophils were purified from human peripheral blood. The cells were activated by SEB in the presence or absence of inhibitors for phospholipases (C A & D) and protein synthesis/ transport and activation was monitored by expression of CD11b; mCD48 and sCD48 were measured by FACS and ELISA respectively. Eosinophils were pre-incubated with sCD48 (5µg/ml, 30 min), activated with anti-2B4 and IL-8 was measured (ELISA).

Results: sCD48 was up-regulated (up to 100pg/ml) in supernatant of SEB activated (up to 18 hrs) eosinophils and was directly correlated to surface expression of CD11b. A cell-associated phospholipase-D (PLD) is involved in the cleavage of mCD48. However, there was no clear-cut direct correlation between mCD48 and sCD48. Eosinophils activated in the presence of Brefeldine-A and Cycloheximide show decreases in mCD48 and sCD48. These results indicated that a CD48 intracellular pool, might be transported to the membrane to maintain nearly constant levels of mCD48. Furthermore new protein synthesis takes part in this process. Interestingly, sCD48 was shown to bind to 2B4, as sCD48 incubated eosinophils were less activated by crosslinking of 2B4 to release IL-8.

Conclusions: Our findings indicate that SEB increases the sCD48 production from eosinophils by a cell associated PLD. sCD48 can down-regulate the 2B4 mediated eosinophil activation. Our data further define the role of CD48 in allergy and also shed light on the cleavage mechanisms of GPI proteins' form cell surface.

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The stealthy fusion nano-machine

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Secretory cells contain small and large granules, but the functional significance of granule size is unclear. We have established a growth and elimination stochastic model for the granule life cycle, which resulted in a simple equation for granule secretion rate *Const*[Ca2+]min(X,M)e-K/2*, in terms of the number of SNAREs (*K*) and the actual (stochastic *X*) and maximal (deterministic *M*) [Ca2+] cooperating ions required for secretion. *K* satisfies the approximate relation *K≈INT(0.9*D0.5)* with granule diameter D (in nm). Based on this model, about 12-15-20 SNAREs are needed for basal secretion of rat mast cell granules (ear, intestinal and peritoneal mast cells respectively). The unit granule is the most probable granule to be secreted under basal secretion, constitutively facilitating newly formed content-updated granules to be secreted. Larger granules, with longer shelf-life, function as inventory to be stored for emergency and secreted under evoked secretion. Evoked fast secretion of 10 granules is tantamount to 0.6-3% of granule inventory, constitutes informative communication between the cell and its environment, supporting the hypothesis that regulated basal secretion of granule content has a role in tissue homeostasis. In contrast, the 40% secretion of granule inventory induced during *in vitro* evoked states mimics anaphylactoid–like reactions and represents an adaptive pathological-physiological condition.

Neutrophils and eosinophils secrete an assortment of proteins, packaged in three and two types of granules respectively, differentiated by size. In spite of the specificity of their cargoes and of their intracellular pathways, these secretory vesicles share similar membrane components, and their exocytosis is mediated by the same SNARE system. Major distinctions happen, dictated by granule size, between the various granule subsets regarding the extent to which these are mobilized, both *in vitro* and *in vivo*. Accordingly, the simple nano-machine performs granule secretion management: small granules containing the substrates (gelatinase positive granules) queue first, while the activating enzymes in azurophil granules are secreted last. To decrease basal secretion of harmful proteins, the mast cells, neutrophils and eosinophils generate oblate granules.

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Mast cells control skin wound infection with Pseudomonas aeruginosa

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Pseudomonas aeruginosa, a ubiquitous environmental Gram-negative bacterium, is one of the leading causes of severe nosocomial skin wound infections. Due to increasing resistance to common antibiotics, this pathogen poses a major thread, especially to immune-compromised patients and raises the urge for new therapeutic approaches. Since mast cells (MC) are situated at the borders to the external environment like the skin their localization at allows them to be among the first inflammatory cells to encounter and act against invading pathogens. The aim of the present study was therefore to examine the effects of MCs have on bacterial skin wound infection with *Pseudomonas aeruginosa*. We report that MCs are beneficial for wound healing and bacterial clearance of PA in a model of physiologically relevant skin wound infections. Upon establishment of murine models for skin wound infection with PA in MC-deficient mouse strains (*KitW/KitW-v*) we found that mice lacking MCs exhibit reduced wound closure and have an increased bacterial burden. The MC dependency of the observed phenotype was confirmed by reconstitution the designated skin areas with bone marrow-derived mast cell and in the non-c-kit dependent Cpa-3Cre; Mcl-$1^{fl/fl}$ mouse model. First analysis of the molecular mechanisms responsible for this antibacterial and wound healing promoting effects of MCs in vitro showed a minor direct antibacterial capacity of mast cells but a crucial interaction between MCs and cells of the epidermal skin compartment resulting in the reduction of bacterial burden. Using a qRT-PCR approach we could identify an upregulation of antimicrobial peptides derived from keratinocytes (KCs) that was enhanced in co-culture with MCs. Multiplex ELISA studies revealed an increased IL-6 production from MCs in responsible for this effect in the co-culture system. To prove that

increased IL-6 levels lead to enhanced antimicrobial response of KCs we applied IL-6 knock-out MCs to the described system and could show that the antibacterial capacity was reduced compared to KCs cultured with wild type MCs Toghether these findings demonstrate an important role of MCs in combating skin wound infection by *Pseudomonas aeruginosa* and support the function of MCs as key players in innate immune responses in a clinically relevant infection model.

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IL-8 derived from mast cells induces epithelial-to-mesenchymal transition and stem cell features in human thyroid cancer cells

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Introduction: We recently demonstrated that mast cell (MC) density in human papillary thyroid carcinomas (PTCs) is increased compared to normal thyroid tissue. MC density correlates with extrathyroidal extension of PTCs. The MC-derived factors and mechanisms responsible for enhanced thyroid cancer (TC) invasiveness remain unidentified. Here, we searched for mediators/mechanisms inducing Epithelial-Mesenchymal Transition (EMT) and stemness of TC cells.

Materials and Methods: Conditioned media (CM) was obtained by human lung MC, HMC-1 and LAD2 cells. We assessed EMT (Immunofluorescence, Western blot, real-time PCR, Wound healing assays) and stemness phenotype (Aldefluor assay, Sphere-formation assay) in thyroid cells (Nthy-ori 3-1, 850-5C and TPC-1). The protumorigenic potential of IL-8 was evaluated by TC cell xenografts in immunocompromised mice (CD1a nude mice). MC density, stemness in human PTCs *vs* normal thyroid tissue were evaluated by immunohistochemistry (IHC).

Results: Upon incubation with MC CM, human-immortalized (Nthy-ori 3-1) and cancerous (850-5C and TPC-1) thyroid cells underwent EMT: acquisition of fibroblast-like morphology, expression of mesenchymal markers, down-regulation of epithelial features and increased ability to migrate. By immune-depletion of MC-derived CM, we found that IL-8 was necessary for EMT induction in TC cells. The IL-8 receptors CXCR1 and CXCR2 were constitutively expressed in TC cells. IL-8 stimulation of TC cells caused increased expression of cancer stem cell (CSC) markers, expansion of ALDHhigh cell fraction, and thyrosphere formation in low-adherence. These features could be reverted by blocking the IL-8/CXCR1/CXCR2 axis. Accordingly, 850-5C engineered to overexpress IL-8 underwent EMT, displayed increased stemness and higher tumorigenic activity with respect to control cells. Analysis of PTC surgical specimens by IHC demonstrated an increased correlation between MC density (tryptase staining) and TC cell stemness features (nuclear OCT4 staining).

Conclusions: Our data indicate that tumor-associated MCs (TAMCs) in TC produce proinflammatory mediators (e.g. IL-8) that induce EMT and expand CSC population. We suggest that targeting IL-8/CXCR1/CXCR2 axis is a possible therapeutic strategy for TC.

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Mast cell-derived IL-1β contributes to uric acid crystal-induced acute arthritis in mice

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Objective: Gouty arthritis is caused by precipitation in joints of crystals of monosodium urate (MSU). While it has been reported that mast cells (MCs) infiltrate gouty tophi, little is known about the actual roles of MCs during acute attacks of gout. This study was undertaken to assess the role of MCs in a mouse model of MSU crystal-induced acute arthritis.

Methods: We assessed the effects of intra-articular (i.a) injection of MSU crystals in various strains of mice with constitutive or inducible MC deficiency, or in mice lacking IL-1β or other elements of innate immunity. We also assessed response to i.a. injection of MSU crystals in genetically MC-deficient mice after i.a. engraftment of wild-type or *IL-1*β*-/-* bone marrow-derived cultured MCs.

Results: We show that MCs can augment acute tissue swelling following i.a. injection of MSU crystals in mice. We report further that IL-1β production by MCs contributes importantly to MSU crystal-induced tissue swelling, particularly during its early stages, and that selective depletion of synovial MCs can diminish MSU crystal-induced acute inflammation in the joints.

Conclusion: Our findings identify a previously unrecognized role for MCs and MC–derived IL-1β in contributing to the early stages of MSU crystal-induced acute arthritis in mice.

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Cannabinoids modulate immune cell-assisted angiogenesis and lymphangiogenesis

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Background: Endocannabinoids are lipid mediators that exert their functions by activating cannabinoid receptor type-1 (CB1) and -2 (CB2). Macrophages play a pivotal role in the orchestration of immune and inflammatory responses by producing a wide spectrum of proinflammatory mediators, angiogenic and lymphangiogenic factors. There is increasing evidence that certain immune cells can express CB1 and/or CB2 receptors. It is unknown whether human primary macrophages express a complete endocannabinoid system. We have investigated endocannabinoid production, CB1 and CB2 expression and the functional effects of CB agonists on the production of angiogenic and lymphangiogenic factors by human lung macrophages (HLM).

Results: HLM constitutively produced 2-arachidonoylglycerol (2-AG) and anandamide. HLM stimulated with LPS (1 µg/ml) selectively overproduced 2-AG. This effect was not accompanied by changes in the expression of 2-AG metabolic enzymes, and was preceded by the elevation of the 2-AG biosynthetic precursor, *sn*-1-stearoyl-2-arachidonoyl-glycerol. HLM constitutively express CB2 and, to a lesser extent CB1, at mRNA and protein level. Activation of CB1 and CB2 with their selective agonists (ACEA and JWH-133, respectively) induced ERK1/2 activation and ROS generation. LPS-activated HLM produced VEGF-A, VEGF-C, angiopoietin 1 (Ang1), angiopoietin 2 (Ang2), cytokines (TNF-α and IL-6) and chemokines (IL-8). ACEA and JWH-133 concentration-dependently (0.03 - 3 µM) inhibited (20 - 90 %) LPS-induced production of VEGF-A, VEGF-C and angiopoietins. The effects of CB1 and CB2 agonists were inhibited by CB1 (AM-251) or CB2 (AM-630) antagonists respectively. Interestingly, ACEA and JWH-133 did not modify the production of TNF-α, IL-6 and IL-8 from LPS-stimulated macrophages.

Conclusions: These results demonstrate that human primary macrophages express a complete endocannabinoid system. CB1 and CB2 activation inhibits the release of both angiogenic and lymphangiogenic factors without modifying the production of cytokines and chemokines. CB receptors could be a novel target to modulate immune cell-assisted angiogenesis and lymphangiogenesis.

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Differences in eosinophilic and mast cell dependent mediator patterns in chronic rhinosinusitis with and without nasal polyps

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Introduction: Chronic Rhinosinusitis (CRS) is characterized by inflammation of the mucosa of the nose and paranasal sinuses. CRS is classified into CRS with and without nasal polyps (CRSwNP/CRSsNP), although it is unclear why some patients develop these and some do not. While it is well known that eosinophils play a role in pathogenesis of the CRS and mast cells are the primary effected cell of IgE mediated histamine release, the modulatory effect of the mast cells is not clearly understood. New evidence shows, that mast cells can interact with eosinophils directly.

Aim of the study: In this study, we examined the quantity and activity of mast cells and also the distribution of eosinophils in biopsy material from controls as well patients with CRSsNP and CRSwNP.

Patients and methods: Nasal biopsies were investigated from patients with CRS (5 CRSsNP, 5 CRSwNP) and inferior turbinates from 5 controls. We used immuno-histochemistry and enzyme-histochemistry techniques on frozen sections to determine the expression of tryptase, chymase and major basic protein (MBP) in CRS. We also determined quantitatively histamine level and tryptase activity in homogenates of the biopsy materials.

Results: The comparison of histamine concentration revealed no significant differences between controls and both groups of CRS samples. But the enzyme-histochemistry results detected a reduced number of mast cells with enzymatic activity (tryptase, chymase) in CRSwNP biopsies in contrast to CRSsNP or controls. In accordance with these results most of the FcR epsilon positive cells in polyps had hardly any tryptase activity. However in CRSwNP samples a higher number of MBP positive eosinophils were seen, while they were low in CRSSNP and in controls.

Conclusion: Our results show that in nasal polyps the number of mast cells present with enzymatic activity are low and limited tryptase activitiy may be part of the pathogenesis of CRS with nasal polyps.

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Yield and quality of basophil RNA are highly dependent on the RNA extraction technique

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Background: Basophil granulocytes comprise < 1% of the blood leukocytes. In addition, they have been suggested to only contain small amount of RNA resulting in low quantities after RNA

purification. These two factors make studies of basophil RNA challenging.

We aimed at comparing RNA yield and quality from basophils versus white blood cells by testing two different RNA purification techniques. RNA quality was assessed by analysing gene expression of FcεRI, β-actin, L-histidine decarboxylase (HDC) and HuPO.

Methods: Peripheral blood mononuclear cells (PBMCs) were purified from buffy coat blood stored over night at 4EC. Basophils (n=8 donors) were purified by negative selection. RNA was purified from cells either snap-frozen in liquid N2 and then treated according to Qiagen RNeasy mini kit or dissolved in Trizol containing glycogen followed by phenol/chloroform extraction. RNA quantity was determined using a Qubit RNA assay kit and RNA quality was investigated using a StepOnePlus real-time PCR.

Results: Basophil purity was 66 % to 97 % (median 90 %). Independent of purity, we obtained enough RNA for quantification from all 8 donors using Trizol-phenol/chloroform (193 ± 90 ng/ml RNA/10^5 total cells). However, with RNeasy only 3 donors had a quantifiable RNA level and the concentration was significant less (mean: 11± 7 ng/ml, p=0.012). Using PBMCs (4 donors), again RNA could be quantified from all 4 donors using Trizol-phenol/chloroform purification (370 ± 101 ng/ml) but only 2 out of 4 with RNeasy, and in addition with a highly reduced concentration (29 ± 15 ng/ml). Comparing the RNA quantity obtained from basophils with the quantity from PBMCs illustrated a 2 fold difference indicating that basophils do not have abnormal low RNA levels.

Real-time PCR revealed significant higher C_T-values (FceRIα, β-actin) or even undetectable gene expression (HDC, HuPO) in the RNeasy purified RNA illustrating a higher quality of Trizol-phenol/chloroform purified RNA.

Conclusion: Quantity and quality of purified RNA from human basophils are highly dependent on the purification technique. Furthermore basophils express comparable levels of total RNA to white blood cells.

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Human basophil-monocyte interaction mediated by IL-3

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Background: Monocytes are important immune effector cells owing to their ability to polarize in response to different inflammatory milieus. Recent *in vivo* evidence suggests that basophil-derived IL-4 is essential for the alternative (M2) activation of mouse monocytes. However, whether this model applies to human basophils and monocytes has not been established. Here, we sought to characterize the monocyte activation status in a human basophil-monocyte co-culture model and identify its molecular determinants.

Method: CCL17, IL-10 and IL-12p70 production was assessed by ELISA and Real Time RT-PCR in basophil-monocyte co-cultures and monocyte cultures. Surface expression of IL-3 receptor α (CD123) and STAT5/STAT6 phosphorylation were assessed by flow cytometry. Monocytes were also isolated from treatment-naïve asthmatic patients with an increased eosinophilic blood count and gender- and sex-matched healthy donors and their expression of CCL17 and CD123 was assessed by flow cytometry and Real Time RT-PCR.

Results: In a basophil-monocyte co-culture model, IL-3 and basophil-derived IL-4 and IL-13 induced monocyte production of CCL17 (a marker of alternative activation). To identify the effect of each cytokine on monocyte activation, we stimulated purified monocytes with IL-3 and/or IL-4. Strikingly, IL-3 and IL-4 had a synergistic effect on CCL17 production. No production of IL-10 and IL-12p70 was observed in these cultures. Although freshly isolated monocytes did not respond to IL-3 stimulation (as assessed by STAT5 phosphorylation) and IL-3 did not modify the response to IL-4 (as assessed by STAT6 phosphorylation), overnight pre-incubation with IL-4 upregulated CD123 and made monocytes responsive to IL-3 stimulation. As IL-3 activates the JAK2-STAT5 pathway, we assessed the ability of JAK2 or STAT5 inhibitors to modulate CCL17 production. Both inhibitors markedly reduced CCL17 production in response to IL-3 and IL-4 but not IL-4 alone, confirming the specific inhibition of the JAK2-STAT5 pathway. Interestingly, monocytes isolated from asthmatic patients exhibited higher levels of CD123 as assessed by flow cytometry and Real Time RT-PCR.

Conclusions: Our data point to an IL-3/STAT5-centered network modulating human basophil-monocyte interaction. We would like to suggest that the coordinated activation of STAT5 and STAT6 may have a major impact on monocyte alternative activation.

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Basophil-derived IL-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation

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Allergic asthma is an inflammatory disease characterized by lung eosinophilia controlled by type 2 cytokines, including IL-4, IL-5, and IL-13. Cysteine proteases are potent triggers of allergic inflammation by causing barrier disruption in lung epithelial cells inducing the elevation of IL-5 and IL-13 from natural helper (NH) cells, a member of ILC2s, which leads to airway remodeling and hyper-responsiveness. In this study, we explored the mechanism of how controls the cysteine proteases induced airway responses. The importance of both basophils and NH cells has been independently reported in allergic responses and protective immunity against helminths. However, collaborative regulation each other remains enigmatic. We found that basophils play a crucial role in NH cell-mediated eosinophilic inflammation

induced by protease allergens. Conditional deletion of basophils caused a resolution of the papain-induced eosinophilia and mucus production. Resolution of eosinophilia was also observed in mice lacking IL-4 production specifically in basophils, indicating that basophil-derived IL-4 enhanced proliferation and expression in NH cells of Ccl11, IL-5, IL-9 and IL-13, attracting eosinophil. These results demonstrate that IL-4 secreted from basophils has an important role in the IL-33-NH cell-IL-13/Eotaxin axis subsequently leading to protease allergen induced airway inflammation.

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Group V secreted phospholipase A_2 mediates the production of angiogenic and anti-angiogenic factors from human neutrophils

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Background: Angiogenesis, the formation of new blood vessels from preexisting ones, plays a prominent role in chronic inflammatory disorders and tumors. This process is sustained by the coordinated production of several angiogenic factors including Vascular Endothelial Growth Factors (VEGFs) and Angiopoietins (Angs). Secreted phospholipases A2 (sPLA2) are multivalent mediators involved in inflammatory diseases and tumors. Human neutrophils (PMNs) are both a source and a target of sPLA2s. These cells release group V sPLA2 and can be activated by sPLA2s to release CXCL8. We have investigated the role of group V sPLA2 in the production of angiogenic factors from PMNs.

Methods: VEGF-A, -B, -C, -D and Angs (Ang1 and Ang2) expression was evaluated by RT-PCR in highly purified (>99%) PMNs. Release of VEGF-A, VEGF-A165b, Ang1 and CXCL8 was evaluated by ELISA.

Results and Conclusions: PMNs constitutively express mRNAs for the proangiogenic molecules VEGF-A165, VEGF-B167, VEGF-B186, and Ang1. mRNA for VEGF-A121, VEGF-A189, VEGF-C, VEGF-D, and Ang2 was not detected. PMNs also expressed mRNA for the anti-angiogenic factor VEGF-A165b. *In vitro* stimulation of PMNs with increasing concentrations (0.1 to 5 □g/ml) of human recombinant group V sPLA2 (hGV) induced the release of VEGF-A (71.5±5.5 pg/10^6 cells), Ang1 (121.2±3.1 pg/10^6 cells) and CXCL8 (311.8±81.2 pg/10^6 cells). hGV also induced the release of VEGF-A165b (6.6±0.5 pg/10^6 cells). hGV-induced release of VEGF-A was significant after 15 min ($p<0.01$) and progressively increased up to 6 hours. Preincubation (30 min, 37°C) of hGV with Me-Indoxam (1 mM), which blocks receptor-mediated effects of sPLA2s, abolished the release of VEGF-A, Ang1 and CXCL8. These results indicate that hGV induced the production of both angiogenic and anti-angiogenic factors from PMNs by a receptor-mediated mechanism. Activation of PMNs by fMLF induced the release of hGV as well as of VEGF-A and CXCL8. Preincubation (10 min, 37°C) of PMNs with Me-Indoxam (1 mM) before stimulation with fMLF (1 mM) significantly inhibited (≈ 55%) the release of VEGF-A and CXCL8. These results are compatible with the hypothesis that endogenous hGV may be involved in fMLF induced release of VEGF-A and CXCL8.

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The fate of IL-4-switched B cells: dynamics of IgE and IgG4 in the hyper-IgG4 syndrome

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The behavior of the post-switch B cell is markedly isotype-dependent. IgE and IgG4 are the human isotypes that are most strongly dependent on TH2 conditions. We postulate that the local environment at the site of switching influences the post-switch fate of the B cell. Since IgE switched B cells are rare, we focused on IgG4-switched B cells. Their fate is still uncertain, largely because there is no close mouse homologue, but also because a reliable detection of membrane-anchored IgG4 was a technical challenge. With the availability of an antibody suitable for FACS procedures, ex-vivo and in-vitro analyses of IgG4-switched B cells are now feasible.

As a significant clinical problem, but also as a model system for TH2-driven immune responses, we are investigating IgG4-related disease (IRD), also known as the hyper-IgG4 syndrome. This is characterized by marked expression of IgG4-positive polyclonal plasma cells in non-lymphoid organs (e.g. pancreas), with severe organ dysfunction due to the tumor-like mass of plasma cells, without evidence of antibody-related damage. Treatment with corticosteroids, methotrexate and/or rituximab rapidly normalizes not only the number of circulating IgG4 B cells, but also plasma IgG4 levels, indicating a high turnover of IgG4 plasma cells. These results suggest a deranged migration, expansion, differentiation and turnover of IgG4-switched B cells in the affected organ(s). However, no plausible antigenic stimulus has been identified to date. Based on the marked reactivity of IgG4 to a mannose-specific lectin from banana, our working hypothesis for this polyclonal B cell activation is that Fab-glycosylation of the B cell receptor makes these B cells susceptible to a tissue-expressed lectin.

This was investigated in IRD patients during therapy by analyzing the dynamics of lectin-positive and lectin-negative IgG4 in plasma. The results indicate that lectin-positive and lectin-negative IgG4 change in parallel during treatment, which does not support the hypothesis that the aberrant IgG4 production is exclusively derived from B cells with lectin-positive IgG4. A highly significant correlation was found between total IgG4 and total IgE. This suggests a significant contribution of the indirect switching pathway of IgE production (from IgM via IgG4 to IgE) in this condition.

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Interleukin-21 induced IgE-synthesis in memory B cells: A role for T follicular helper cells in Seasonal Allergic Rhinitis

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Background: T follicular helper T (Tfh) cells are a distinct CD4 T cell subset with modulatory properties. Distinguishing features of Tfh cells are the expression of Bcl6 as a master regulator of Tfh cells differentiation; CXCR5, PD-1, ICOS and they secrete interleukin (IL) -21. We hypothesised that IL-21+ Tfh cells are differentially expressed in patients with seasonal allergic rhinitis compared to non-atopic controls and IL-21 induces IgE production from memory B cells and contributes toward immunopathophysiology of Th2 allergic response.

Method: PBMCs were isolated from 12 grass pollen allergics (SAR) and 12 non-atopic controls (NA) during the grass pollen season. IL-21$^+$CD4$^+$CXCR5$^+$PD-1$^+$BCL6$^+$ T cells were quantified by flow cytometry. PBMCs obtained from SAR were stimulated with anti-CD40L (100ng/mL), IL-4 (100ng/mL) in a presence or absence of IL-21 (100ng/mL). Total IgE levels were measured by ImmunoCAP system.

Results: Grass pollen allergics had higher total rhinoconjunctivitis symptom scores compared to NA ($p<0.001$). The frequency of IL-21$^+$CD4$^+$CXCR5$^+$PD-1$^+$BCL6$^+$ Tfh cells were elevated in SAR compared to NA ($p<0.001$). The proportion of CD4$^+$CXCR5$^+$PD-1$^+$BCL6$^+$ Tfh cells remained unchanged in SAR compared NA. IL-21 gene expression and protein levels were increased in SAR compared to NA ($p<0.001$). Furthermore, IL-21 induced a dose-dependent increase in IgE production in PBMCs stimulated with anti-CD40L (100ng/mL) and IL-4 (100ng/mL). Interestingly, IL-21, anti-CD40L and IL-4 augmented IgE production in PBMCs compared to anti-CD40L and IL-4 or IL-4 alone. IL-21$^+$CD4$^+$CXCR5$^+$PD-1$^+$BCL6$^+$ Tfh cells in SAR correlated with sIgE levels ($r=0.59$, $p<0.05$).

Conclusions: Our preliminary findings suggest that IL-21$^+$CD4$^+$CXCR5$^+$PD-1$^+$BCL6$^+$ Tfh cells are elevated in SAR compared to NA. IL-21-derived from Tfh cells is associated with IgE-production from PBMCS. Their role in the pathophysiology of grass pollen allergic rhinitis remain to be further studied.

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HLA-class II peptide tetramers vs. allergen-induced proliferation for identification of allergen-specific CD4 T cells in the model of mugwort pollen allergy

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Background: Fluorescence-labeled MHC class II/peptide tetramer complexes are considered as optimal tool to identify peptide-specific CD4$^+$ T cells. However, this technique is costly and restricted to frequently expressed HLA-class II molecules and the knowledge of immunodominant epitopes. Mugwort pollen-allergy is an ideal candidate to apply this tool in allergy research. Art v 1, the sole major mugwort allergen contains only one single immunodominant epitope (Art v 125-36), which is mainly restricted by HLA-DR1.

Methods: We used the HLA-DRB1*01:01/Art v 119-36 tetramer to investigate its specificity and sensitivity for allergen-specific T cells and compared tetramer detection with CFSE-dilution of proliferating T cells in response to Art v 125-36.

Results: In PBMC from HLA-DR1-positive mugwort-allergic or healthy individuals only very few, respectively no tetramer$^+$ T cells could be detected *ex vivo*. After *in vitro* expansion, tetramer$^+$ T cells could be detected in 83% of Art v 125-36-reactive T cell lines (TCL) from mugwort-allergic individuals, but not in TCL from healthy individuals. The tetramers defined a Th2-biased subset at an intermediate to late differentiation stage (CD45RO$^+$ CD27$^-$ CCR7$^-$ PD-1$^+$). CD4$^+$ T cells that showed a proliferative response to allergen-stimulation (CFSE^{lo}) were less mature and contained considerable numbers of bystander cells with very low proportions of Th2 cells. Unexpectedly, only 44 % of Art v 125-36-specific T cell clones showed detectable tetramer reactivity.

Conclusion: While allergen-induced proliferation defined also bystander CD4$^+$ T cells, the Art v 1/DR1 tetramer showed high specificity, but a low sensitivity for T cells reactive with the immunodominant Art v 125-36 cognate peptide. Although, pMHCII tetramers are still the most accurate tool to identify allergen-specific CD4$^+$ T cells for assessment of functional aspects, their low sensitivity renders their use much more problematic compared to pMHCI tetramers.

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Increased expression of periostin and IL-33 in chronic rhinosinusitis with nasal polyps

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Background and purpose: CRS with nasal polyps (CRSwNP) is often associated with asthma and characterized by markedly increased numbers of eosinophils, Th2 type lymphocytes, fibroblasts, goblet cells and mast cells. The inflammation leads to a proliferative response in the extracellular matrix (ECM). Previously, we demonstrated high expression of thymic stromal lymphopoietin (TSLP) in good correlation with IgE and eosinophil cationic protein (ECP) and decreased levels of FOXP3+ cells in CRSwNP. Periostin is an ECM protein induced by Th2 cytokines and known to play a role in tissue remodeling in inflammatory diseases. IL-33 is an IL-1-cytokine family member that plays a role in regulating Th2 inflammation. Here we investigated the expression of periostin, IL-13 and IL-33 in nasal polyps (NP) from atopics and non-atopics and the nasal mucosa (NM) from patients with allergic rhinitis (AR).

Methods: NP from atopic and non-atopic patients and NM from AR patients were obtained at surgery done for the treatment. Immunohistochemistry using the peroxidase-based Avidin-Biotin Complex (ABC) method was used to analyze the expression of periostin and IL-13. Real time PCR was performed to evaluate the mRNA expression of periostin and IL-33. The number of positively stained cells was analyzed using an objective micrometer and the density of immunoreactivity was quantified by Image J analysis system.

Results: Periostin was mainly expressed in the basement membrane and the density of expression was significantly higher in NP than in the NM of AR patients. Periostin expression was higher in NP from atopic than non-atopic patients. IL-13+ cells were greater in NP than in the NM and there was a good correlation between the number of IL-13+ cells and the expression of periostin in NP. IL-33 mRNA expression was also higher in NP than NM and there was a good correlation between periostin and IL-33.

Conclusions: Based on the findings of the high expression of periostin, IL-33 and IL-13 in NP and the correlation of periostin to IL-13 and IL-33, periostin and IL-33 may play potentially crucial roles in the pathogenesis of nasal polyps.

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Effects of corticosteroid on cytokine-induced periostin production by primary human lung and dermal tissue cells

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Background: Periostin, a matricellular protein originally isolated from an osteoblast cell line, is reported to be induced by each of IL-4, IL-13 and TGF-β in airway epithelial cells, and lung and skin fibroblasts. Increasing evidence indicates that epithelial cell- and/or fibroblast-derived periostin is involved in the pathogenesis of allergic diseases, including asthma and atopic dermatitis, as a component in subepithelial fibrosis. Overproduction of periostin despite adequate inhaled corticosteroid treatment plays a key role in the chronicity of allergic inflammation seen in corticosteroid-refractory lung fibrosis in asthma. However, an earlier study demonstrated that IL-13-induced periostin expression by airway epithelial cells was completely inhibited by dexamethasone treatment. Therefore, we hypothesized that some tissue cells other than epithelial cells must produce periostin in a corticosteroid-insensitive manner.

Methods: Primary human normal lung and dermal cells (microvascular endothelial cells and fibroblasts) were stimulated *in vitro* with IL-4, IL-13 or TGF-β in the absence and presence of dexamethasone (Dex). Periostin mRNA and protein levels were quantified by qPCR and ELISA.

Results: IL-4 and IL-13 each induced comparable, significant levels of periostin production by both kinds of tested cells. When added to the fibroblasts, Dex completely inhibited IL-4/13-induced periostin production, but it did not affect TGF-β-induced production. In contrast, Dex induced periostin production by microvascular endothelial cells, and a synergistic effect was shown by Dex plus Th2 cytokines. TGF-β did not induce periostin production by microvascular endothelial cells. RU-486, a corticosteroid receptor antagonist, completely abrogated both the enhancing and inhibitory effects of Dex on periostin production.

Conclusion: Our present findings may help explain how overproduction of periostin is promoted in subepithelial or dermal tissues. They also suggest that IL-4/13-induced microvascular endothelium-derived and/or TGF-β-induced fibroblast-derived periostin might play a pivotal role in corticosteroid-refractory tissue fibrosis, leading to chronic allergic inflammation in the lung and/or skin.

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Platelets constitutively express active interleukin-33 protein

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Background: Recent studies revealed that platelets are activated during asthma exacerbation and that depletion of platelets

in a mouse model of asthma improves airway inflammation. However, the precise mechanisms of how platelets—despite being devoid of a nucleus—regulate allergic inflammation are not fully understood. IL-33, an IL-1 family cytokine, has recently attracted attention as a critical cytokine in the development, exacerbation and prolongation of allergic diseases. We thus hypothesized that platelets contain and release active IL-33 protein and play a pivotal role in IL-33-dependent airway inflammation.

Objective: To determine whether platelets express IL-33 protein.

Methods: IL-33 protein in human platelets and a megakaryocyte cell line, MEG-01, and in bone marrow-derived mouse megakaryocytes was detected by Western blot analysis and fluorescent immunostaining. Papain inhalation (25mg/day x 3 days)-induced IL-33-dependent airway inflammation (Oboki K. et al. PNAS 2010) was compared between platelet-intact and platelet-depleted (by injection of anti-CD41 mAb) groups.

Results: Human platelets and anucleated fragments of mouse bone-marrow-derived megakaryocytes expressed full-length IL-33 protein, which is biologically active. Cytosol, but not nuclear, fractions of MEG-01 and mouse megakaryocytes also expressed full-length IL-33 protein. Depletion of platelets resulted in a significant decrease in eosinophilic, but not neutrophilic, inflammation in papain-treated mouse airways.

Conclusions: Our novel findings suggest that platelets constitutively express active IL-33 protein and that activation of platelets plays a pivotal role in IL-33-dependent type II airway inflammation. Further understanding of the precise molecular mechanisms may lead to platelets' becoming an attractive new target in asthma treatment.

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Cytokine secretion profiles in the tears of patients with chronic allergic conjunctivitis

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Background: Atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC) are chronic or chronically relapsing ocular inflammatory diseases that threaten vision. The clinical characteristics of these diseases include T helper-cell 2 (Th2 cells)-dominant conjunctival inflammation that manifests as proliferative changes in the conjunctiva, such as giant papillae. These are the most important complications and often cause corneal damage. Giant papillary tissues show preferential accumulation of mononuclear cells and granulocytes, especially eosinophils, under the epithelium. Increased vascularity and excess fibrous connective tissue also occur. Although abundant expression of Th2 cytokines and eosinophil-recruiting chemokines has been reported, the precise mechanisms of how the above proliferative changes occur remain unsolved. To elucidate the tissue remodeling mechanisms of severe allergic conjunctivitis, we investigated the cytokine secretion profiles in the tears of patients with various types of allergic conjunctivitis.

Methods: Tear samples were obtained from 39 patients with AKC, 6 patients with VKC, 15 patients with seasonal allergic conjunctivitis (SAC) and 16 healthy volunteers (control). The concentrations of various cytokines in the cell-free tears were analyzed using a multiplex cytokine array system.

Results: The concentrations of pro-inflammatory cytokines (IL-1beta, IL-6, TNF-alpha) and Th2 cytokines (IL-13, IL-5) were significantly higher in tears from the AKC/VKC patients compared with the controls. In addition, Th1 cytokines (IFN-gamma, IL-12p70), a Th17 cytokine (IL-17) and other cytokines (IL-10, IL-9) were also significantly elevated in the AKC/VKC patients, but not in the SAC patients. The concentrations of IL-13 correlated strongly with the concentrations of IFN-gamma, IL-17, TNF-alpha and IL-9.

Conclusions: Our data strongly suggest that not only Th2 cells, but also Th1 cells and Th17 cells, are activated concurrently and may be involved in the pathogenesis of proliferative changes in the conjunctiva of AKC and VKC. The mechanisms underlying multi-lineage T-cell activation might be suitable therapeutic targets for the treatment of severe allergic conjunctival diseases.

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In vitro effects of Interleukin-17 on cultured human bronchial epithelial cells

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Background: Asthma is now considered a heterogeneous disease, presenting with several distinct phenotypes. Although mild to moderate asthma is characterized by eosinophilic inflammation, some severe patients are known to have neutrophilic inflammation and increased numbers of IL-17-producing cells in the airway.

Objective: The aim of this study was to evaluate the effects of IL-17 on cultured normal human bronchial epithelial cells (NHBE).

Methods: NHBE were treated with TNF-α and IL-17, either alone or in combination, for 24 hours. Expression levels of the mRNA and protein of neutrophil-associated chemokines/cytokines were measured by qPCR and ELISA, respectively.

Results: Although IL-17 alone induced almost no chemokines/cytokines in NHBE, in combination with TNF-α it robustly enhanced TNF-α-induced expression of such neutrophil-associated chemokines/cytokines as GRO-α, IL-8, IL-6 and G-CSF. Conversely, that combination significantly attenuated TNF-α-induced expression of eosinophil-, lymphocyte- and monocyte-associated chemokines, such as RANTES and IP-10.

Conclusions: IL-17 can modulate the TNF-α-regulated signaling cascade, resulting in selectively enhanced expression of neutrophil-associated chemokines/cytokines by airway epithelial

cells. This action of IL-17 may be closely associated with the neutrophilic inflammation seen in the airway of some severe patients with asthma.

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Escherichia coli heat-labile detoxified enterotoxin modulates dendritic cell function and attenuates allergic airway inflammation

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Background: Various mutant forms of Escherichia coli heat-labile enterotoxin (LT) have been used as a mucosal adjuvant for vaccines, as it enhances immune responses to specific antigens including antigen-specific IgA antibodies when administrated intranasally or orally. We hypothesized that a detoxified mutant form of LT, LTS61K, could modulate dendritic cell (DC) function and alleviate allergen-induced airway inflammation.

Methods: Two protocols, preventative and therapeutic, were used to evaluate the effects of LTS61K in a Dermatophagoides pteronyssinus (Der p)-sensitized and challenged murine model of asthma. LTS61K or Der p-primed bone marrow-derived dendritic cells (BMDCs) were also adoptively transferred into Der p-sensitized and challenged mice.

Results: Intranasal inoculations with LTS61K or LTS61K/Der p decreased allergen-induced airway inflammation and alleviated systemic TH2-type immune responses. Bronchoalveolar lavage fluid (BALF) and sera from LTS61K/Der p-treated mice also had higher concentrations of Der p-specific immunoglobulin (Ig) A than those of other groups. In vitro, BMDCs stimulated with Der p underwent cellular maturation and secreted proinflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF) α. In contrast, Der p-stimulated BMDCs that were pretreated with LTS61K showed decreased IL-6 and TNFa production and were less mature. Intratracheal adoptive transfer of LTS61K- or LTS61K/Der p-primed BMDCs into Der p-sensitized mice reduced inflammatory cell infiltration and TH2-type chemokines in BALF and alleviated airway inflammation in treated mice. LTS61K influenced DC maturation and decreased inflammatory cytokine production. Moreover, LTS61K/Der p induced increased Der p-specific IgA production to decrease allergic TH2 cytokine responses and alleviated airway inflammation in Der p-sensitized mice.

Conclusions: These results suggest that the immunomodulatory effects of LTS61K may have clinical applications for allergy and asthma treatment.

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In depth comparison of Monophophoryl Lipid A (MPLA) and Lipopolysaccharide (LPS): immune activation, adjuvant capacity and application as part of a MPLA:allergen fusion protein

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Monophosphoryl lipid A (MPLA), a non-toxic TLR4 ligand derived from *Salmonella minnesota R595* (Re) lipopolysaccharide (LPS) by chemical modification, is clinically used as an adjuvant for cancer treatment (Ceravix®), hepatitis vaccination (Fendrix®), and allergen-specific immunotherapy (Pollinex® Quattro). Nevertheless, its mechanism of adjuvant activity is not clear. In this study, we compared the immune modulating capacities of MPLA and LPS.

In mouse bone marrow-(BM-) derived myeloid dendritic cells (mDCs), LPS and MPLA induced a similar pattern of cytokine secretion (IL-1β, IL-6, IL-10 and TNF-α), albeit requiring higher concentrations of MPLA. Using Trif-deficient mDCs, cytokine secretion had greater Trif-dependency with MPLA than with LPS. Stimulation with high doses of either agent resulted in comparable mDC activation (i.e., CD40 and CD69 upregulation), albeit with different thresholds of activation (MPLA vs. LPS: 100 vs. 1 ng/ml). In BM-derived cultured mast cells (BMCMCs), LPS but not MPLA induced IL-6 secretion but neither MPLA nor LPS had any effect on DNP-induced ß–hexosaminidase release. Compared to stimulation with Ova alone, co-administration of a mixture of MPLA and Ova resulted in enhanced IL-5 secretion from DO11.10 $CD4^+$ T cells co-cultured with mDCs, an effect not observed with LPS plus Ova. Moreover, stimulation with MPLA coupled to Ova (MPLA:Ova) resulted in enhanced cytokine secretion from both mDCs (IL-1β, IL-6, TNF, IL-10, IL-12) and DO11.10 CD4 T cells (IL-5, IL-13, IL-2, IFN-γ, IL-17) compared to equimolar amounts of a mixture of MPLA and Ova. In an *in vivo* endotoxin shock model (ESM) in mice and a human *ex vivo* monocyte activation test (MAT), MPLA induced increased levels of the same cytokines as did LPS (ESM: IL-6, IL-12, TNF- α; MAT: IL-1ß, IL-6, TNF- α), albeit at lower levels.

In summary, using a variety of different assay systems, we observed that MPLA induced similar immune responses compared to LPS, but less potently. Stimulation of $CD4^+$ T cells with MPLA coupled to an allergen boosted allergen-specific production of TH1-, TH2-, and TH17-associated cytokines. Although MPLA is considered to be safe in humans, further studies should critically assess the adjuvant capacity of MPLA in order to evaluate possible non-desired immunological effects.

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The persistence of asthma requires the establishment of multiple interconnected feedback circuits involving airway epithelial cells and type 2 innate lymphoid cells but not adaptive immune cells

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Background: Although the mechanism of development of acute asthma has been extensively studied, the mechanism of persistence of asthma is poorly understood. We developed a mouse model where asthma persists for a remarkable 6 month period after cessation of allergen exposure. We utilized a combination of microarray, genetic, phosphoproteomic and pharmacologic approaches to dissect the mechanism of persistence of asthma.

Methods: Chronic asthma is induced through twice weekly intranasal exposure to a combination of allergens (dust mite, ragweed, and Aspergillus) for six weeks. Immune ablation was accomplished by lethal irradiation three weeks after the allergen exposure. Bone marrow from naïve, rag1-/-, or rag2-/-γc-/- were transferred following lethal irradiation. Airway resistance to inhaled methacholine (by Flexivent), immunohistological features, and lung cytokine production was measured 6 weeks after irradiation. The gene expression profile in acute and chronic asthma was compared by microarray. Activation of signaling molecules was studied by a phosphoprotein screen (Kinexus, Inc.). Airway hyperreactivity and lung cytokine production was measured 2 weeks after intranasal IL33 delivery to WT and ERK1-/- mice. Bronchoalveolar lavage (BAL) cells and fluid from asthmatic patients and disease controls were studied by flow cytometry and ELISA for type 2 innate lymphoid cells (ILC2) and IL33, respectively.

Results: We showed that repetitive allergen exposure established multiple interconnected feedback and feedforward circuits in the chronic asthma model: 1). Allergen-› epithelial ERK-› spry2-› Fyn-› ERK-› epitheial IL33. 2). Epithelial IL33-› ILC2-› IL13-› epithelial IL33. 3). Epithelial IL33-› ILC2-› IL13-› epithelial IL33 receptor -› IL33 autoinduction. The consequence of these feedback circuits is a persistent increase in epithelial ERK1/2 signaling, IL33 production and lung ILC2 (Lin-CD25+IL7Rα+c-kit+ Sca1+ST2+CRTH2+IL5+IL13+ cells) generation. Lethal irradiation eliminated antigen-specific T cells while bone marrow transfer from naïve, rag1-/-, or rag2-/-γc-/- mice demonstrated a requirement for ILC2 but not T cells in maintaining airway hyperreactivity, remodeling and inflammation once chronic asthma is established. Exogenous delivery of IL33, adoptive transfer of ILC2, antibody blockade of IL33 and IL13, and genetic and pharmacologic inhibition of ERK1/2 have shown that each component of this feedback mechanism is essential for persistence of asthma. Surprisingly, antigen-specific T cells were redundant for persistence of asthma, although they contributed to its severity. In agreement with mouse data, IL33 and ILC2 (Lin-FcεRI-CD25+IL7Rα+ST2+CRTH2+IL5+IL13+ cells)were increased in BAL from asthmatic patients as compared to disease controls and were correlated with disease severity (FEV1).

Conclusion: We conclude that an ILC2-facilitated and ERK1/2-driven positive feedback mechanism of epithelial IL33 generation is essential for persistence of asthma.

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PGI_2 signaling inhibits IL-5 and IL-13 protein expression by group 2 innate lymphoid cell in response to inhaled aeroallergen

Stokes Peebles

Background: Group 2 innate lymphoid cells (ILC2) produce large quantities of IL-5 and IL-13, cytokines central to the asthma phenotype. Very few pharmacologic agents and no prostaglandins are known to inhibit cytokine production by ILC2 cells.

Methods: ILC2 were isolated from wildtype (WT) and mice lacking the prostaglandin (PG)I2 receptor IP (IP KO) and activated with IL-33 in the presence or absence of the PGI2 analog cicaprost. WT and IP KO mice were challenged via the airway for 4 consecutive days with *Alternaria alternata* allergen extract (Alternaria extract). IL-5 and IL-13 were assayed from lung homogenates by ELISA and ILC2 cells were identified by flow cytometry. Human ILC2 were purified from red blood cells filters obtained from the American Red Cross and activated with IL-33 in the presence or absence of the PGI2 analog cicaprost.

Results: Cicaprost inhibited IL-5 and IL-13 protein expression from IL-33-stimulated ILC2 purified from mouse bone marrow in a manner that was dependent on signaling through the PGI2 receptor IP. In addition, PGI2 induced ILC2 apoptosis in an IP-dependent fashion. Endogenous PGI2 signaling significantly inhibited Alternaria extract-induced lung IL-5 and IL-13 protein expression and reduced the number of lung IL-5 and IL-13 expressing ILC2, as well as the mean fluorescence intensity of IL-5 and IL-13 staining. In addition, exogenous administration of cicaprost inhibited *Alternaria* extract-induced lung IL-5 and IL-13 protein expression, and reduced both the number of lung IL-5 and IL-13 expressing ILC2, in addition to the mean fluorescence intensity of IL-5 and IL-13 staining. Finally, a PGI2 analog inhibited IL-5 and IL-13 expression from human ILC2 that were stimulated with IL-2 and IL-33.

Conclusion: These results suggest that PGI2 is a therapeutic target to reduce the innate allergic response to protease containing aeroallergens such as *Alternaria alternata*. Inhaled PGI2 analogs are currently approved for the treatment of pulmonary hypertension and their use could be extended for treatment of allergic airway diseases such as asthma.

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Symbiotic microbiota regulates type 2 immunity through RORgt⁺ Tregs

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Background: The hygiene hypothesis postulates a correlation between the lack of exposure to infectious agents, microorganisms or parasites in early childhood and an increased susceptibility to allergic disease. Antibiotic treatment early in life predisposes to allergic pathologies both in man and mice, and germfree mice produce elevated levels of serum IgE, suggesting that the symbiotic microbiota regulates the development of type 2 immunity. Recent data show that regulatory Foxp3⁺ T cells (Treg) generated in the periphery and depending on the Foxp3 enhancer conserved non-coding sequence (CNS)1 control allergic responses. Furthermore, intestinal Tregs express TCRs specific for the intestinal microbiota arguing for a dominant role of the microbiota for induction of intestinal regulatory T cells.

Method: We use germfree, antibiotic treated and ex-germfree mice and a set of knockout and conditional knockout mice to analyse various subsets of intestinal lamina propria cells and to test their effect for immunopathology.

Result: We show that Tregs induced by the intestinal microbiota co-express RAR-related orphan receptor gamma t (RORgt), a transcription factor required for the development of lymphoid cells expressing IL-17 or IL-22, even though RORgt⁺ Tregs express high amounts of IL-10, no or low levels of IL-17 and are regulatory. While being completely absent in the thymus, RORgt⁺ Tregs constitute the majority of Tregs in the adult colon and start to populate the intestine around weaning suggesting an important role for the developing microbiota during induction of RORgt⁺ Tregs. Furthermore, we demonstrate a critical role for the major histocompatibility complex class II, dendritic cells, interleukin 6 and cell-intrinsic expression of the signal transducer and activator of transcription 3 for induction of RORgt⁺ Tregs. RORgt-deficient mice and mice with a deficiency for RORgt specifically in regulatory T cells show an increase in Gata-3⁺ Tregs and Th2 cells, elevated levels of serum IgE and type 2 immunopathology.

Conclusions: RORgt⁺ Tregs link the symbiotic microbiota with regulation of type 2 immunity, and their loss leads to deregulated pro-allergic responses. Therefore, RORgt⁺ Tregs might serve as a cellular basis for the hygiene hypothesis.

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Let-7f miRNA differentially regulates IL-17A protein expression in Th17 cells from women compared to men

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Background: Prior to puberty, males have an increased prevalence of severe asthma; however, after puberty, females have a greater prevalence, suggesting a role for sex hormones. The mechanisms that regulate the age-related gender differences in severe asthma prevalence are unknown. IL-17A is secreted by CD4+ Th17 cells, and is increased in the bronchoalveolar lavage fluid of patients with severe asthma compared to milder asthma phenotypes. Th17 cell differentiation requires IL-23/IL-23 receptor (R) signaling for maximal IL-17A protein expression, and IL-23R is negatively regulated by the Let-7f miRNA. We hypothesized that 17b-estradiol (E2) and progesterone (P4) increase IL-17A protein expression in Th17 cells by decreasing Let-7f miRNA expression and increasing IL-23R expression.

Methods and Results: Naïve T cells from healthy men and women (ages 18-45) not taking exogenous sex hormones were differentiated to Th17 cells. IL-17A protein expression and IL-23R surface expression was significantly increased and Let-7f miRNA expression was significantly decreased in Th17 cells from women compared to men. Inhibition of Let-7f at the time of Th17 cell differentiation increased IL-17A+ CD4+ Th17 cells in T cells from both women and men. However, addition of 17b-E2 or P4 during Th17 cell differentiation had no effect on IL-17A protein expression, suggesting an *in vivo* epigenetic mechanism for sex hormones on Th17 cell differentiation. Using an *in vivo* BALB/c mouse model, we treated ovariectomized female mice with 17b-E2, P4, 17b-E2 plus P4, or placebo for 21 days. As controls, male and female mice receiving sham surgery were administered placebo pellets. Naïve T cells were isolated from spleens and differentiated to Th17 cells. IL-17A protein expression and IL-23R mRNA expression was significantly increased and Let-7f miRNA expression was decreased in the Th17 cells from ovariectomized mice administered 17b-E2 plus P4 compared to Th17 cells from ovariectomized mice administered placebo, suggesting ovarian hormones regulated Let-7f miRNA expression in Th17 cells.

Conclusions: Combined, our results suggest 17b-E2 plus P4 regulate IL-17A protein expression by decreasing Let-7f miRNA expression and increasing IL-23R expression in Th17 cells. Our results are imperative to identify potential therapeutic targets in the Th17 pathway for patients, in particular women, with severe asthma.

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TSLP induces corticosteroid-resistance in the IL-33/natural helper cell pathway

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Background: Asthma is a chronic airway disease characterized by persistent eosinophilic inflammation in the airways dependent on type-2 cytokines such as IL-5 and IL-13. Although inhaled corticosteroids can effectively control the symptoms and airway inflammation in the majority of patients, ~5% of patients with asthma respond poorly to high dose of inhaled corticosteroids. Elucidating the mechanism of corticosteroid resistance in the patients with severe asthma is critical for the development of highly effective, novel pharmacotherapy. Recent studies demonstrate some evidences showing that an IL-1 family cytokine, IL-33, is relevant to the pathophysiology of severe asthma. In response to IL-33, group 2 innate lymphoid cells such as natural helper (NH) cells proliferate and produce large amounts of IL-5 and IL-13. However, it is unknown whether IL-33/NH cell pathway is involved in the corticosteroid-resistant airway inflammation.

Methods and Results: We found that intranasal injection of IL-33 induced innate type-2 immune response accompanied by airway eosinophilia, goblet cell hyperplasia, and accumulation of NH cells, identified as lineage-negative, $Thy1^+c\text{-}Kit^+Sca\text{-}1^+CD25^+IL\text{-}7R^+ST2^{dim}$ cells, in the lungs. Although either the IL-33-induced innate response or acquired type-2 immune response in a classic antigen-induced asthma model was efficiently despaired by intraperitoneal injection of dexamethasone, airway inflammation induced by the addition of IL-33 to antigen-induced asthma model was resistant to dexamethasone, suggesting that an inflammatory milieu in the asthmatic airways can provoke corticosteroid-resistance in NH cells. We isolated NH cells from fat-associated lymphoid cluster or from the lungs, and applied a panel of cytokines *in vitro* to examine the corticosteroid sensitivity of NH cells. Thymic stromal lymphopoietin (TSLP) was identified as a molecule that can dampen the effect of dexamethasone to induce apoptosis in NH cells. The effects of TSLP on NH cells were mediated by controlling phosphorylation of STAT5 and expression of Bcl-xL. We confirmed that TSLP expression was elevated in the asthmatic airways, and the blockade of TSLP/STAT5 signaling with a neutralizing antibody against TSLP or low molecular-weight STAT5 inhibitors such as pimozide, an anti-psychotic drug, restores corticosteroid sensitivity.

Conclusions: TSLP-STAT5 pathway could be a new therapeutic target in severe, corticosteroid-resistant asthma.

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Immune suppression in severe atopic dermatitis mediated by myeloid-derived suppressor cells

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that is nearly always covered with and triggered by *Staphylococci*. The skin is constantly exposed to bacteria and cutaneous innate immune sensing plays a crucial role in shaping immune responses.

Surprisingly we found a strong immune suppression after cutaneous exposure to TLR2/6 ligands and living *S. aureus* in AD mouse models. Investigating underlying mechanism, we found $Gr1^+CD11b^+$ myeloid-derived suppressor cells (MDSCs) to be responsible. Using appropriate knock-out and chimeric mice, we identified TLR2 on skin resident cells and induction of IL-6 responsible for MDSC accumulation, MDSC recruitment to skin, and T-cell suppression. To take our findings further, we next analyzed AD patients, in which cutaneous TLR2 is constantly activated by *Staphylococci* derived substances. We observed a significant increase of human MDSCs in the blood in AD (n=33) in comparison to healthy individuals (n=30), especially in patients with eczema herpeticum, a severe complication of herpes simplex virus infection in AD due to immune suppression. Further investigations of peripheral blood mononuclear cells (PBMCs) revealed in AD patients a significant down-regulation of T-cell receptor ζ-chain, known to be a characteristic of MDSCs-mediated immune suppression. Furthermore, depletion of $CD11b^+$ cells from PBMCs increased T-cell proliferation in AD patients and not in controls, which demonstrates that MDSCs in AD are immunosuppressive. In addition, we found significantly elevated arginase activity in plasma of AD patients and detected a distinct $iNOS^+$ population of $CD11b^+CD11c^-$ cells in AD patients. To investigate whether MDSCs accumulate in the skin we performed FACS amd three colour fluorescence immunohistology of human skin and found MDSCs significantly elevated in AD skin compared to controls. Thus, we conclude that severe bacterial colonization/infection in AD and subsequent skin inflammation causes induction of suppressive MDSCs, which then accumulate in the skin and exert their suppressive activity allowing e.g. herpes viruses to spread.

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Human novel effector and regulatory subsets of memory B cells

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Background: Regulatory B cells (Breg), which produce IL-10 suppress immune responses and the lack of regulatory B cells leads to exacerbated symptoms in mouse models of autoimmunity, transplantation and chronic infections. IgG4 is a characteristic antibody isotype induced in many human high dose

antigen tolerance models and corresponds with IL-10 secreting Breg cells.

Methods: In the present study, we isolated human inducible IL-10-secreting Breg cells and investigated their link to the isotype of the immunoglobulin that they produce after developing into plasma cells. Human IL-10-secreting peripheral B cells were labeled using a cytokine secretion assay and purified by fluorescence-activated cell sorting. IL-10-secreting and non-IL-10 secreting B cells were phenotypically and functionally characterized by whole genome expression analysis, flow cytometry, T cell suppression assay and antibody production analysis. B cells were immortalized by retroviral transduction with Bcl-2 and BclxL and expanded in the presence of IL-21 and CD40 stimulation, and human memory B cell subsets were further characterized.

Results: Human IL-10-producing Breg cells showed high CD25, CD71, and CD274, and low CD73 expression on their surface, and suppressed antigen-specific CD4+ T cell proliferation. The class of the immunoglobulin produced by human Breg cells isolated from healthy individuals is selectively confined to IgG4. In addition to these findings, B cells isolated from beekeepers as well as after allergen-specific immunotherapy, which are specific for bee venom phospholipase A2 have increased expression of IL-10 and IgG4. Immortalized human primary B cells specific for tetanus toxoid and bee venom phospholipase A2 showed cytokine profiles similar to effector T cell subsets that can be classified as Breg cells (IL-10 and IL-1ra dominant), B inflammatory cells (IL-6 and/or TNF-alpha dominant), B2 cells (IL-13 dominant).

Conclusion: Human B cells play a role in allergen tolerance and functional regulatory and effector memory B cell subsets, such as Breg, B inflammatory and B2 cells similar to Treg and T helper cell subsets, exist in humans.

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Generation and characterization of allergen-specific B cell clones from tolerant and allergic individuals

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Background: Allergen-specific immunotherapy (SIT) can induce tolerance to certain allergens. During SIT allergen-specific IgG4 levels are frequently increased. Beside changes in circulating allergen-specific immunoglobulins there is little known about the regulation of B cell responses during SIT. The aim of this study is to investigate the effect of high-dose antigen exposure on the function and phenotype of allergen-specific memory B cells.

Methods: We immortalized memory B cells by introducing Bcl-6 and Bcl-xl into peripheral blood memory B cells. This leads to formation of highly proliferating, cell surface B cell receptor (BCR)-positive, immunoglobulin-secreting B cells. IgA^-IgM^- memory B cells (including IgG- and IgE-switched cells) were isolated from non-allergic individuals, Beevenom (BV)-allergic patients before and after BV-SIT and beekeepers. Using labeled phospholipase A2 (PLA), we measured the frequency of PLA-specific memory B cells and generated PLA-specific B cell clones.

Results: Non-allergic individuals did not have detectable PLA-specific memory B cells, whereas frequencies of PLA-specific cells in beekeepers and BV-allergic patients reached up to 0.4%. Beekeeper-derived PLA-specific B cells mainly produced PLA-specific IgG4 and expressed mostly surface BCR of the IgG4 isotype. The frequency of IgG4+ cells within PLA-specific B cells from BV-allergic patients was < 1% before BV-SIT and increased significantly after BV-SIT. Furthermore, IgG4-switched B cell clones showed increased expression of surface HLA-DR and CD86 when compared to IgG1-switched clones. Secretion of TNF-alpha, RANTES, IL-6, IP-10 and CCL4 ware significantly reduced in IgG4-switched clones.

Conclusions: Here we show the isolation and culture of human in vivo switched allergen-specific memory B cells. This approach will allow us to gain more insight into the mechanisms at the B cell level that drive induction of immune tolerance to allergens. Allergen-specific B cells showed an increased frequency of IgG4-switched cells after SIT. We found that CD86 and HLA-DR were upregulated in IgG4-switched memory B cells indicating that these cells may be efficient antigen-presenting cells. Furthermore IgG4-switched B cell clones show reduced production of pro-inflammatory cytokines and chemokines. This indicates that SIT induces highly efficient antigen-presenting IgG4-switched B cells that secrete low levels of pro-inflammatory cytokines upon activation.

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Follicular helper T cells mediate IgE antibody production in response to airborne allergens in mice

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Background: Follicular Helper T (Tfh) cells develop when animals are infected with helminths. However, little is known about the roles for Tfh cells in allergic airway diseases. The goal of this project was to examine development of Tfh cells in response to airborne allergens and to elucidate their roles in IgE antibody production.

Methods: Naïve mice were exposed intranasally to endotoxin-free ovalbumin (OVA) with or without IL-33 or IL-1β or to extracts of natural allergens. Th2 cell- and Tfh cell-mediated adaptive immune responses to OVA were analyzed by using IL-4 reporter mice, gene-deficient animals, and adoptive transfer approaches.

Results: Airway exposure of naïve mice to OVA plus IL-33 induced OVA-specific IgE and IgG1; when challenged with OVA, these mice developed robust Th2 cytokine response (IL-4, IL-5, and IL-13) and airway eosinophilia. Exposure of naïve mice to OVA plus IL-1β induced comparable levels of anti-OVA IgE/IgG1 and IL-4, but minimal IL-5, IL-13 and eosinophilia. Isolation of IL-4-positive $CD4^+$ T cells and microarray analysis showed that IL-33 induces

antigen-specific Th2 cells and Tfh cells and that IL-1β induces mainly Tfh cells. Mice deficient in ICOS ($Icos^{-/-}$), which is critical for development of Tfh cells, developed Th2 cells and anti-IgE/IgG1 antibodies normally when exposed to OVA plus IL-33. In contrast, when exposed to OVA plus IL-1β, $Icos^{-/-}$ mice showed decreased numbers of Tfh cells and reduced levels of anti-OVA IgE/IgG1. Adoptive transfer of Tfh cells to T cell-deficient animals restored production of anti-OVA IgE/IgG1 antibodies. Finally, airway exposure of mice to natural airborne allergens, such as house dust mite, induced Tfh cells as well as Th2 cells in draining lymph nodes.

Conclusions: IL-1-family cytokines and natural allergens trigger development of both antigen-specific Th2 cells and Tfh cells in the airway. The roles for Tfh cells in IgE antibody production and allergic diseases in humans need to be investigated.

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Influence of natural pollen exposure on local and peripheral IgE repertoires in allergic rhinitis revealed by next-generation sequencing

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Background: Immunoglobulin (Ig) repertoire analysis has been an important approach to gain insights into IgE-mediated allergic responses although previous studies are often limited in the analytic depth by the use of low-throughput Sanger sequencing. With rapid advances in sequencing technology over the recent years, next-generation sequencing (NGS) has revolutionised our ability to probe the Ig repertoire and subsequently addressed previously unapproachable questions related to infectious diseases and autoimmunity. The power of NGS technology in combination of single-cell antibody expression techniques has further led to the discovery of neutralising HIV antibodies. However this NGS approach has not yet been explored in the setting of allergic diseases. Here we have employed NGS technology to characterise Ig repertoires, with a focus on IgE, in allergic rhinitis in order to gain a better insight into the underlying mechanisms relevant to the pathophysiology and disease mechanisms.

Methods: The 454 GS FLX+ System was employed to pyrosequence Ig heavy-chain (*IGH*) genes in the blood and nasal biopsies from healthy subjects (n=3) and allergic rhinitis patients take in season (n=3) or out of season (n=4).

Results: A total of 97610 *IGH* (including 8135 IgE) sequences were analysed. *IGHV1* and *IGHV5* subgroups were more abundant than other antibody classes ($p < 0.05$), regardless of atopic status. Seasonal pollen exposure significantly ($p<0.005$) increased hypermutations and intraclonal diversification for in-season IgE clones, particularly those in nasal biopsies, as compared to out of season samples and normal nonatopic controls. IgE clones in seasonal allergic rhinitis were subjected to stronger positive selection ($p <0.05$) and had a higher degree of repertoire diversity ($p <0.005$). We also observed intraclonal diversification via hypermutations and class switching for IgE clones isolated from the nasal mucosae. Our lineage tree analysis revealed that IgE sequences in nasal biopsy and blood samples were linked by the similar molecular footprints, and most importantly demonstrated the clonal relatedness between IgE and IgG in allergic rhinitis, which was absent in healthy controls ($p=0.05$). In addition, seasonal changes extended into non-IgE repertoires in allergic rhinitis.

Conclusions: Our high-throughput repertoire analysis has revealed that the overabundance of *IGHV5* may be a repertoire characteristic of IgE rather than a result of allergen-driven selection that was previously suggested. Positive effects of allergen exposure on hypermutations and selection may be associated with elevated level of high-affinity IgE antibodies seen in allergic individuals. Finally our data have further provided evidence of affinity maturation of IgE within the respiratory mucosal tissues in allergic rhinitis.

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MicroRNA-155 deficiency affects allergen-induced $CD4^+$T cell plasticity

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Rationale: MicroRNAs (miRs) are small noncoding RNAs that regulate gene expression and are emerging as modulators of immune responses. Studies suggest that miRs play a role in both the development and function of $CD4^+$ T cells. We have recently shown that miR-155 deficiency results in decreased eosinophilic inflammation, T_H2 cells and T_H2 cytokine production locally in the lung in a model of allergic airway inflammation. In addition, T_H17 cells and Treg cells were also decreased in allergen challenged miR-155 deficient mice compared to wild type (WT) mice, whereas T_H1 cells were unaffected. The aim of this study was to investigate whether miR-155 plays a role for T cells ability to co-express several $CD4^+$ T cell master transcription factors, which may be an indicator of T cell plasticity.

Methods: WT and miR-155 deficient (miR-155 KO) mice were sensitized and airway challenged to OVA. TH cell populations and co-expression of master regulatory transcription factors (T-bet,

GATA-3, RORγt and FOXP3) in lung CD4+ T cells were determined by means of flow cytometry.

Results: The different CD4+ T cell subtypes varied by expressing only one single transcription factor and up to co-expressing all four transcription factors. Of the larger subsets, we found significant decreased numbers of CD4+ T cells co-expressing all four transcription factors (T-bet+GATA-3+RORγt+FOXP3+ cells) and cells co-expressing T-bet+GATA-3+RORγt+ in allergen challenged miR-155 KO mice compared to WT mice.

Conclusions: Our data suggests that CD4+ T cells obtained from lung tissue after allergen challenge displays ability of plasticity. This ability may at least in part be dependent on miR-155.

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Diversity of allergen-specific IgG4 responses during birch pollen immunotherapy

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Background: Allergen-specific immunotherapy (SIT) induces high titers of allergen-specific IgG4 antibodies (Ab). However, data on the diversity of such allergen-specific IgG4 responses are still limited.

Method: We employed competitive immunoscreening of phage displayed-peptides to elucidate the diversity of Bet v 1-specific IgG4 in sera collected after 6 and 36 months of SIT with birch pollen (BP) from five BP-allergic patients who significantly improved their respiratory symptoms to natural BP exposure and skin prick test reactivity to BP extract. IgE-blocking activity was determined in facilitated antigen-binding assays.

Results: For each individual and time point, 4-8 peptides were isolated predicting in total 36 (6 months) and 39 (36 months) conformational epitopes. Every epitope found at month 6 was patient-wise compared for aa identity with every epitope predicted at month 36 to elucidate whether epitope specificites were already established after 6 months of BP-SIT. 37/39 epitopes (95%) found after 36 months of BP-SIT partially overlapped with the IgG4-epitopes found after 6 months. This observation suggests that the diversity of BP-SIT-induced Bet v 1-specific IgG4 Ab did not significantly increase with prolonged treatment. Only one of the five studied BP-SIT-treated patients displayed a larger number of IgG4-epitopes after 36 than after 6 months of BP-SIT. The serum of this individual showed a higher blocking capacity after 36 months than the serum collected after 6 months of treatment. In contrast, the IgE-blocking activity of sera from all other patients reached on average 90% after 6 months of BP-SIT and did not increase remarkably thereafter. All predicted IgG4-epitopes found at both time points were located within surface domains on Bet v 1 that had previously been shown to contain IgE-epitopes. This overlap of our predicted IgG4-epitopes with published IgE-epitopes is further supported by the high capacity of IgG4-Ab to inhibit IgE-binding to Bet v 1.

Conclusion: We provide first evidence that the Bet v 1-specific IgG4 repertoire does not broaden with long time BP-SIT by development of IgG4 specificities that recognize epitopes different from IgE Ab.

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Seasonal increases in peripheral innate lymphoid type 2 cells (ILC2s) are inhibited by grass pollen immunotherapy

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Background: Type 2 innate lymphoid cells (ILC2) are morphologically similar to lymphocytes, but lack T-cell, B-cell, natural killer cell or other cell lineage markers. They constitutively express prostagladin D2 receptor (CRTH2), IL-7R and ST2 receptors and produce substantial amount of type 2 cytokines, particularly IL-5 and IL-13. Although immunotherapy has been shown to suppress allergic inflammation and type 2 cytokines from T cells, its effects on ILC2 have yet to be determined.

Method: In a prospective controlled study conducted in and out of the grass pollen season, we recruited 12 subjects with seasonal allergic rhinitis (SAR), 9 grass pollen subcutaneous immunotherapy-treated subjects (SCIT) and 11 non-atopic (NA) controls. Peripheral blood samples were collected from all participants both in and outside the grass pollen season. Clinical symptoms were evaluated using self-reported visual analogue scales (VAS). ILC2 were immunophenotyped using multiparameter flow cytometry. Lineage-negative lymphocytes that expressed CRTH2 and IL-7 receptor (CD127) and ST2 were considered ILC2.

Results: ILC2 were increased in SAR during the pollen season compared to out of season (58%, p=0.007). In contrast, there was no change in ILC2 over the same time period for SCIT and NA groups. During the pollen season, the proportions of ILC2 were elevated in SAR (median (IQR), 14.8 (2.99)) compared to SCIT (9.34 (3.45); p=0.03) and NA (7.34 (4.86); p<0.001). In contrast to a previously published study, we report that peripheral blood ILC2 during the pollen season are elevated in SAR compared to NA patients (2-fold; p<0.001). Furthermore, IL-13+ILC2+ cells were elevated in SAR (3.06 (0.78)) compared to both SCIT (1.78 (0.62), p<0.001) and NA (1.77 (0.30), p<0.001) groups. SCIT-treated subjects reported markedly less seasonal symptoms compared to untreated SAR. Moreover, in allergic individuals, there was a correlation between VAS and the proportion of ILC2 during the pollen season (r=0.52, p=0.02).

Conclusions: Our findings support a role for ILC2s in the pathology of SAR and indicate that their suppression during SCIT might contribute to clinical and immunological tolerance.

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Facilitated induction of Tregs by anti-IL-4: A RDBPC immunotherapy under the umbrella of anti-IL-4

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Allergy is characterized by allergen sensitization that is associated with a Th2-biased T cell phenotype. Non-symptomatic responses against allergens are associated with regulatory responses mediated by regulatory T cells that are characterized by the expression of FOXP3. The differentiation of pro-allergic Th2 cells is dependent on the expression of GATA3. At the CIA meeting in 2006 we presented data demonstrating that IL-4-induced GATA3 is a potent inhibitor of FOXP3 and consequently of Tregs. We hypothesized that IL-4 represents a critical negative regulator of immune tolerance. Although this hypothesis is supported by multiple studies in murine systems, we sought to translate this finding into clinical application, seeking to induce Tregs in the frame of allergen specific immunotherapy by neutralizing IL-4 systemically. A randomized, double-blind, three-group parallel design trial was initiated that included 37 otherwise healthy grass pollen allergic individuals. The subjects received either grass-pollen specific immunotherapy (30.000 SQ) in combination with a humanized anti-IL-4 antibody (VAK694), grass-pollen SCIT (30.000 SQ) plus placebo or double placebo (placebo-SCIT & placebo-Antibody) over the course of 13 weeks (updosing phase) prior to the grass pollen season. The primary endpoint was the 24h late phase skin response to allergen at 3, 6 and 18 months after treatment. Secondary endpoints included the assessments of frequency of IL-4$^+$ and IL-10$^+$ cells as measured by fluorospot, induction of facilitated antigen-binding (FAB), allergen-specific IgG4, Th2 effector and regulatory T cells.

Treatment with VAK694 and suboptimal SC doses of allergen (to leave room for improvement) lead to a sustained reduction of allergen-specific IL-4 producing cells and the induction of IL-4/IL-10 producing cells. Both active treatments led to significant and substantial reduction of the LPR without additional reduction of the LPR by the VAK694 treatment over suboptimal SCIT alone. The study demonstrates that the co-injection of allergen under the umbrella of a biological therapy during the up-dosing phase led to sustained modulation of T cell responses and may pave the way for rational vaccine design of SIT.

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Safety and tolerability of multiple food oral immunotherapy with and without Omalizumab

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Context: Up to 30% of patients with food allergies have clinical reactivity to more than one food allergen. Although there is currently no cure, oral immunotherapy (OIT) is under investigation. The objective of this study was to evaluate the safety and dose tolerability of two Phase 1 Single Site OIT protocol to desensitize up to 5 food allergens simultaneously with or without omalizumab.

Methods: Fifty (50) participants were enrolled in the protocols (median age 7 years). For each included food, participants had failed an initial double-blind placebo-controlled food challenge at a protein dose of 100 mg or less. Home reactions were recorded with diaries. Twenty-five (25) subject were administered omalizumab for 8 weeks prior to and 8 weeks following the initiation of an accelerated OIT schedule. Subjects underwent an initial escalation visit to desensitize to up to 6mg or 1250mg of a combination of 2 to 5 years, without or with omalizumab, respectively, followed by home dosing and biweekly updosing visits.

Results: During the studied period, a total of 7,530 and 12,030 home doses were administered in the omalizumab and non-omalizumab groups, respectively. Reaction profile was similar with both protocols (median 3.2 reactions per 100 doses). Participants reached their maintenance dose of 4,000 mg protein per allergen at a median of 18 and 85 weeks with and without omalizumab respectively.

Conclusion: These phase 1 data demonstrate that simultaneous OIT to multiple foods is feasible and can be performed safely. A protocol with 16 weeks of omalizumab allows for a faster desensitization. Phase 2 randomized controlled trials are needed to better define safety and efficacy parameters of multi-OIT experimental treatments with and without omalizumab.

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Vaccination with SG100 attenuates aeroallergen-induced early and late phase asthmatic responses in house dust mite sensitive non-human primates

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Rationale: House dust mite (HDM)-sensitive nonhuman primates (NHP) exhibit hallmark features of allergic asthma including bronchoconstriction, nonspecific bronchial hyper-responsiveness, airway inflammation, and nocturnal apnea and hypopnea. This

study was performed to assess whether treatment with a new vaccine SG100 attenuates airway reactivity to aerosolized house dust mite antigen (aero-HDM) in these chronically allergic animals.

Methods: HDM-sensitive NHP were anesthetized, intubated, and instrumented to record lung resistance, dynamic compliance, SpO2, HR and blood pressure before and after acute challenge with aero-HDM (early asthmatic response, EAR) and aerosolized methacholine (aero-Mch) 24 hours after aero-HDM challenge (late asthmatic response, LAR). Baseline responses were recorded, the animals were immunized with either SG100 or placebo, and then EAR and LAR were assessed 9 and 13 weeks later.

Results: 9 weeks post-immunization, EAR was unchanged in both SG100 and placebo animals, but 24 hours after the aero-HDM challenge, reactivity to aero-Mch (i.e., LAR) was reduced in 7 of 8 animals treated with SG100. Only 2 of 7 animals treated with Placebo exhibited reduced reactivity (difference in nonspecific airway reactivity SG100 vs. Placebo, $p<0.05$).

13 weeks post-immunization, after additional contact with HDM, EAR was reduced in the SG100-treated group. 7 of 7 animals exhibited lower aero-HDM-induced lung resistance. Compared to 4 weeks prior, these animals required fewer rescues with supplemental oxygen to maintain SpO_2 above 70% following acute aero-HDM exposure. In contrast, 3 of 6 evaluable Placebo-treated animals exhibited greater acute responses to aero-HDM. LAR recorded in both groups was similar to that observed 4 weeks earlier.

Conclusions: SG100 vaccine is safe and efficacious in this chronic NHP model of allergic asthma, leading to an early and prolonged reduction in nonspecific airway reactivity (i.e., LAR) followed by a more slowly developing reduction in EAR with repeated allergen exposure.

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A recombinant mutant of the major fish allergen parvalbumin shows reduced allergenic activity in skin tested fish allergic children and reduces allergic symptoms in a mouse model of fish allergy by blocking antibodies

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Fish is a frequent elicitor of IgE-mediated food allergy and may cause severe anaphylactic reactions. Beside avoidance, there is currently no therapy available. Parvalbumins were identified as highly cross-reactive major allergens responsible for allergic symptoms after fish ingestion. Recently a hypoallergenic mutant of the carp parvalbumin Cyp c 1 was developed for specific immunotherapy of fish allergy by introducing four point mutations into the calcium binding sites of the molecule. The resulting Cyp c 1 mutant showed reduced IgE-reactivity and induced high levels of Cyp c 1-specific IgG antibodies upon immunization of rabbits, which inhibited patients' IgE-binding to wildtype Cyp c 1. In order to investigate the therapeutic effect of vaccine-induced IgG, a mouse model of fish allergy closely mimicking the human disease was developed. C3H/HeJ mice sensitized to recombinant Cyp c 1 by intragastric gavage, developed Cyp c 1-specific IgE and T cell responses. Epitope mapping of Cyp c 1-specific IgE responses in sensitized mice and humans revealed that mainly conformational epitopes were recognized. Vaccine-induced IgG antibodies or for control purpose Phl p 1-specific IgG antibodies were applied to sensitized mice before challenge with Cyp c 1. Blood samples before and after antibody application were analyzed by ELISA and allergic symptoms were recorded. We found that rabbit IgG antibodies induced by immunization with the mutant inhibited IgE-binding to Cyp c 1 and Cyp c 1-specific effector cell degranulation in mice and suppressed symptoms of fish allergy after Cyp c 1 challenge.

In addition we evaluated the *in vivo* allergenic activity of the Cyp c 1 mutant in humans. Wildtype Cyp c 1 and the Cyp c 1 mutant were compared by skin testing in 12 fish allergic children, who were sensitized to parvalbumin and developed allergic reactions after ingestion to fish. Skin testing was performed with four concentrations of wildtype Cyp c 1 and the Cyp c 1 mutant and revealed a more than 16-fold reduced *in vivo* allergenic activity of the mutant compared to the wildtype molecule.

Thus the Cyp c 1 mutant represents a candidate molecule for the treatment of fish allergy.

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Clinical review of 18 patients with late-onset anaphylaxis after ingestion of Bacillus subtilis-fermanted sybeans (natto)

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Background & Purpose of study: Since we reported the first case of late-onset anaphylaxis to the subtilis-fermented soybeans (natto) in 2001, many cases similar to this case have been reported in succession. The mechanism of late-onset anaphylaxis to natto is hypothesized to be due to delayed release or absorption of the relevant allergen into the bowel rather than an immunologic phenomenon, but it is still unclear what is causative allergenic molecule in this unique anaphylaxis. Then, we analyzed the clinical symptoms and signs, laboratory data, and results of skin test and provocation with natto in 18 cases (including 9 cases which we experienced) collected from the reported cases of late-onset

anaphylaxis to natto, in order to clarify the clinical features of this unique natto allergy and to characterize its allergenic molecules.

Results & Discussion: The 18 patients collected aged from 7 to 58 years old (mean age, 29.6 years old). 17 out of 18 patients collected were man (94.4%) and only one patient was woman (5.6%), this is the first case of woman, who we recently experienced. The 18 patients all developed generalized urticarial with or without dyspnea, loss of consciousness, collapse, vomiting, and diarrhea after ingestion of natto, which are supposed to be IgE-mediated allergic reactions. The interval between ingestion of natto and onset of symptoms was 4 to 15 hours, mean 10.0 hours. The results of skin prick test (SPT) were positive for natto as is in all patients tested (16/16) but not for soybean extract and Bacillus natto. Interestingly, the SPT with poly (γ-glutamic acid) (PGA), which is a major constituents of natto mutilage and is also known as a water-soluble, biodegradable biopolymer (MW 200,000-6000,000), showed strongly positive reactions in 5 out of 6 patients skin tested (83.3%), and the positive SPT reactions to PGA were prolonged until about half a day after skin testing. In 6 patients orally challenged with natto, late-onset anaphylactic reactions were confirmed and in one out of these 6 patients, a peak of histamine in blood was observed together with late-onset anaphylactic reactions 13 hours after its ingestion. All patients (6/6) in which ELISA was measured showed elevated levels of specific IgE antibodies to natto extracts. From these results, firstly, this late-onset anaphylactic reactions to natto of fermented soybeans are presumed to be mediated by specific IgE antibodies to natto. Secondly, PGA itself having the sustained-release activity is presumed to be a strong candidate of allergenic molecules of natto, because of positive SPT reaction to PGA with high frequency (5/6) and its prolongation. Recently PGA has been applied to a wide range of products such as foods, cosmetics and medicines, and then patients with late-onset anaphylaxis to PGA of natto should avoid not only natto but also other products containing PGA. Thirdly, since some patients with late-onset anaphylactic reactions to natto are surfer, who have chance to be stung by a jellyfish containing PGA, the sting by a jellyfish may result in development of late-onset anaphylaxis to PGA of natto.

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The usefulness of thymus and activation-regulated chemokine (TARC) in early diagnosis of typical DIHS, in which reactivation of HHV-6 is observed

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Background & Purpose of Study: Drug-induced hypersensitivity syndrome (DIHS) is well-known as a serious acute drug rash with fever, lymphadenopathy and several visceral dysfunctions in Japan. This disease is supposed to be almost similar to drug rash with eosinophilia and systemic symptoms (DRESS), which is well-accepted in Europe. Reactivation of HHV-6 is considered as an important factor in criteria for diagnosis of DIHS in Japan but not in criteria for diagnosis of DRESS in Europe. Eosinophilia is a common characteristic hematological abnormality in DIHS and DRESS, indicating that the Th2-type immune response is involved in the both disease. Also, thymus and activation-regulated chemokine (TARC) is a family of CC chemokine, which is well-known to play an important role in Th2-mediated immune inflammatory reactions. Then, we measured the serum TARC levels by ELISA in the sera obtained from 2 patients with typical DIHS and 2 patients with atypical DIHS and examined the relationship between their TARC levels, clinical symptoms and various hematological parameters, in order to investigate a probable role of TARC in patients with typical or atypical DIHS in which reactivation of HHV-6 is observed or is not.

Results & Discussion: The results demonstrated that the serum TARC levels in patients with typical DIHS were remarkably higher than those in patients with atypical DIHS during the acute phase, and then correlated with the disease activity of typical DIHS. Therefore, the serum TARC levels are presumed to be very useful in early diagnosis of DIHS with reactivation of HHV-6 and also in early assessment of its disease activity.

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A quantitative multiplex immunoassay for food allergen proteins

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Background: In order to help allergic patients manage often severe symptoms, food manufacturers are required to list allergens on their products. Researchers are working to improve diagnostic products and develop effective immunotherapies. Existing tools (generic ELISA) do not provide precise identification of specific food allergens. Our goal was to develop an accurate, sensitive and high throughput assay that would enable simultaneous quantification of multiple food allergens in foods and in allergen preparations used for oral food immunotherapy (OIT).

Methods: Fluorescent microspheres coupled to allergen specific monoclonal antibodies were used to develop a multiplex immunoassay for simultaneous quantification of major food allergens from peanut (Ara h 1, Ara h 2 and Ara h 6), milk (Bos d 5), egg (Gal d 1), and shrimp tropomyosin. Target allergens were detected using biotinylated monoclonal or polyclonal antibodies and streptavadin conjugated fluorochrome. The array was quantified using highly purified natural allergens as standards. Allergen content was measured in NIST Standard Reference Material (SRM) peanut butter, diagnostic extracts, spiked food samples, 'real foods' and extracts used for OIT. The results were compared to ELISA.

Results: There was a high correlation between the multiplex array and allergen specific ELISA (R^2= 0.82 to 0.98) and the sensitivity of the array was increased by up to 40-fold compared to ELISA,

demonstrating accuracy as low as 20 pg/ml. Significant differences were found between specific peanut allergen levels (Ara h 1 : Ara h 2 : Ara h 6 ratio) in NIST SRM peanut butter (4:1:1) compared with peanut flour extracts used for OIT (1:3:3) and diagnostic peanut extract (1:5:6).

Conclusions: These data demonstrate the development of an accurate and sensitive multiplex immunoassay for the detection of multiple food allergen proteins. The flexibility of the microsphere technology allows for expansion of the array and will enable addition of further allergens to produce a comprehensive array for most important food allergens. Ultimately, this quantitative multiplex technology is expected to reduce the risk of inadvertent contamination of food, and will enable more effective assessment of the potency of food extracts used for immunotherapy and allergy diagnosis.

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Allergen food matrix interaction – impact on allergenicity

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Background: A number of relevant food allergens are already identified. However, our current understanding of allergen-food matrix interactions and the potential impact on the allergenicity is limited. Tree nuts, peanuts and seeds are highly allergenic foods often inducing severe allergic symptoms. Their unique food matrix, rich in lipids, may contribute to the immunogenic activity of these plant foods. The aim of this study was to assess the allergenic activity of purified allergens with and without the lipid fraction using hazelnut as a model food.

Methods: The non specific Lipid transfer protein (nsLTP), Cor a 8, and a 2S albumin, Cor a 14, were purified from raw hazelnut extracts by standard precipitation and chromatographic methods. Final batches of Cor a 8 and Cora 14, were identified by mass spectrometry. Total hazelnut lipid fraction was obtained by n-hexane extraction. Sera from hazelnut allergic patients were used for ELISA and tested for IgE binding activity to Cor a 8 and Cor a 14, both under native and reducing conditions. In a rat basophil beta-hexosaminidase release (RBL) assay Cor a 8 and Cora 14 were tested with and without addition of lipids.

Results: Mass spectrometry analysis confirmed the integrity of both final batches of Cor a 8 and Cor a 14, respectively. IgE binding to native and denatured Cor a 8 and Cor a 14 investigated by ELISA showed, that linear as well as conformational epitopes were recognized by the allergic patients sera. In the RBL assay, Cor a 8 and Cor a 14 induced dose dependant mediator release. Addition of the lipid fraction to Cor a 8 induced a considerably higher release as compared to Cor a 8 alone. This effect was less pronounced when testing Cor a 14.

Conclusions: Detailed physicochemical characterization of allergens is required for reliable allergen specific allergy diagnosis. However, the interaction of individual allergens with lipid fractions is less well understood. Our data suggest that the lipid protein interaction has an effect on the allergenic activity of hazelnut proteins.

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Detection of food allergen-specific immunoglobulin free light chains in serum of food-intolerant patients

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Introduction: A considerable percentage of patients suffering allergic diseases such as food allergies, rhinitis and asthma have a pathophysiology that does not involve IgE. On the other hand, these allergies are associated with an increase in mucosal inflammatory cells and physiological responses compatible with a local allergic-type immune response. We have shown that immunoglobulin free light chains (FLC) can trigger immediate hypersensitivity-like responses via antigen-specific mast cell activation. Substantially increased local or systemic concentrations of FLC are found in upper and lower airway diseases, (food) allergies, and other inflammatory diseases such as rheumatoid arthritis, and increases in FLC levels appear to correlate with clinical disease activity. Thus far, no assays are available to detect antigen-specific FLC. In this study, we introduce an ELISA-based assay to detect allergen-specific FLC.

Methods: Serum antigen-specific FLC were measured in antigen-coated ELISA 96-well plates. The plates were coated with different antigens such as milk protein concentrate (MPC), whey, casein, gliadin, ovalbumin, and betalactoglobulin, Binding of antigen-specific FLC to coated antigen was detected with anti-kappa FLC and anti-lambda FLC specific antibodies.

Results: FLC specific for different antigens can be measured using a flexible ELISA-based binding assay. Increased binding was found with increasing antigen concentrations. Serial dilution of serum samples produced typical titration curves. Specificity of the assay was shown by a lack of effect from depletion of complete immunoglobulins. For casein, antigen-specific binding of FLC`s is progressively lost with increased hydrolysis of this protein. Also, premixing of serum samples with antigen inhibited antigen-specific binding by light chains in a dose dependent manner. Preliminary data indicate that titers of antigen-specific kappa and lambda FLC`s are higher in food intolerant subjects.

Discussion: Here we provide a novel assay to detect antigen-specific FLC in human serum. We show that FLC specific for different food antigens such as milk protein concentrate, whey, casein, gliadin, betalactoglobulin and ovalbumin can be detected

in human serum. Preliminary data suggest that circulating allergen-specific FLC may be an underlying factor in food intolerance. The assay for allergen-specific FLC may be a very useful tool to further determine whether FLC are involved in IgE-independent allergies.

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Periostin as a novel clinical biomarker of disease severity and chronicity in patients with atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic, highly pruritic inflammatory skin disease accompanied with skin barrier damage and allergic skin inflammation with a dominant Th2-type immune response. Recent findings indicate that periostin, an extracellular matrix protein induced by Th2 cytokines, plays a critical role in the pathogenesis of AD. Although periostin is known as a useful biomarker for bronchial asthma, whether differential levels of periostin are associated with the severity and clinical phenotype of AD has not been evaluated.

Objective: To determine whether periostin level is associated with the severity and clinical phenotype in adult AD patients.

Methods: An enzyme-linked immunosorbent assay was performed to evaluate serum periostin levels in 257 adult AD patients, 66 patients with psoriasis vulgaris (PV) as a disease control and 25 healthy control subjects. Serum periostin levels were analysed together with clinical characteristics and laboratory parameters including thymus and activation-regulated chemokine (TARC), lactate dehydrogenase (LDH), blood eosinophil count and total immunoglobulin E (IgE). Immunohistochemical analysis was performed to examine periostin expression in association with various clinical phenotypes of AD. The effect of treatment on serum periostin level was also assessed.

Results: Serum periostin was significantly higher in AD patients than in PV patients and healthy control subjects. The periostin level was positively correlated with disease severity, TARC level, LDH level and eosinophil count, but not IgE level. Higher serum periostin level was observed in extrinsic AD patients compared to intrinsic AD patients. Robust expression of periostin was detected in the dermis of AD patients with erythroderma, lichenification and, to a lesser extent, scaly erythema. Serial measurement of serum periostin revealed reduced levels of periostin after treatment for AD.

Conclusions: Our results suggest that periostin may play a critical role in disease severity and chronicity in the pathogenesis of AD. Serum periostin is a novel biomarker that can accurately reflect disease activity and treatment efficacy. This study is *in press* of the British Journal of Dermatology.

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Systems biology to understand the reaction of the allergenic pollen from common ragweed (Ambrosia artemisiifolia) to air pollutions and climate change

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Background: Common ragweed (*Ambrosia artemisiifolia*) is an invasive neophyte from North America, which is now spreading throughout Europe. This annual herb produces huge amounts of pollen grains that have a very high allergenic potential. Climate change and air pollution will affect the allergenic potential of pollen, either by changes of the pollen season or the pollen amount [1, 2], by directly increasing the transcripts and the expression of allergenic proteins or by a changed surface exine, and interactions with biologically important ligands, e.g., flavonoids.

Aim: The aim of the project is to investigate the impact of different natural and anthropogenic environmental and climatic parameters on the induction of potentially allergenic components in ragweed pollen.

Methods & results: We used a systems biology approach to get a better understanding of molecular mechanisms in ragweed pollen under global change scenarios. For this we have grown our plants in climate chambers under controlled conditions upon elevated CO2 (380ppm vs. 700ppm), NO2 (filtered air vs. 80ppb) and drought stress. NO_2-treatment resulted in increased amounts of allergenic proteins as well as in stress-related proteins. In addition, nitrosylation of ragweed major allergen (Amb a 1) isoforms was observed that may also influence the allergenic potential. Upon elevated CO_2 plus drought stress conditions it has been shown by HPLC that individual flavonoid metabolites were increased. Transcriptomic analyses via SuperSAGE showed changes in allergen-encoding transcripts upon elevated CO_2 and drought stress. Treatment of mice B cells with pollen-extracts from CO_2-fumigated and/or drought stressed plants augmented the IgE enhancing effect, indicating an impact on allergic diseases.

Conclusion: The data highlight an influence of climate change on the transcript and protein level of pollen allergens and support the idea of a direct influence of air pollution on allergens.

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Natural variability of allergen release from plants (olive, birch, and grass pollen) and animals (cats)

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The majority of type I allergic individuals suffer from airborne allergens from pollen, house dust mites and animal dander. We determined the natural variability of the release of Bet v 1 from birch pollen, Phl p 5 from grass pollen, Ole e 1 from olive pollen, Fel d 1 from cat hairs and the adjuvant factor endotoxin in different size fractions of ambient air.

Methods: Ambient air was collected daily in 10 countries across Europe for several years. Ole e 1, Bet v 1, Phl p 5 and endotoxin were determined in two fractions of ambient air: Particulate Matter (PM)>10µm (larger than coarse) and 10µM>PM>2.5µm (coarse fraction). Fel d 1 was extracted from pulled neck hairs. All allergens were determined using ELISA, endotoxin with the Lonza-rFc-method®.

Results: All biological components, pollen, Bet v 1 , Phl p 5, Ol e 1 and endotoxin were predominantly detected in PM>10µm. This fraction of ambient air is mostly discarded, except in pollen counters. In addition, a large natural variability in allergen release from each source was detected. From plants, olive pollen released >12-fold different amounts of Ole e 1 depending on day and location. For birch and Bet v 1 this was 10-fold, for grass pollen and Phl p 5 this was >20-fold. For Fel d 1, a 100-fold difference between individual cats was detected. Endotoxin was >50% detected in the PM>10µm fraction, indicating that bacteria in ambient air stick to larger particles.

Conclusion: Using proxies for airborne allergen exposure like pollen or number of animals can introduce a variability of 20 to 100-fold, depending on the allergen. We urgently need to determine if differences in allergen exposure leads to differences in allergy symptoms and sensitizations.

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Low molecular weight factors from pollen mediate aggravation of the allergic immune response to pollen allergen in humans

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Background: Besides allergens, pollen release bioactive, low molecular weight compounds lacking intrinsic allergenic potential but shown to modulate and/or stimulate cells of the immune system. *In vivo* relevance of these substances has not been demonstrated so far.

Objective: This study aimed at elucidating the effect of a non-allergenic, low molecular weight fraction from pollen (APE<3kDa) on the human allergic immune response *in vivo*.

Methods: Birch and grass pollen-allergic individuals were skin-pricked with allergen alone, allergen plus APE<3kDa, or allergen plus candidate substances, and wheal size was recorded. In a nasal provocation study, healthy and pollen allergic individuals were subjected to repeated intranasal provocations with either allergen alone or with allergen plus APE<3kDa. Local cytokine and IgE production was measured, and whole transcriptome was assessed in nasal specimen of a patient subgroup. Clinical end-points were nasal secretion weights, obstruction, and symptom scores.

Results: Patients pricked with pollen allergens developed larger wheals when allergens were administered together with the low molecular weight fraction (APE<3kDa). A lipid-enriched fraction of aqueous pollen extracts and isolated linoleic acid derivatives had a similar effect. In nasal secretions of allergic patients challenged with allergen plus APE<3kDa, IL-8 and IgE were significantly increased. These patients also showed increased nasal discharge and reported more severe rhinorrhea than the allergen-only group. In nasal specimens of two patients, differential regulation of serotonin receptor genes was observed after challenge with allergen only or allergen plus APE. Finally, the aggravating potential of serotonin was assessed in skin prick tests and serotonin was found to increase the cutaneous wheal-and-flare reaction to pollen allergen.

Conclusions: APE<3kDa aggravates the allergic immune response in the skin and nose. In the skin, pollen-associated lipid mediators and serotonin boost the wheal-and-flare reaction. In the nose, pollen-derived serotonin might play a role in differential aggravation of symptoms.

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House dust mite allergens manipulate barrier integrity and immune balance

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Background and Aims: 22,5% of 207 allergic patients tested by ISAC immunoCAP showed IgE to any house dust mite molecules, 12,1% to group1/2, 5,8% to group1, and 4,8% to group2 allergens only. We aimed to investigate the molecular sensitization mechanisms of Der p 1 or Der p 2 via the skin.

Methods and Results: Recombinant Der p 1 or 2 were topically applied to mouse skin. Both induced eczema and specific allergic sensitization. Treatments with the cysteine protease Der p 1 facilitated sensitization to Der p 2. To study the impact of enzymatic function for percutaneous sensitization, papain was used as cysteine protease surrogate. 30 min incubation to primary human keratinocytes degraded tight junction proteins, and in C57BL/6 mouse skin led to an increase of transepidermal water loss; after 1h, vascular leakage followed by efflux of neutrophils was seen in vivo in 2-photon microscopy. E64 enzyme inhibitor could abolish these effects. In repeatedly papain-treated mice the numbers of CD3+ and mast cells in the skin increased and papain-specific IgE and IgG1 were formed. Therefore, the inflammatory, but not the specific sensitization capacity depended on the enzymatic function of Der p 1 and homologue papain.

We next addressed whether Der p 2 via molecular mimicry to MD2, as in bronchi, also in the skin interacts with the TLR4 complex. Against our expectations, TLR4 expression did not impact the outcome of percutaneous Der p 2 sensitization in TLR4-/- versus WT mice. The data thus argue against a TLR4-dependent sensitization mechanism for Der p 2 when encountered via the skin. In search for alternative mechanisms and based on recent own work, we addressed a potential capacity of Der p 2 to bind Fe-binding ligands. Docking calculations predicted that inside its narrow beta-barrel-like cavity, Der p 2 can harbor catechols and that upon Fe-binding their affinity increases by more than 200-fold. This may point towards a ligand-dependent immunomodulatory activity of Der p 2 similar to Bet v 1.

Conclusion: Der p 1 and Der p 2 independently, but synergistically support primary sensitization, Th2 bias and IgE formation by manipulating barrier and immune function.

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Bet v 1, a lipocalin-like allergen, whose function depends on iron

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Background: It is hypothesized that allergens are at the borderline of self and non-self and, through as yet elusive circumstances, mount a Th2-response for allergic sensitization. Interestingly, the majority of animal-derived respiratory allergens belong to the lipocalin family. On the other hand, also our human body harbors a great number of lipocalin proteins, with the human lipocalin-2, LCN2, having immune-regulatory function. LCN2 is highly expressed in the lung and that its immune-regulatory properties depend whether it carries iron via siderophores or not. In this respect, we investigated the major allergen Bet v 1, which is considered the prototype for the PR-10 protein family, for its structural and biological resemblance with LCN2.

Methods: Structural analysis of Bet v 1 was performed using FATCATflex, Combinatorial extention algorithm and Template Modeling. Docking calculations of ligands were performed with AutoDock Vina. PBMCs of 10 human subjects were activated and stimulated for 18h with apo- or holo-Bet v1 or controls. Subsequently, cells and supernatants were analysed by flow cytometry and their cytokine-content.

Results: We give structural evidence that Bet v 1 is a lipocalin-like protein with a striking resemblance to human lipocalin 2 *in silico*. We demonstrate that similar to lipocalin 2, Bet v 1 is capable of binding iron via catechol-based siderophores. Thereby, calculated Kd-values of 66nM outpassed affinities to known ligands nearly

by a power of ten. Moreover, we give functional evidence of the immune-modulatory capacity of Bet v 1 being dependent on its iron-loaded state. When incubated to human immune cells, only the apo-form of Bet v 1, but not the holo-form, was able to promote Th2 cells secreting IL13.

Conclusion: Bet v 1 is a lipocalin-like protein, which is capable of binding iron via siderophores. Moreover, we give for the first time evidence that the form of application (apo- or holo-) is decisive for the subsequent immune response. The apo-form promotes Th2 cells, whereas the holo-form appears immunosuppressive. These results provide for the first time a functional understanding on the allergenicity of Bet v 1 and a basis for future allergen immunotherapies counteracting Th2 immune responses on a molecular basis.

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Structure-function analysis of the highly immunogenic protein tropomyosin – a major cross-reactive pan-allergen in allergic sensitization

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Background: Recent developments in molecular techniques made it possible to measure IgE antibodies to specific allergen components. This 'component-resolved diagnostics' (CRD) has the potential to stratify persistence and clinical severity of reactions, and have shown utility in the diagnosis and management of peanut, egg and milk allergy. Such diagnostics are not yet developed for prawn allergy and require in-depth molecular characterisation of prawn allergic subjects. As the Black Tiger prawn (*Penaeus monodon*) is the most widely cultured prawn and available crustacean species worldwide, the aim of this study was to characterise the most allergenic proteins using different immunological and molecular techniques and compare the allergen components between paediatric and adult patients, as well as clinical relevant linear and conformational IgE-epitopes on the major allergen tropomyosin.

Method: IgE binding proteins proteins were analysed by immunoblotting and sequences identified by mass spectroscopy (MALDI-TOF and LC-MS) using 20 individual sera of confirmed adult and paediatric patients. Allergenic proteins were characterised and compared by molecular structure analysis. Recombinant tropomyosin (TM) was generated in *E. coli* and 20-mer and 50-mer peptides synthesized for IgE epitope studies by immunoblotting, ELISA-inhibition, dot blotting and NMR analysis. Basophil stimulation with raw, heated prawn extract and purified TM was measured by flow cytometry for two patients.

Results: Heat processing enhanced the overall patient IgE binding to prawn extracts and increased recognition of a number of allergen variants and fragments of prawn allergens. Allergens identified on molecular level include tropomyosin, myosin light chain, sarcoplasmic calcium binding protein, and three putative novel allergens; triose phosphate isomerase, aldolase, and titin. Children demonstrated a different allergen recognition profile compared to adults, with myosin light chain and titin being the major allergen, in addition to tropomyosin.

Conclusions: Heating enhances the IgE reactivity to a variety of allergenic proteins and fragments thereof, with children differing in major allergen recognition. 3-dimensional homology modelling identified five of the seven characterised allergens as polymers, possible explaining the allergenic potential of these proteins.

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Identification of the muscle protein, myosin light chain 1, as an important chicken meat allergen

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Chicken meat is a source of high-quality proteins, vitamins and important micronutrients and contains a relatively low amount of fat. Therefore, chicken meat is highly recommended as an essential part of a healthy diet. However, meat of chicken and other poultry can also induce severe IgE-mediated allergic reactions and it is estimated that about 0.6 to 5% of food allergic subjects are affected by this disease. Diagnosis of meat allergy is currently still based on poorly standardized extracts with unsatisfactory sensitivity and specificity. Chicken meat allergic patients can be categorized in two groups: those who display an associated hypersensitivity to egg and bird feather (bird-egg syndrome) and those who are only allergic to poultry meat. For the first group, chicken serum albumin (Gal d 5) has been identified as important cross-reactive allergen. For the second group, the nature of the sensitizing allergens has not yet been thoroughly investigated.

With the aim to get a better understanding of the allergens causing genuine chicken meat allergy and to develop tools for improved diagnosis of meat allergy, we analysed in patients only sensitized to poultry meat the IgE reactivity profiles to protein extracts from raw and cooked chicken muscle tissue. Those protein bands that were recognized by IgE antibodies from the majority of patients were subjected to peptide mass fingerprinting. This allowed to identify a 20 kDa IgE-reactive protein, which was predominantly recognized in cooked meat, as the muscle protein myosin light chain 1.

Using specific primers a cDNA clone coding for chicken myosin light chain 1 was isolated from chicken muscle mRNA.

Overexpression of the cDNA in *Escherichia coli* resulted in an IgE-reactive recombinant protein with immunological features comparable to its natural counterpart. This molecule could now be used for diagnosis of genuinely sensitised chicken meat allergic individuals. Circular dichroism analysis further revealed that recombinant myosin light chain 1 represents a folded, α-helical protein with remarkable thermal stability and refolding capacity. This finding might explain why, despite cooking and exposure to the gastrointestinal tract, myosin light chain 1 has the potential to sensitise allergic individuals.

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Analysis of Can f 5 content in urine and fur samples from male and female dogs

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Background: Some dog allergic patients report tolerance to certain dogs while reacting to others. The major allergen Can f 1 has been found to vary in amount between dogs but to be present in fur samples of all breeds examined. A proportion of dog allergic patients are monosensitized to Can f 5, an allergen first isolated from urine of male dogs. Previous studies have shown that expression of this protein is regulated by androgens. In this study, the presence of Can f 5 in urine and fur of male and female dogs was investigated.

Methods: A sandwich assay using Can f 5-specific monoclonal antibodies was established in the ImmunoCAP platform and designed to obtain a measuring range of 1-500 ng Can f 5/mL, suitable for analysis of diluted or undiluted urine samples and undiluted fur extracts. Urine samples were collected from 35 poodles (10 intact and 1 castrated male, 24 females) and 15 German Sheperds (8 intact males/7 females) and analysed for Can f 5 content, as were fur extracts from 29 additional dogs of different breeds (7 intact and 4 castrated males, 18 females). From 14 of the latter 29 (2 intact and 1 castrated male, 11 females), a urine sample was also obtained and analysed in parallel.

Results: All intact male dogs (n=20) displayed high levels of urinary Can f 5, ranging from 8 to 164 µg/mL. In contrast, no Can f 5 could be detected even in undiluted urine from any of the female dogs (n=42) or two castrated male dogs. Consistent with absence of Can f 5 in urine, no Can f 5 was found in fur extracts from female dogs. Can f 5 was detected in fur extract from 3 of 7 intact male dogs but at much lower concentrations than in urine. Interestingly, a low level of Can f 5 was detected in fur extract from one castrated male dog, despite its absence in urine from the same individual.

Conclusion: Can f 5 appears to be produced exclusively in intact male dogs and patients monsensitised to Can f 5 may therefore tolerate female and possibly castrated male dogs.

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In vitro immunomonitoring of Hymenoptera venom-allergic patients

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Objective: Sting challenge is the gold standard method to evaluate the therapeutic efficiency of allergen specific immunotherapy (ASIT) in hymenoptera venom allergic patients. Unfortunately, this method is risky, expensive and time consuming. Therefore, the development of an *in vitro* method is desirable. Recently, the basophil activation test (BAT) performed with natural venom and recombinant allergens have been shown to be a promising method for *in vitro* diagnosis of allergic patients.

Aim: to evaluate if natural venom and the recombinant allergens Ves v 1, Ves v 2, Ves v 3 and Ves v 5 applied to BAT may be useful as predictive test for successful ASIT

Methods: BAT was performed in 83 patients with hymenoptera venom allergy. Patients were evaluated before and one year after starting ASIT. Successful immunotherapy was evaluated by sting challenge.

Results: Patients without systemic allergic reactions after the sting challenge during wasp venom immunotherapy showed a significantly less activation of basophils towards natural venom ($p<0.05$). In keeping with these results basophil activation towards recombinant allergens tended to be lower. Three patients had systemic allergic reactions after the sting challenge. Two out of these three patients showed increased basophil activation during ASIT towards rVes v 5 or natural venom.

Conclusions: BAT performed with natural venom and rVes v 5 is a promising *in vitro* method to predict successful immunotherapy. Moreover, our data indicate a potential allergen sensitization against single allergens upon ASIT.

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IgE and IgG4 profiles to a panel of recombinant CCD free honeybee venom allergens in honeybee venom allergic patients

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Background: Detection of IgE to recombinant Hymenoptera venom allergen Api m 1 has suggested that additional HBV allergens might be of relevance. Here we analyzed sensitization

profiles of patients with HBV allergy to a panel of recombinant HBV allergens devoid of cross-reactive carbohydrate determinants (CCD).

Methods: Diagnosis of HBV allergy was based on history, skin test results, and allergen-specific IgE levels to HBV. Recombinant allergens were produced in insect cells and their IgE reactivity was analyzed by ImmunoCAP.

Results: IgE reactivity to rApi m 1, rApi m 2, rApi m 3, nApi m 4, rApi m 5, and rApi m 10 was detected in 72%, 47%, 50%, 22%, 58%, and 61% of the patients with HBV allergy, respectively. Positive results to at least 1 HBV allergen were detected in 94%. IgE reactivity to Api m 3, Api m 10, or both was detected in 68% and represented the only HBV allergen-specific IgE in 5% of the patients. IgE profiles of bee venom allergic patients before and after at least 6 months of VIT showed a more than 2 fold increase in IgE reactivity to rApi m 1, rApi m 2, rApi m 3, nApi m 4 and rApi m 10 in 18 %, 21 %, 7%, 14%, and 8%, respectively. Analysis of sIgG4 demonstrated that VIT induced a pronounced IgG4 response to HBV, Api m 1 and Api m 4, while only moderate induction of IgG4 to Api m 3 and Api m 10 was observed.

Conclusion: Analysis of a panel of CCD-free HBV allergens identified additional major allergens, and revealed presence of a broad spectrum of sensitisation profiles. In addition, it demonstrates a robust induction of specific IgG4 responses during VIT to high abundance allergens and a relative lack to allergens that have been reported to be absent or underrepresented in therapeutic HBV preparations.

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Investigating the delayed adverse reactions to non-steroidal anti-inflammatory drugs by an *ex-vivo* stimulation test: a new safety approach to support the drug allergy diagnosis

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Background: Adverse drug reactions are classified into predictable (80%) and unpredictable. The latter includes intolerance and "allergic" reactions. Type IV,delayed hypersensitivity reactions (DHR), according to Gell&Coombs, are mediated by drug-specific T lymphocytes. Due to the heterogeneity of T-cells, type IV reactions were subclassified in IVa–IVd. IVa are mediated by Th1-cells; IVb corresponds to Th2 immune responses;IVc occurs directly by effector-T-cells;IVd is characterized by neutrophilic-inflammation T-cells-driven.To support the diagnosis of DHR we have only the Lymphocyte Transforming Test (LTT) quantifying the proliferation of T-cells to drugs, by a long (6-7 days), complex(radioactive isotope) and not-well-reproducible method.

Method: We selected 13 patients (age 22-61) referring DHR to NSAIDs (ASA, Paracetamol) from our Allergy Unit, and five healthy-subjects (age 21-59) as controls. We recover white cells (WBC) from peripheral blood by centrifugation and resuspended them with a Calcium/Brefeldin-A enriched buffer. Afterwards we added the NSAIDs dissolved in PBS, or only PBS (Background Control,BC), or phytohemagglutinin-L (PHA-L) (Positive Control,PC). Samples were incubated at 37°C for 12-16 hours and then fixed-permeabilized and labeled by fluorescent anti-human-CD4/CD69/IL4 mAbs. Finally we analysed samples by flow cytometry to determine the percentage of TH cells expressing intracellular-IL-4 (int-IL-4) and surface-CD69. The ratio between the percentage of activated TH-cells in NSAIDs-stimulated samples and BC,defined Stimulation Index (S.I.) was calculated and considered positive if S.I.≥2.

Results: No healthy subjects showed positivity to NSAIDs. S.I. between mean of PC and BC was 4,03 (CD69) and 2,56 (int-IL-4). We observed TH activation by CD69 (S.I.≥2) in 2 subjects for ASA (n=7;28.57%) and 1 for Paracetamol (n=8;12,5%),whereas TH2 activation by int-IL-4 was detected in 2 subjects for ASA (n=7;28,57%) and 1 for Paracetamol (n=8;12,5%). Interestingly simultaneous activation by CD69/IL-4 was detected in 1 subject for ASA.

Conclusions: Due to the different T-cell-phenotypes and activation-pathways it is wrongly to consider a single test as LTT useful for DHR to drugs.By this method we could explore type IVb and IVc reactions. We confirmed in 6 patients out of 13 (46,13%) the diagnostic hypothesis of physicians. Simultaneous activation CD69/IL-4 could mean that in some cases we have a dual activation-pathway. Our preliminary results are very promising and these test could represent a useful support for the diagnosis and prevention of DHR.

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Basophil activation and skin tests with cetuximab in patients sensitized to Galactose-alpha-1,3-Galactose

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Introduction: In patients with allergy to mammalian meat, sensitization to alpha-Gal can be demonstrated by specific IgE. However, skin tests with commercially available test solutions, and prick-to-prick and scratch tests with raw meat have a low sensitivity. With cetuximab standardized skin tests and basophil activation tests can be performed.

Patients and methods: As previously described in four patients (1), we performed skin prick tests with undiluted and intradermal tests with a 1:100 dilution of cetuximab in another two male patients (60 and 52 years) with a history of anaphylaxis to meat. In both also skin prick tests with commercial meat tests, and scratch tests with raw meats including pork kidney were performed. Determination of specific IgE to mammalian meats and alpha-Gal was done by ImmunoCAP (Thermo Fisher Scientific Inc., Waltham, USA). Basophil activation test was done with cetuximab, rituximab and raw pork kidney.

Results: Both patients have repeatedly experienced severe anaphylactic reactions after consuming red meat, particularly in association with alcohol. One was sensitized to tree pollen. In both skin prick tests with commercial meat extracts were negative or equivocal, scratch tests with raw meats were clearly positive to pork kidney and positive to different other meats. IgE to alpha-Gal was 71.1 kU/l and 5.61 kU/l, respectively. Specific IgE to mammalian meats ranged between <0.35 kU/l and 0.84 kU/l.

In one patient, basophils were non-responsive to anti-IgE but highly responsive to fMLP, a chemoattractant and degranulating agent. Stimulation with cetuximab, rituximab and pork kidney extract was negative. In the second patient, anti-IgE and fMLP were positive, and cetuximab showed a concentration-dependent activation and degranulation, rituximab was negative. Stimulation with fresh pork kidney was positive in the highest concentration only.

Conclusions: The use of cetuximab for skin and basophil activation tests permits diagnosing patients with anaphylaxis from meat in a standardized manner. Approximately 20% of a tested population, are non-responsive to anti-IgE or fMLP excluding basophil activation as diagnostic tool.

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Allergy to formaldehyde: basophil histamine-release test with a modification is useful for diagnosis

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Background: Formaldehyde has long been recognized as both an allergen to sensitized subjects and an irritant to the general population. The reliability of basophil histamine-release test is not high for this allergen. Here, we report a 28-year-old female case of urticaria induced by paraformaldehyde used during root canal treatment. Formaldehyde-specific IgE assessed by ImmunoCAP was strongly positive (>100 UA/ml), but her basophils showed no histamine-release reaction in response to diluted formalin or paraformaldehyde. We thus believed that there must be some underlying mechanisms that would explain the discrepancy between the basophil test results and the presence of serum formaldehyde–specific IgE.

Methods: Following days to weeks of storage of a mixture of formaldehyde plus albumin, the solutions were serially diluted and tested for basophil stimulation, without any dialysis step.

Results: We found that mixtures stored for 3 days to 2 weeks were potent inducers of histamine release from sensitive basophils, indicating that binding of formaldehyde to albumin is a slow process. Protein-unbound formaldehyde showed no effect on basophil activation, since formaldehyde solution added at 300 or 1000 ng/ml before stimulation with formaldehyde-albumin conjugates failed to modify histamine release from basophils.

Conclusions: Formaldehyde induced basophil histamine release only when it had been pretreated with albumin for days to weeks, suggesting that this molecule's slow interaction with proteins may affect the usefulness of this *in vitro* test. These results may expand the academic scope of molecular allergology; the chemical and/or biological properties of allergens, especially small-molecular-weight allergens, should be taken into consideration in order to improve our understanding of the pathogenesis and pathophysiology of clinical allergy.

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Basophil activation test as a biomarker in allergic patients to platins undergoing rapid desensitization

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Background: Rapid Drug Desensitization (RDD) has become a cornerstone of the management of immediate hypersensitivity reactions (HSRs) to chemotherapeutic agents, including biologicals. It is the only effective procedure for overcoming HSRs to first-line therapy, thus representing an important advance in patients' treatment and prognosis. Biomarkers to assess drug sensitization and monitor RDD safety and efficacy are lacking. Preliminary data suggested that in addition to skin testing, basophil activation test (BAT) could be used to assess patient's IgE sensitization to platins and provide markers of activation to evaluate the response to RDD.

Method: We studied 12 patients with gastrointestinal and OBGYN cancers who presented hypersensitivity reactions to platins and 6 healthy volunteers who had never been exposed to platins. Skin testing and BAT were done before RDD to platins, and the expression of activation markers CD203c and CD63 was evaluated on HLADR-/CD41-/CD123+ basophils. Most patients were evaluated during 2 or more RDD procedures.

Results: BAT was positive in 9/12 patients (75%), with increased expression of CD203c (9 patients) and CD63 (4 patients). The BAT positivity was 66,7% (6/9) for carboplatin and 100% (3/3) for oxaliplatin. Subsequent BAT analysis in different RDD procedures showed that the test remained positive before each procedure with an even greater expression of CD203c and CD63, indicating temporary tolerance during RDD, which was lost after each exposure. In an attempt to correlate reactions during RDD with specific BAT markers, we observed an association between CD63 expression and the severity of the reactions. All controls had negative tests. Because all patients had positive skin tests further studies are needed to determine the predictive values of BAT in patients with platins hypersensitivity.

Conclusions: We standardized a BAT to platins with good sensitivity and which can predict patients with severe reactions during RDD. RDD to platinum drugs does not induce persistent hyporresponsiveness on basophils, highlighting the need to maintain repeated RDD in allergic patients to platins.

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IgE-mediated sensitization against storage pests: relevance of cross-reactivity and improvement of diagnostics

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Background: Although storage pests e.g. of the insect order Coleoptera, Blattodea, and Lepidoptera are not direct carriers of pathogens they can induce secondary infestation with pathogenic hygiene pests or molds and cause allergic sensitization. Particularly in working environments with high exposure to organic material like grain and flour, sensitizations to storage pests seem to be more frequent. Commonly parallel sensitizations against different insect species occur and the source of the primary sensitization is often unclear. The objective of the study was to analyze different storage and hygiene pests in respect to IgE-binding capacity of different development stages and antibody cross-reactivity among arthropods.

Method: Protein extracts of the development stages larva, pupa and imago of *Tribolium confusum, Tenebrio molitor, Sitophilus granarius, Ephestia kuehniella, Plodia interpunctella, Oryzaephilus surinamensis,* and *Cryptolestes ferrugineus* were prepared. Protein concentrations, IgG or IgE-cross-reactivity were quantified by Bradford assay, immunoassays based on rabbit IgG-antibodies against *T. molitor* larvae, *T. confusum* pupa and the mites *D. pteronyssinus* and *D. farinae*, or IgE-ImmunoCAP-inhibition tests with sera from multi-insect-sensitized individuals, respectively. Protein composition and IgE and IgG binding were analyzed by SDS-PAGE and immunoblotting.

Results: Whereas rabbit anti-mite-IgG showed only minute reactivity with the various storage pest extracts and reactivities of the *T. molitor* and *T. confusum* immunoassays to different species were in the range of 0.001%-37%, IgE-inhibition experiments demonstrated a high degree of cross-reactivity between the different insect species. Mean IgE-inhibition rates were between 76% and 90% with *T. confusum* as the strongest inhibitor. Using extracts of the different developing stages of *T. confusum*, *S. granaries* and *E. kuehniella* coupled to ImmunoCAPs, the pupa extracts of *T. confusum* and *S. granarius,* and the imago extract in the case of *E. kuehniella* showed the highest IgE-binding capacity, even higher than the particular commercial ImmunoCAP. Tropomyosin as suggested arthropoda panallergen seemed not to be responsible for the observed cross-reactivity between the different storage pests as it rarely bound IgE.

Conclusions: This study suggests that storage pest extracts produced from different development stages exhibit variable IgE-binding capacity and we recommend the application of the allergen extract with the best outcome to improve allergy diagnostics.

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Immune mechanisms of induction and long-term maintenance of allergen tolerance in patients allergic to hymenoptera venom

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Background: While the clinical efficacy of allergen-specific immunotherapy (ASIT) in patients with hymenoptera venom (HV) allergy is well documented, the underlying immune mechanisms are not yet fully understood. Furthermore, little is known about how allergen tolerance is maintained after cessation of treatment.

Methods: Patients with IgE-mediated allergy against HV were analyzed at different time points before, during and up to 11 years after finishing ASIT with either wasp or bee venom extract. Different HV-specific T-cell subsets were quantified by FACS analysis and ELISPOT assay. Furthermore, specific IgE and IgG antibodies against either HV-extract or the recombinant major allergen Ves v 5 (common wasp) or Api m 1 (honey bee) were determined. The *in vitro*-capacity to block allergen-IgE complex formation was measured by the recently established cell-free HV-ELIFAB (enzyme-linked immunosorbent facilitated antigen binding) assay. Results were related to the clinical efficacy of ASIT, determined by the outcome of sting challenge during and field stings after therapy.

Results: While neither IL-10-secreting type-1 (Tr1) or $CD4^+CD25^+CD127^{low}$ Treg cells nor IFN-γ+ (Th1) cells showed substantial changes during ASIT, a continuous and long-term decrease of IL-5-producing (Th2) cells was noticed, which started to rise again several years after stopping ASIT. Allergen-specific IgG/IgG4 antibodies were induced already within the first month and continued to increase during ASIT. In addition, allergen-blocking capacity, which could be abrogated by depletion of HV-specific IgG, was enhanced. Furthermore, toleration of sting challenge was accompanied by significant increases in both allergen-specific IgG/IgG4 antibody concentrations and inhibition of allergen-IgE complex formation. Interestingly, these parameters started to decline again several years after finishing ASIT.

Conclusions: Induction and maintenance of tolerance against HV seem to be dependent on both the loss of allergen-specific Th2 responses and the synthesis of HV-blocking IgG antibodies. Since these parameters gradually return to pre-treatment values many years after termination of ASIT, not all patients might retain ASIT-induced HV tolerance for prolonged time frames. Thus,

identification of these patients would be important for improved long-term therapeutic management of IgE-mediated HV-allergy.

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Grass pollen immunotherapy: suppression of nasal symptom scores after nasal allergen challenge correlates with nasal fluid IL-9 concentrations

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Background: Nasal allergen provocations are useful in investigating the pathophysiology of allergic rhinitis. They may also be helpful in assessing response to treatments, including specific-allergen immunotherapy. We aimed to use grass pollen nasal challenge (NAC) to investigate the effects and mechanisms of allergen immunotherapy.

Methods: We conducted a cross-sectional study of untreated grass-pollen allergics (n=14), immunotherapy-treated allergics (n=18, 14 currently-treated, 4 completed treatment), and non-atopics (n=14). Volunteers underwent a standardised NAC with 2,000 BU *Timothy grass* allergen (equivalent to 1.3µg major allergen, Phl p5). Nasal fluid was collected using polyurethane sponges before and at intervals up to 8 hours after NAC, and later analysed by ImmunoCAP and multiplex assays. Nasal mucosal biopsies were taken 8 hours after challenge; tissue sections were later immunostained for T cells, eosinophils, mast cells and basophils. Clinical response to challenge was assessed by symptom scores and peak nasal inspiratory flow (PNIF). Cutaneous allergen response was measured by intradermal allergen injection. Seasonal symptom questionnaires were also completed.

Results: Immunotherapy-treated patients had lower symptom scores (p=0.04) and higher PNIF (p=0.02) after challenge than untreated-allergics. They had reduced early (15 minutes, p=0.0007) and late (8 hours, p<0.0001) skin responses, and lower seasonal symptom scores (p=0.003). Nasal challenge response correlated with seasonal symptom scores (symptoms: r=0.52, p<0.003; PNIF: r=-0.57, p<0.001), as did skin late phase response (r=0.63, p=0.0001). Immunotherapy-treated patients had reduced nasal fluid IL-4, IL-9, and eotaxin at 8 hours (all p<0.05) and tryptase at 30 minutes (p=0.05). Nasal fluid IL-9 correlated with symptom scores after challenge in immunotherapy-treated patients (r=0.47, p=0.049). In untreated allergics, early phase tryptase correlated with late phase IL-4, IL-5, IL-9, and eotaxin (all r>0.5, p<0.03). Nasal fluid allergen-specific IgE levels were similar amongst immunotherapy-treated and untreated allergics; no increase was seen after challenge. There was a trend to lower nasal eosinophils after challenge in immunotherapy-treated patients (p=0.09), but levels of mast cells, T cells, and basophils were similar.

Conclusions: Local assessments after nasal allergen challenge are sensitive in the detection of the clinical and biological effects of allergen immunotherapy. Local IL-9 suppression may be linked to effective immunotherapy.

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Frequency of CD39+ cells in the upper airways – a marker of tolerance in hayfever?

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Mechanisms of SIT target different levels including the induction of T-regulatory cells (Tregs). In clinical contexts only allergen challenges and symptom assessments during natural exposure reveal acceptable data to assess relevance of or tolerance to allergen. Markers of efficacy and prognosis are urgently needed, thus assessment and therapeutic monitoring of nasal Treg frequencies and T-cell activation at the site of disease may prove useful.

The cell-surface ecto-nucleoside triphosphate diphosphohydrolase CD39 was initially identified as B-cell activation marker and is increasingly used as a Treg marker. CD39 mediates suppressive effects by modulation of pericellular levels of adenosine, while the adenosine receptor A2A activates an immuno-inhibitory loop that differentially regulates Th1 and Th2 responses. *Cd39*-null mice spontaneously develop features of autoimmune diseases associated with Th1 immune deviation.

We monitored prospectively n=57 grass-pollen allergics, n=31 patients with hayfever on symptomatic treatment and n=20 non-allergics in and out of the grass-pollen season, upon nasal allergen challenge and immune responses during a course of pre-seasonal SCIT. We assessed QOL, disease activity and performed nasal FACS assessment at different timepoints. Nasal curettages were taken and immediately analysed in a 7-parameter FACS staining panel. Aim of study was to evaluate surrogate markers of T-cell activation and regulation to assess the allergic status quo in the upper airways and nasal mucosa at the site where allergic disease occurs.

The study reveals that most cellular markers, e.g. nasal CD3 cells increased during peak-season. Interestingly SCIT-mediated T-cell activation characterized by integrin-alpha-E CD103 (mucosal homing) increased, whereas CD69 displayed a distinctive pattern: overall events of nasal CD69 as cellular activation marker increased upon SIT, while gated for T-cells the frequency decreased. Frequency of nasal CD39+ cells as marker of immune regulation and suppression showed a significant time-treatment pattern thus culminating in a greater than 50% increase at end of SCIT.

We therefore conclude that local mucosal responses offer great potential for immune-monitoring of Treg induction. This patient cohort will be continued on long-term.

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Nasal inhibitory IgG4 antibodies: potential biomarkers for monitoring grass pollen immunotherapy

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Background: Grass pollen subcutaneous allergen immunotherapy (SCIT) is associated with induction of serum IgG4-associated inhibitory antibodies that prevent IgE-facilitated allergen binding to B cells. We hypothesised that local nasal IgG4 antibodies with inhibitory activity are induced following SCIT and correlate with clinical efficacy.

Methods: In a prospective controlled study conducted in and out of the grass pollen season, Nasal fluid and sera were obtained from 9 SCIT-treated patients, 13 untreated allergics and 10 non-atopic controls. Specific IgE and IgG4 to *Phleum pratense* (Phl p) components were measured by ISAC microarray biochip technology. Total IgE was measured by immunoCAP system. Inhibitory activity of nasal fluid and serum was measured by IgE-FAB assay.

Results: Nasal and serum rPhl p 1 and rPhl p 5 specific IgE were elevated in SAR compared to NA ($p<0.05$). Levels of sIgE in SAR and AIT-group remained unchanged in the nasal fluid and serum during the pollen season. Grass pollen rPhp 1 and r Php 5 specific IgG4 levels and associated inhibitory activity in nasal fluid and in serum as measured by IgE-FAB assay was significantly increased in AIT when compared to SAR group (nasal fluid, $p=0.0002$; serum, $p=0.0002$). Interestingly, the magnitude of the inhibitory activity was more profound with neat nasal fluid when compared to serum (91% IgE-FAB inhibition for nasal fluid,38% for serum). The relative allergen-IgE complex binding to B cells in nasal fluid ($r= -0.66$, $p<0.001$) and in serum ($r= -0.49$, $p=0.01$) correlated with global seasonal symptoms scores

Conclusion: Functional Local IgG4 antibodies in nasal fluid have potential as a surrogate biomarker for monitoring clinical response to allergen immunotherapy.

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Birch pollen immunotherapy in mice: inhibition of Th2 inflammation is not sufficient to decrease airway hyper-reactivity

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Background: In allergen immunotherapy, it remains a matter of debate which changes of the immune system are decisive for reaching beneficial effects. It is important to establish the sequence of events that occur in the path towards effective treatment. The aim of this study was to optimize a mouse model for birch pollen (BP) immunotherapy. This preclinical model can be used to elucidate underlying mechanisms that contribute to improvement of clinical symptoms leading to improvement of allergen immunotherapy.

Methods: BP-sensitized and challenged mice received 8 weekly subcutaneous immunotherapy injections (SCIT) with BP extract adsorbed to 1 mg alum. The effect of different doses of BP (0.01, 0.1, 0.3 and 3 mg) adsorbed to a fixed concentration of alum on the suppression of airway inflammation and airway hyperresponsiveness (AHR) was determined. Next, the impact of the amount of SCIT injections was investigated. Mice received 2, 4, 6 or 8 injections of 0.3 mg BP, the dose that gave a suboptimal effect in the dose-finding experiment. Mice were re-challenged on 3 constitutive days (98, 99 and 100) with BP. The hallmarks of allergic asthma were evaluated.

Results: Suppression of immunological parameters by SCIT was dependent on the dose of BP. The highest dose of 3 mg resulted in the strongest suppression of allergic symptoms. In the second study, investigating the amount of necessary injections to suppress allergic symptoms, 2 injections were sufficient to significantly suppress IL-4, -5, -13, -10 and IFNγ production, eosinophil recruitment and peribronchial inflammatory infiltrates. BP-specific IgE, IgG1 and IgG2a were upregulated. Although many features of allergic asthma were already affected after only 2 injections, 8 injections were needed to significantly suppress AHR. This was related to the degree in which other allergic features were affected. In particular, the gradual reduction in AHR was inversely associated with the increase of BP IgG2a.

Conclusions: In this model SCIT induced a fast initial suppression of the allergic symptoms after challenge. However, amelioration of AHR was delayed. Most likely, protective BP immunoglobulins (especially IgG2a) need to be sufficiently induced, together with suppression of the Th2 response, to affect AHR.

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Observations on skin test reactivity and allergen-specific IgE in ragweed-allergic patients

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Introduction: Selection of subjects for allergen immunotherapy trials has traditionally been based on clinical history and objective demonstration of allergen sensitivity. Recent clinical studies of ragweed (RW) SLIT-tablets (Creticos; Nolte) indicate that puncture skin test (PST) + allergen-specific serum IgE (sIgE) are critical determinants in identifying symptomatic patients. Furthermore, in a 5-grass SLIT study (Cox), 11% of subjects met PST inclusion criteria, but had undetectable timothy-specific IgE (< 0.1 kU/L) at screening -this subgroup was ~asymptomatic during the grass season. We report here our findings on PST sensitivity, RW-specific sIgE, and clinical symptomatology (RCSS) in subjects participating in a DBPC RW sublingual immunotherapy (RW-SAIL) trial [1].

Methods: Post-hoc analyses were performed, including correlation between PST (defined by sum of erythema), sIgE, and RCSS during the ragweed pollen season.

Results: This DBPC trial resulted in a robust clinical benefit in RCSS in subjects treated with RW-SAIL vs placebo [42%↓ entire; 41%↓ peak season]. However, of the 419 subjects that met the inclusion criteria for the trial, 77 subjects (18.4%) had <0.35 kU/L sIgE. Among the placebo-treated subjects, the RCSS was 2.91 in the subgroup with baseline sIgE < 0.35 kU/L (n=36) and 4.29 in those with baseline sIgE > 0.35 kU/L (n=157) [p = 0.04]. Among all subjects with sIgE < 0.35 kU/L , the estimated SLIT treatment effect was -0.246 with respect to placebo (p=0.750, n=69). For subjects with sIgE >0.35 kU/L, the treatment effect was -0.974 (p = 0.012, n=315) and for all subjects, -0.830 (p=0.017, n=384).

Conclusions: We found that skin test-positive, sIgE-negative subjects had lower RCSS during the pollen season. We looked for a correlation between sIgE and RCSS among the placebo-treated subgroup: This relationship was not significant (p=0.343) when seasonal RCSS were used, but was significant (p=0.002) when analyzed by baseline-adjusted scores, because sIgE-negative subjects had significantly higher pre-seasonal RCSS as compared to sIgE-positive subjects. Our findings highlight the benefit of defining patients by PST erythema and the incorporation of sIgE as part of the subject inclusion criteria to detect a more robust immunotherapy treatment effect.

[1] Creticos PS, Esch RE, Couroux P, Gentile D, D'Angelo P, Whitlow B, Alexander M, Coyne TC. Randomized, Double-Blind, Placebo-Controlled Trial of Standardized Ragweed Sublingual-Liquid Immunotherapy for Allergic Rhinoconjunctivitis. J Allergy Clin Immunol 2014. 133:751-58.

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Neuraminidase-coated, allergen-loaded microparticles are a safe and efficient novel treatment option for food allergy

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Background: We have reported that Neuraminidase (NA) from *Vibrio cholera* represents a promising functionalization substance of allergen-loaded Poly (D,L-lactide-co-glycolide) (PLGA) microparticles (MPs) due to specific interactions with intestinal epithelial cells and enhanced uptake via M-cells. Here we aimed to assess the safety of orally applied NA-coated MPs in naïve animals as well as the therapeutic potential of these MPs in a mouse food allergy model.

Methods and Results: For safety evaluations six oral administration cycles with MPs were performed. Naïve animals received ovalbumin (OVA)-loaded MPs, either uncoated or coated with NA, *Aleuria aurantia* lectin (AAL), wheat germ agglutinin (WGA). A control group was left untreated. The OVA-specific immune response of animals was evaluated subsequently. OVA-specific serum IgA levels increased significantly after NA-coated MPs administration, whereas all other antibody subclasses remained at the baseline. MP gavages did not induce IL-4, IL-10 or IFN-γ production. To evaluate the therapeutic potential of MPs, mice were sensitized intragastrically (ig.) using OVA under concomitant gastric acid suppression followed by intraperitoneal (ip.) injection of OVA adsorbed to Al(OH)3 to induce sustained systemic response. Subsequently mice were split in groups for six cycles of oral immunotherapy with OVA-loaded MP either uncoated or coated with NA, AAL, WGA. OVA ig. treated, untreated or animals left naïve for the entire study period served as controls. After last treatment mice were systemically challenged with OVA and rectal body temperature was recorded. Drop of body temperature was attenuated in the groups having received NA-coated MPs or OVA ig. In intestinal fluids total IgA titers were significantly elevated in animals treated with uncoated MPs, NA-coated MPs or OVA ig. In the latter group significantly stronger OVA-specific mucosal IgA and systemic IgG1 and IgA responses were measured. Stimulation of spleen cells from animals treated with NA-coated MPs with OVA induced significantly higher levels of the regulatory cytokine IL-10 than controls.

Conclusion: Our data suggest that NA-coated MPs with entrapped allergen are a safe and efficient treatment option in a mouse model of type I food allergy.

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Peanut allergy can be cured by oral immunotherapy

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Background: Active treatment approach for peanut allergy using oral immunotherapy (OIT) has been shown to obtain desensitization. The possibility of recovery has to be evaluated.

Methodology: An open-label individualized oral immunotherapy (OIT) enrolling 78 children with persistent peanut anaphylaxis (PA) started in 2007 and is still in progress. Diagnosis was based on prick tests, specific IgEs to peanut, Ara h 2, Ara h 1 and 3, and oral challenges (SBOC). According to the severity of reactions, an asthmatic background, the level of sensitization and the threshold, they followed an escalation of doses during 17, or 36, or 60 weeks. Maintenance was set by the daily intake of MM's (median dose:

555 mg of peanut proteins) Regular controls every 12 weeks included prick tests and specific IgEs and IgG4 to Ara h1, 2, 3. Immunoblotting to peanut for sIgG4 was performed at the onset of OIT and at the end-point.

Results: Primary failures during the escalation led to discontinue the OIT in 8 cases (10.2%). Desensitization was obtained in 70 children tolerating a daily dose of 500 mg of peanut proteins (initial threshold: 125 mg). In 11 children, with an initial threshold of 269±290 peanut proteins (pp) (med 125), maintenance (median : 2.5 years) was stopped over 8 weeks prior to an open OC from 3250 mg to 5000 mg of pp. 8 children (4 males and 3 females, median age 8,5 y.) passed the challenge. Mild symptoms occurred in the other three. Initial sIgG4 to peanut (mgA/L) were 0.08±0.09 (median 0.07), and raised to 2.1±1.9 (median 1.5). initial sIgG4 to Ara h 2 (mgA/L) were 0.11±0.15 (median :0.07) and raised to 5.7±7 (median 2.6). Only two factors were correlated with the recovery: a lower age (8.5y. instead of 12.5y, a lower level of sIgEs: (3± 4.3 vs 23.5±25 KUA/L)

Conclusion: A complete recovery was observed in 10.2% of treated children despite the persistence of sIgEs, linked to a continuous decrease of the sIgE/sIgG4 ratio, and a large array of sIgG4 shown by immunoblotting. Starting peanut OIT at earlier age, less than 5 years old, should be considered.

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Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3)

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The mechanisms contributing to clinical immune tolerance following oral immunotherapy for food allergies remain incompletely understood. Our overall objective was to study laboratory changes associated with clinical immune tolerance in antigen-induced T cells, basophils, and antibodies in subjects undergoing oral immunotherapy (OIT) for peanut allergy. The following results provide evidence for specific immune mechanisms that are associated with a model of operationally defined clinical tolerance.

In a phase 1 single-site study, we studied participants (n = 23) undergoing peanut OIT and compared them with age-matched allergic control subjects (n = 20) undergoing standard of care (abstaining from peanut) for 24 months. Participants were operationally defined as clinically immune tolerant (IT) if they had no detectable allergic reactions to a peanut oral food challenge after 3 months of therapy withdrawal (IT, n = 7), whereas those who had an allergic reaction were categorized as nontolerant (NT; n = 13).

Changes in mean peanut specific IgE or IgG4 and basophil activation measurements did not statistically differentiate between NT versus IT participants at different timepoints from baseline through 27 months. However, T-cell function and demethylation of forkhead box protein 3 *(FOXP3)* CpG sites in antigen-induced regulatory T cells were significantly different between IT versus NT participants. When IT participants were withdrawn from peanut therapy for an additional 3 months (total of 6 months), only 3 participants remained classified as IT participants, and 4 participants regained sensitivity along with increased methylation of *FOXP3* CpG sites in antigen-induced regulatory T cells.

In summary, modifications at the DNA level of antigen-induced T-cell subsets might be predictive of a state of operationally defined clinical immune tolerance during peanut OIT.

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Nanoparticles as new adjuvant for peanut oral immunotherapy

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Abstract: Peanut allergy is involved in the majority of anaphylaxis and fatal reactions. There is a great need to develop safe Immunotherapy strategies. Nanoparticles-based allergen delivery systems (NP) have received much focus as a novel and promising strategy for allergen vaccines.

In this study, we used the synthetic copolymer between methyl vinyl ether and maleic anhydride (PVM/MA) (Gantrez®),that offers good bioadhesive mucosal properties with low oral toxicity and a low cost polymer.

NPs were prepared by the solvent displacement method.. After purification, NPs were dried by lyophilization [LF] (208 nm) or spray-drying [SD] (291 nm) with less than 0.3 polydispersity, reflecting NP of uniform size.

ICR mice (n=5/group), were sensitized intragastrically by 4 weekly 1mg peanut butter plus 5 µg of cholera toxin in PBS solution doses. One week after the last sensitization dose, three of the sensitized groups received the vaccine intragastrically, consisting in three doses of whole free roasted, (PE-LF) or (PE-SD) encapsulated peanut extract. One week later, mice were challenged intraperitoneally with 2 mg of roasted PE hydrophilic fraction. Blood and fecal samples were taken and total IgE, peanut-specific IgG1, IgG2a, IgE, and IgA was measured. Mice spleens were extracted and IL-2, IL-4, IL-6, IFN-γ, TNF, IL-17A and IL-10 released from the splenocytes in vitro re-estimulated with concanavalin A or PE was measured.

Specific IgG1 and IgG2a during immunotherapy were high and persistent, whereas total IgE levels decreased until challenging. Unsensitized control mice did not show symptoms of anaphylaxis after challenge. The sensitized untreated group presented the highest mortality rate (67%), similar to the free PE treated group. In contrast, the anaphylactic death rate was reduced in the nanoparticle-treated groups, reaching 33% in the PE-NP-LF group

Challenge surviving mice splenocytes released higher levels of IFN-γ and TNF-α than the ones from the dead mice. In contrast,

splenocytes from dead mice produced more IL-10 than survivors suggesting that a Th1 response is induced in protected mice.

Conclusions: Oral immunotherapy with PE-loaded nanoparticles was able to reduce anaphylaxis in previously peanut sensitized ICR mice. Further studies are required in order to determine the efficacy of different patterns of this immunotherapy.

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Oral immunotherapy for cow's milk and egg allergy with heat modified milk and egg derivatives

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Purpose of the study: Patients with severe cow's milk allergy (CMA) or egg allergy (EA) have high incidence of accidental reactions and reduced probability of allergy resolution. The Food Allergy Referral Centre initiated a pilot study to evaluate efficacy of oral immunotherapy (OIT) with baked products containing cow's milk or egg.

Methods: We enrolled 80 patients with CMA, aged 3–26 years, and 64 patients with EA, aged 2-24. All patients had self-injectable adrenaline prescription because of previous anaphylaxis. They underwent Oral Food Challenges (OFC) with extensively heated CM and E derivatives, defining thresholds of tolerated protein dose (TPD). Up-dosing was scheduled every month at the hospital, with products of increasing protein content, progressively less cooked. After 18-24 months patients underwent a final OFC with raw milk or egg.

Results: To date 20 patients with CMA (25%) concluded the protocol with liberalization. The median sIgE for casein was 3,19kU/L at recruitment and 1,11kU/L at the final OFC. At recruitment TPD ranged from 0,0117 to 1,65g CM protein (median 0.04); at the final OFC all patients tolerated 10 g of protein in raw CM.

21 patients with EA (32,8%) concluded the protocol, but only 10 (15,6%) were liberalized. In the latter, the median of sIgE (kU/L) at recruitment was 1,08 for egg white and 0,75 for ovomucoid, and 0,325 and 0,14, respectively, at the final OFC. At recruitment TPD ranged from 0,15 to 2,88g EP (median 0,84); at the final OFC it was 6g (boiled and raw egg).

The remaining 11 patients (not liberalized) had a median sIgE (kU/L) of 2,13 for egg white and 1,71 for ovomucoid at recruitment, and of 1,1 and 0,78 respectively, at the final OFC.

The TPD at recruitment ranged from 0,06 to 1,58g EP (median 0,52); Patients reacted to boiled or raw egg, but were all liberalized for baked egg derivatives.

Conclusion: In this pilot CMA patients obtain better results from the OIT compared to EA pts. Not liberalized EA patients show higher sIgE at recruitment, with a lower decrease during the protocol. Nonetheless their TPD increased, so baked products could be introduced and quality of life improved.

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Peptide immunotherapy is not associated with deletion of allergen-specific T cells, or modulation of chemokine receptor expression

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Background: Peptide immunotherapy using synthetic peptide immunoregulatory epitopes (development name: Cat-PAD) of Fel d 1 has been shown to reduce symptoms in cat allergic subjects and is currently undergoing evaluation in a Phase III clinical trial (6 nmol dose). The mechanisms underlying clinical benefit remain partially understood, but may include regulation of chemokine receptor expression and clonal deletion. A recent study of whole allergen immunotherapy demonstrated deletion of allergen-specific $CD27^{neg}$ effector Th2 cells.

Purpose: To conduct a pilot analysis of the frequency and functional phenotype of $tetramer^+$ T cells, before and after treatment.

Hypothesis: We hypothesized that Fel d 1 peptide immunotherapy would reduce the frequency of Fel d 1-specific CD4 T cells and decrease expression of chemokine receptors associated with recruitment to sites of Th2 inflammation.

Methods: Cryopreserved PBMC samples were obtained from an earlier randomized, double-blind, placebo-controlled trial in which 24 cat allergic subjects received 8 x 3nmol Cat-PAD at two week intervals and 24 subjects received placebo. PBMC were frozen at baseline (0wks) and 6wks after treatment. Fel d 1-specific cells were identified using MHC class II tetramers. Expression of CD27 and chemokine receptors CCR3, CCR4, CCR5, CXCR3 and CRTh2 was evaluated by flow cytometry. Due to lack of available data, the statistical power of this study design to detect differences in frequency and phenotype of cells could not be determined prior to analysis.

Results: The frequency of $CD4^+tetramer^+$ T cells was not significantly altered by treatment, but variability in frequencies was large (active baseline: 94.91 $tetramer^+$ cells per 10^6CD4^+ (sd. 96.96) vs. post-treatment: 94.17 (sd. 143.8); placebo baseline: 126.5 (sd. 93.42) vs. post-treatment: 183.5 (sd. 197.0). No significant changes were observed in expression of chemokine receptors and CD27.

Conclusions: At the 3nmol dose examined in this small study, peptide immunotherapy did not lead to deletion of allergen-specific T cells, nor did it alter chemokine receptor expression. This may correlate with the lack of long-term clinical efficacy reported for the 3nmol dose. Further studies are required to investigate the effect of the 6 nmol dose which has been reported to achieve a disease modifying effect.

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Development of a new SQ HDM SLIT-tablet for house dust mite allergy immunotherapy

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The drug substance for house dust mite (HDM) immunotherapy products has traditionally been based on aqueous extraction of purified mite bodies or whole mite culture. Using such raw materials the ratio between major allergens is difficult to control. The objective of the present study was to establish a highly reproducible drug substance offering independent control of the major allergens by fractionation of the source material and use this material to establish a strong evidence base for a new SQ-HDM SLIT-tablet in a comprehensive clinical trial development program.

Dermatophagoides farinae (Der f) and *Dermatophagoides pteronyssinus* (Der p) HDM were grown separately under controlled conditions and were harvested and separated into fractions by mechanical sieving. Two fractions were isolated from each mite species containing primarily mite bodies and mite faecal particles, respectively. Extracted fractions were subsequently mixed to achieve a constant ratio between the 4 major allergens, Der f 1/2 and Der p 1/2.

The normalized mean and standard deviation of the content of dry matter and protein in 20 independent batches was 100.0% ± 7.2% and 100.0% ± 12.0%, respectively. The total IgE-binding potency relative to the in house reference preparation (IHRP) was 1.06 ± 0.17 by solid phase immune-assay, while the contents of major allergens relative to the IHRP by radial immune-diffusion were 1.15 ± 0.13 (Der f 1), 0.98 ± 0.09 (Der p 1) and 1.12 ± 0.18 (Der f 2 + Der p 2), respectively, demonstrating high consistency in the ratio between major allergens. All measured variation coefficients between batches were less than 12%. Phase III clinical trials demonstrated that the SQ HDM tablet was well tolerated and effective in reducing symptoms and medication scores in allergic rhinitis, and reduce risk of exacerbations in allergic asthma patients after inhaled steroid reduction.

In conclusion, the production process for the SQ HDM SLIT-tablet based on fractionating of the HDM source material enables independent control of the major allergens and high reproducibility of the drug substance. The new SQ HDM SLIT-tablet demonstrated relevant clinical efficacy in randomised double blind placebo controlled clinical trials including more than 3,300 patients.

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AAAAI membership experience with allergen immunotherapy safety in patients with special medical conditions varies according to practice characteristics

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Background: Little data exists on (AIT) in patients with specific medical conditions. Results of a large survey among practicing allergists were presented last year. We here present sub-group analyses.

Methods: A survey (Surveymonkey®) was sent out to all AAAAI members in and outside US to explore their experience with AIT in patients with specific medical conditions. We analyzed sub-group differences with Pearson's X2-tests (95% CI,p< 0.05).

Results: Response rate: 21.1% (N=5148). Practices: 86-14% US-outsideUS; 44% urban, 51% suburban and 5% rural; 31% academic; 54% had clinical experience >16y; small, middle and large practices equally represented. 97.8% dosed within Practice Parameter dosing intervals. More high dosing in medium+large practices (p = 0.006) and >11y working experience (p = 0.0006). Equal % of high dosing in academic/non-academic or urban/rural. Starting immunotherapy during pregnancy is generally considered a contra-indication (CInd); experience with such patients is very low. Physicians in urban centers consider ´continuing AIT once a patient gets pregnant´, severe asthma, hypertension, coronary artery disease, arrhythmias and cerebrovascular disease more frequently a CInd for AIT, than allergists working in sub-urban/rural clinics (p< .001). Consequently, these allergists have much more experience than urban centers with AIT in this kind of patient (p< 0.008). Physicians in academic centers consider certain medical conditions a CInd for AIT much more frequently than non-academic centers (depending on condition: p=.03 to p< 0.00001). Consequently non-academic allergists have more experience with AIT in patients with certain medical conditions than academics (p = 0.027 - p< 0.00001, exceptions: solid organ transplantation or HIV). Physicians with a practice experience of 11-15y more often contraindicate AIT in severe asthma and cerebrovascular disease. Physicians working longer in practice (11+ years) contraindicate IT more in patients after BM transplantation, with HIV or auto-immune diseases. Physicians of small clinics (< 100 patients on AIT) considering many of the medical conditions more often a CInd for immunotherapy (p=0.002 to p< 0.00005). Allergists from medium and large clinics have much more experience with AIT in patients with almost all medical conditions (p = 0.003 - p < 0.00001).

Conclusion: Allergists with more experienced in AIT consider severe asthma, cerebrovascular and autoimmune diseases more often a CInd to AIT.

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Natural clinical tolerance to peanut in African patients is caused by poor allergenic activity of peanut IgE

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Background: In the westernized world peanuts may cause life-threatening allergic reactions but in Africa, where peanuts are frequently consumed, reports of severe allergic reactions are rare. The aim of the study was to investigate immunological pattern of tolerance to peanut in peanut sensitized patients from central Africa compared to Swedish peanut allergic vs peanut tolerant patients.

Methods: Sera from allergic patients (n=54) from Zimbabwe with IgE to peanut but without symptoms to peanut, and sera from peanut allergic (n=25) and peanut sensitized but asymptomatic (n=25) patients from Sweden, were analyzed for total IgE and a detailed analyses of IgE, IgG and IgG4 reactivity profiles towards allergen molecules from the most important allergen sources including six peanut allergens (Ara h 1, 2, 3, 6, 8, 9) using microarray. Allergenic activity was investigated by basophil activation assays. IgE recognition to Ara h 2 peptide epitopes was analyzed.

Results: All 54 African patients had IgE antibodies to peanut extract and 10 showed IgE levels higher than 15 kUA/L. Notably, 46% of the African and all peanut allergic Swedish patients showed IgE towards at least one of the highly allergenic peanut allergens (Ara h 1,2,3,6,9). However, 48% of the African patients had IgE to cross-reacting carbohydrates with low allergenic activity and 60% of the Swedish asymptomatic patients against the PR-protein Ara h 8. Peanut IgE from both peanut asymptomatic patient groups showed very poor allergenic activity compared to IgE from the peanut allergic patients. IgG and IgG4 specificities and levels could not discriminate between the African asymptomatic and Swedish peanut-allergic patients. Asymptomatic patients almost completely lacked IgE reactivity to Ara h 2 peptide epitopes which were recognized by peanut allergic patients.

Conclusion: Natural clinical tolerance to peanut in the African and Swedish patients could be explained by exclusive IgE reactivity to low allergenic peanut components such as profilins, CCD and/or PR-10 proteins and by poor allergenic activity of peanut-specific IgE.

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Bet v 1 binds lipids from birch and grass pollen but not from peanuts

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Background: In a real life setting the immune system never encounters allergens in their pure form. In many cases, lipids accompany or are directly bound by the allergen and thus contribute to the microenvironment in which sensitization takes place. Such lipids originate either from the allergen source or from bacterial contaminations. Lipids from the pollen coat and pollen-associated lipid mediators are co-delivered with allergens and can modulate the immune response of predisposed individuals by interacting with the innate immune system and invariant natural killer T cells. Bet v 1 was shown to possess a promiscuous binding activity for fatty acids, flavonoids and cytokinins. A recent publication describes quercetin-O-sophoroside as the natural ligand of Bet v 1. We sought to analyze the ability of Bet v 1 to bind lipids extracted from birch pollen, grass pollen, and peanuts.

Method: The probe 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS) is essentially non-fluorescent in water and becomes fluorescent with a maximum emission wavelength at 484nm when binding to the hydrophobic cavity of Bet v 1. rBet v 1.0101 (10µM final concentration; 174µg/mL) was incubated with different concentrations (3µg/mL,15µg/mL and 30µg/mL) of lipid extracts from birch and timothy grass pollen and peanuts. The binding of lipids to Bet v 1 was monitored by adding 10µM 1,8-ANS and measuring the decrease in 1,8-ANS fluorescence intensity resulting from their competition to enter the hydrophobic cavity after 5 minutes at 484nm.

Results: In this study supported by the Austrian Science Fund SFB-F4608, we saw that Bet v 1 was able to bind lipids from both pollen species, whereas it did not bind lipids from peanuts. These results indicate that the prime lipid ligands are most likely not straight chain fatty acids as they are present in both pollen and in large quantities in peanuts. These binding characteristics are in contrast to some previous studies.

Conclusions: Pollen lipids other than straight chain fatty acids may skew the immune response towards a Th2-dominated phenotype. The next question to be addressed will be whether the lipids bound by Bet v 1 have any effect on the outcome of the sensitization process.

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Serum periostin is a biomarker reflecting tissue remodeling in bronchial asthma

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Background: Tissue remodeling including fibrosis is a histological characteristic of bronchial asthma and is thought to be closely related to steroid resistance. However, thus far, no suitable biomarker detecting tissue remodeling has been available. Periostin, an extracellular matrix protein, is a downstream molecule of IL-13. As a surrogate biomarker for Th2 inflammation, periostin can thus predict the efficacy of Th2 antagonists such as anti-IL-13 and anti-IgE antibodies. However, the significance of serum periostin in monitoring tissue remodeling in bronchial asthma has remained unclear.

Methods: We enrolled 224 asthma patients and conducted hierarchical Ward's cluster analysis using variables that were essential and measurable in the daily practice. We investigated the correlation between serum periostin levels analyzed by the ELISA system (SS18A and SS17B) and annual changes in FEV1, functional consequences of airway remodeling, in our population.

Results: The asthma patients fell into four subgroups: Cluster 1 (low blood eosinophils and neutrophils), Cluster 2 (medium eosinophils and low neutrophils), Cluster 3 (high eosinophils and low neutrophils), and Cluster 4 (medium eosinophils and high neutrophils). Cluster 3, characterized as the late onset and eosinophil-dominant type, showed the highest serum periostin levels (101.6 ± 35.9 ng/ml) among the subgroups. Furthermore, annual changes in FEV1 were significantly different between the high serum periostin (≥ 95 ng/ml, -23.3 ± 33.8 ml/yr) and the low periostin groups (< 95 ng/ml, -1.7 ± 35.6 ml/yr) in Cluster 3, whereas there were no clear differences in the high and low periostin groups in the other clusters.

Conclusions: Serum periostin is useful in predicting steroid resistance or hypo-responsiveness in eosinophil-dominant asthma, which may indicate that in bronchial asthma, periostin is a biomarker reflecting tissue remodeling in addition to Th2 inflammation.

Nagasaki T *et al*. J Allergy Clin Immunol, in press

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Live imaging of the skin immune responses: idenfication of inducible skin associated lymphoid tissue (iSALT)

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Background: Antigen presentation to peripheral memory T cells is a key step in the prompt elicitation of acquired immune responses. In the submucosal areas, specific sentinel lymphoid tissues called mucosa-associated lymphoid tissue (MALT) are served as antigen presentation sites. For the skin, the concept of skin-associated lymphoid tissue (SALT) has been proposed. Consistently, dendritic cell (DC) and T cell accumulation is observed after the elicitation of human contact dermatitis. However, the formation of SALT has not been well characterized.

Methods: Here, we identified inducible lymphoid clusters in the skin, which we called inducible SALT (iSALT), using a murine contact hypersensitivity model.

Results: Upon first hapten application, DCs formed clusters in the dermal perivascular areas. In the elicitation phase, DCs formed clusters and interacted with skin-infiltrating memory T cells for several hours. This sustained interaction was essential for memory T cell proliferation and activation *in situ* in antigen and integrin LFA-1-dependent manners. Intriguingly, DC clustering in the dermis was abrogated by depletion of skin macrophages, and DCs were attracted toward macrophages both *in vivo* and *in vitro*. Taken together, our findings suggest that dermal DCs and memory T cells form lymphoid clusters, iSALT, in the skin via macrophage activation.

Conclusions: This sustained conjugation between DCs and memory T cells is essential for establishment of the effector phase in cutaneous acquired immunity.

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Targeting IgE production using antibodies against the M1 prime segment of human membrane IgE

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Background: Allergen-specific IgE antibodies are pathogenic in asthma and other allergic diseases, and inhibiting IgE antibody production may be efficacious for the treatment of these diseases. IgE exists in two forms – a membrane B cell receptor form found on IgE-switched B cells, and a soluble secreted form that is produced by IgE plasma cells. In humans but not mice, a small extracellular domain segment, referred to as M1 prime, is found on membrane IgE but not secreted serum IgE.

Methods: We have used a genetically modified IgE reporter mouse that also contains the human M1 prime domain to study in vivo IgE production. In addition, we have generated antibodies against the human M1 prime domain that enable the specific targeting of IgE-switched B cells, including a humanized anti-M1 prime therapeutic antibody called quilizumab that has been used in clinical studies.

Results: Our studies of IgE production in mice indicate that IgE is produced through a germinal center pathway that generates short-lived plasma cells and membrane IgE-positive memory B cells. In addition, an antibody against M1 prime abrogates IgE responses in mouse models. In human clinical studies, quilizumab inhibits the generation of new allergen-specific IgE, induces a

prolonged reduction of total serum IgE levels, and reduces early and late airway responses upon allergen challenge of asthmatic patients.

Conclusions: Our studies in mice have clarified a pathway of IgE production and memory and indicate that targeting IgE-switched cells may be an effective approach for preventing IgE production. Clinical studies using quilizumab indicate that anti-M1 prime antibodies are a novel approach for disrupting IgE production and may have significant clinical utility for the treatment of allergic diseases.

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Critical role for IL-27 in induction of Foxp3- regulatory T cells by retinoic acid-differentiated dendritic cells, and their role in reversal of anaphylaxis sensitivity

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Background: Regulatory dendritic cells (DCreg) can induce T cell tolerance in both human and murine model systems. Most inducible DCreg activate classical Foxp3+ regulatory T cell (Treg) responses, but others instead switch on IL-10-expressing Foxp3- Tr1 cell responses, at least *in vitro*. Thus, IL-10-differentiated DCreg (aka, DC10) convert human asthmatic effector Th2 cells (Teff) into Foxp3+ Treg, and this is seen also in mouse models of asthma and multiple sclerosis. DC that differentiate in the gut do so under the influence of retinoic acid (RA) and TGFβ, and thus induce tolerance to innocuous gut commensals and food proteins (reviewed in *Front. Immunol 5:7, 2014*). We wished to know whether food allergen-presenting RA-exposed DC could be used to reverse anaphylaxis sensitivity and, if so, the mechanisms that mediate these effects.

Methods: We differentiated mouse DC in the presence of retinoic acid (DC-RA), exposed the cells to maturational signals and loaded them with specific food allergen. We characterized their cell surface and secretory profiles (FACS, qRT-PCR, ELISA) and assessed their tolerogenic activities *in vitro* and *in vivo*, in mouse models of OVA and peanut allergen-induced anaphylaxis.

Results: DC-RA expressed CD103 (αE integrin), MHCII and co-stimulatory markers, as well as elevated levels of the tolerogenic markers PDL1 & -L2, ICOSL, TGFβ, IL-27 and the RAmetabolizing enzyme Aldh1A2. They induced anaphylactogenic Th2 cells to take on a Tr1 phenotype, such that they suppressed stimulatory DC-induced Teff cell responses. DC-RA immunotherapy reversed anaphylaxis responses to oral allergen challenge in mice sensitized to either OVA or peanut, including clinical scores, diarrhea, mast cell activation (mMCP-1 release) and Th2 responses, and lowered serum allergen-specific IgE and IgG1 levels. DC-RA generated from the marrow of IL-27-/- mice were largely therapeutically ineffective in this model.

Conclusions: Allergen-presenting DC-RA can induce food allergen tolerance by activating CD25+Foxp3- Tr1 phenotype responses in an IL-27-dependent fashion. This data indicates that, like DC10, DC-RA can also be used for allergic disease immunotherapy, but that these two populations of DCreg induce distinct Treg responses, and this potentially provides us with therapeutic options in terms of the type of Treg that we may wish to induce clinically.

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Development of oral plasma kallikrein inhibitors for prophylactic treatment of hereditary angioedema

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Background: Hereditary and acquired forms of angioedema are diseases of dysregulation of the plasma kallikrein-bradykinin axis, a major arm of the contact activation system. Hereditary angioedema (HAE), a rare autosomal dominant disorder due to mutations of regulatory or coding elements of the *SERPING-1* gene that codes for the major natural kallikrein inhibitor, plasma protease C1 inhibitor, is characterized by episodic swelling of the skin, pharynx, larynx, GI tract, genitals and extremities. Current treatments that inhibit either kallikrein or the bradykinin-B2 receptor are limited to IV or SC routes of administration. Plasma kallikrein is a serine protease, and its active enzymatic site shares homologous elements to other serine proteases. These structural features provide major challenges to discovery of small molecule inhibitors of adequate specificity using traditional screening and hit-to-lead medicinal chemistry.

Methods: We describe the development of a series of first and second generation plasma kallikrein inhibitors using structure-based drug design with a focused structural biology and medicinal chemistry approach, supported by specific and sensitive plasma-free and plasma-based kallikrein enzymatic activity assays. In the plasma-based assay, contact activation is initiated by ellagic acid, and specific kallikrein amidolytic activity is measured by cleavage of N-carbobenzyloxy-phenylalanine-arginine-7-amino-4-methylcoumarin (Z-FR-AMC).

Results: The inhibitors displayed high potency against plasma kallikrein (IC50 < 1nM in isolated enzyme assay, EC50 <10 nM in plasma-based assay) and high specificity versus other serine proteases, with selectivity index against trypsin of > 10,000-fold. The first generation compound, BCX4161, demonstrated 15-fold higher potency for suppressing kallikrein amidolytic activity in HAE patient plasma compared to plasma protease C1 inhibitor. In a Phase 1 study in healthy subjects, BCX4161, the first oral kallikrein inhibitor to be tested in the clinic, demonstrated acceptable safety and tolerability, drug exposure that met or exceeded the target range predicted for efficacy as a prophylactic HAE treatment, and exposure-related inhibition of plasma kallikrein (r = 0.93,

Emax model). Second generation compounds showed improved bioavailability in the rat, with plasma concentrations 24 hours after dosing > EC80 in the plasma-based assay.

Conclusions: A structure-based drug design approach succeeded in discovering potent, specific, and orally bioavailable small molecule inhibitors of plasma kallikrein.

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Delayed anaphylaxis to red meat and the nature of the IgE response to Galactose Alpha-1, 3-galactose

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Patients with delayed urticarial or anaphylactic reactions to meat or organs derived from mammals have been identified in Australia, Europe and the USA. They present many features that are different from other forms of food allergy including; onset in adult life; delay of 2-6 hours after eating meat; high titer IgE specific for an oligosaccharide; and in many cases relatively rapid spontaneous recovery. Evaluating 200 cases who had presented acutely to an ED we found: 50% had tolerated meat for 40 years before becoming allergic; 25% of the cases presented after midnight; the prevalence of inhalant allergy was less than 50%; and that in 60% the serum IgE ab to alpha-gal was ≥10% of the total IgE.

Evidence that tick bites induce the IgE response to alpha-gal has been reported from Australia, Sweden, and the United States. In three patients, we followed the time course of the IgE response, changes in IgG to alpha-gal and the histology of skin responses to tick bites. IgE ab increased in each case, in one case from 23 IU/ml to 290 IU/ml during the 5 weeks following 30 larval tick bites. The dermal infiltrate included CD3+ve/CD45RO+ve cells accompanied by CD-20+ve B cells. In allergic subjects eosinophils and basophils were also present. By contrast, bites in non-allergic individuals had T cells and B cells but no eosinophils or basophils. To assay IgG antibodies, protein A on beads was used to capture IgG, followed by radiolabelled alpha-gal bound to human serum albumin. Although the quantity of IgG antibodies to alpha-gal varied widely between cases the rises following tick bites were generally less than two fold.

The kinetics of IgE responses to alpha-gal have more in common with IgM responses to an oligosaccharide than with IgE responses to proteins. Furthermore, it is known that switch to IgE can occur outside germinal centers and IgE responses to other parasites do not include extensive somatic hyper-mutation. Our evidence supports a model in which tick saliva induces a direct switch to IgE outside germinal centers in areas where IL-4 could be derived from CD-4 cells, basophils, or iNKT cells.

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Structure-function relationships that govern FcεRI signaling by allergens

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Background: Multivalent antigens trigger robust FceRI-dependent signaling responses in mast cells and basophils. Most prior studies have relied on chemically heterogenous artificial ligands, rending it difficult to predict receptor cluster size and orientation and to translate the observations to clinical relevance.

Methods: This work focuses on the use of structurally defined polyvalent antigens to crosslink IgE-FceRI, including a symmetrical trivalent ligand (DNP3-fibritin) and natural allergen (shrimp tropomyosin). *In silico* docking and computer simulation methods were used to build structural models of ligand-IgE-FceRIα ectodomain complexes and to estimate aggregation properties, including distances between individual receptors after crosslinking. Signaling responses were studied in "humanized" RBL-2H3 cells, that express the human FceRIα, as well as in human basophils from well-characterized allergic subjects. High resolution imaging approaches were employed to provide additional insight into the kinetics of crosslinking and the nanoscale organization of FceRI signaling.

Results: These ligands stimulate degranulation and calcium responses in a dose-dependent manner. The bell-shaped secretory response is shown to be linked to the balance of positive (Syk-mediated) and negative (SHIP-mediated) signaling, as well as to differences in aggregate size, complexity and time course.

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Importance of mast cell Prss31/transmembrane tryptase/ tryptasegamma in lung function and experimental chronic obstructive pulmonary disease and colitis

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Background: Mouse and human mast cells (MCs) store three tryptases in their secretory granules, one of which is protease serine member S31 (Prss31)/transmembrane tryptase/ tryptasegamma. Due to its membranespanning domain, Prss31 is preferentially retained on the outer leaflet of the plasma membrane of activated MCs. Thus, MCs use the tryptase to regulate those cell types that they physically contact. Although its physiologic function has not been deduced, we previously showed that recombinant Prss31 induced cultured T cells to markedly increase their expression of IL-13 and many other biologically active proteins.

Methods: Using a homologous recombination approach we created a novel Prss31/ C57BL/6 mouse line to evaluate the importance of this conserved MC tryptase.

Results: Prss31-null mice exhibited no obvious developmental abnormality, contained normal numbers of granulated MCs in their tissues, and did not compensate for their loss of the membrane tryptase by increasing their expression of other granule proteases. When Prss31-null MCs were activated with calcium ionophore or by their high-affinity IgE receptors, they degranulated in a pattern similar to that of wild-type MCs. Prss31null mice had increased baseline airway reactivity to methacholine, but reduced experimental chronic obstructive pulmonary disease (COPD) and colitis, thereby indicating both beneficial and adverse functional roles for the tryptase. In a cigarette smokeinduced model of COPD, WT mice had more pulmonary macrophages, higher histopathology scores, and more collagen in their small airways than similarly treated Prss31-null mice. In a dextran sodium sulfate-induced colitis model, WT mice lost more weight, had higher histopathology scores, and contained more Cxcl1 and IL-6 mRNA in their colons than similarly treated Prss31-null mice.

Conclusion: The accumulated data raise the possibility that inhibitors of this membrane tryptase might be of therapeutic benefit in the treatment of humans with MC-dependent inflammatory.

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Inhibition of allergen-induced colitis by activated regulatory T cells in a humanized mouse model

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Background: Recently we developed a humanized mouse model of allergen-induced IgE-dependent gut inflammation in PBMC-engrafted immunodeficient mice. In the present study we investigated the role of regulatory T cells (Treg) in this model.

Method: NOD-*scid*-gc$^{-/-}$ mice were injected intraperitoneally with human PBMC from allergic donors together with the respective allergen or with NaCl as control in the presence or absence of different concentrations of CD4^{+}CD25^{+} Treg of the same donor. After an additional allergen boost (i.p.) one week later, mice were challenged with the allergen rectally on day 21 and gut inflammation was monitored by a high resolution video mini-endoscopic system by a blinded investigator (B.W.).

Results: Allergen-specific human IgE in mouse sera, which was only detectable in PBMC plus allergen-treated mice, was strongly inhibited by co-injection of Treg at a ratio of at least 1:10. The presence of Treg also reduced allergen-specific proliferation and cytokine production of human CD4^{+} T cells recovered from spleens at the end of the experiment. Furthermore, the allergen-induced endoscopic score evaluating translucency, granularity, fibrin production, vascularity, and stool after rectal allergen challenge was significantly decreased by Treg. Activation of Treg prior to injection further increased all inhibitory effects which could be prevented by blockade of the Treg activation marker GARP.

Conclusion: These results demonstrate that allergen-induced gut inflammation in human PBMC-engrafted mice can be prevented by human autologous Treg providing direct evidence that Treg activity is of key importance for control of gut inflammation in an allergen-driven setting.

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IL-31 regulates skin barrier and antimicrobial function through IL-1 signaling

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Background: Atopic dermatitis (AD), a chronic inflammatory skin disease with increasing prevalance, results in skin barrier defects. The expression of IL-31 is increased in skin lesions and serum of AD patients and correlates with disease severity.

Methods: Expression and functional studies were performed using human 3D skin equivalents and a novel epidermal mouse model based on cell-sorted skin equivalents.

Results: We found that IL-31 affected the expression of genes whose products are relevant for establishing and maintenance of the mechanical skin barrier. Functional studies revealed that IL-31 attenuated the skin barrier, as shown by increased penetrability to allergens, small molecular weight compounds and irritants. Furthermore *in vivo* studies employing cell-sorted skin equivalents demonstrated enhanced transepidermal water loss following subcutaneous administration of IL-31. We identified the IL-1 cytokine network as a key downstream effector of the IL-31/ IL-31 receptor axis. Antagonizing the IL-1 receptor with anakinra was sufficient to rescue IL-31 effects on skin differentiation and barrier formation. The IL-1 cytokine network also augmented the expression of antimicrobial peptides, even at low levels of IL-31, incapable of altering the skin barrier.

Conclusions: Together, IL-31 damages the mechanical skin barrier while simultaneously promoting the antimicrobial barrier and this should be a consideration when targeting IL-31 for therapeutic purposes.

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Benign versus pathologic aeroallergen-specific Th2-immunity: maturation of disease-associated immunophenotypes involves parallel changes in both humoral and cellular response profiles

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Background: Aeroallergen sensitization is common and risk for inflammatory airway disease increases with specific IgE titres. Paradoxically however, only a minority of sensitized/exposed subjects become symptomatic, suggesting that the potentially pathogenic sequelae of IgE triggering are usually efficiently endogenously controlled. In occupational allergy and immunotherapy, IgG co-produced with IgE has been invoked as a possible modulator of IgE-associated airways inflammation, but this concept has attracted scant interest outside these areas. In particular the potential role of specific IgG in modulating airways inflammation in atopic children has not been systematically evaluated.

Methods: We have addressed this issue employing a series of birth cohorts from Australia and UK via analysis of relationships between HDM/DerP1 and Cat/FelD1-specific IgE and IgG and susceptibility to asthma and corresponding Rye/PhloemP1-specific antibodies and rhinitis expression, and parallel analysis of associated Th-memory responses.

Results: The likelihood of symptoms amongst sensitized children increases with IgE titres, however risk is markedly attenuated in the presence of high levels of corresponding specific (total) IgG or IgG1 but not IgG4 i.e. when specific IgG:IgE ratios are high. This is observed consistently with respect to presence/absence of current symptoms, symptom severity scores, and persistence of symptoms over time. Strikingly, the lowest specific IgG:IgE ratios are seen in highly sensitized children recruited following hospitalization for severe asthma exacerbations. This apparent "IgE-sparing" effect in atopic children with high IgG:IgE ratios is frequently mirrored by attenuation of SPT responsiveness, exemplified by a subset of children who remain SPT^- despite underlying specific serum IgE levels in excess of 30kU/L. This suggests that the underlying mechanism(s) include reduction of effective engagement of allergen with FcεR1 on granulocytes. Consistent with this, titration of DerP1-specific IgG onto stripped basophils re-armed with HDM-specific IgE revealed clear evidence of concentration dependent IgG-mediated inhibition of DerP1-dependent basophil activation. Additionally, analysis of banked data on HDM-specific Th-memory responses from 540 sensitized cohort members revealed that increasing specific IgE:IgG ratios are mirrored by increases in Th2-effector cytokine production.

Conclusion: Development of disease-associated atopic immunophenotypes over time involves parallel maturation of mechanisms that drive both the immediate and late phase components of the host response to allergens.

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Targeted inhibition of IgE:FcεRI signals during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells

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Background: Food allergy has emerged as a major health issue with a steady increase in food induced anaphylactic reactions. Currently, individuals with food allergy are counseled to avoid allergenic foods. This is necessary for safety, but paradoxically deprives them of the chance to attain oral tolerance via ingestion, leaving them trapped in a sensitized state. Existing strategies for desensitization therapy are effective in many patients, but come with a substantial risk of adverse reactions. The anti-IgE biologic omalizumab is being tested as an adjunct to desensitization therapy in an attempt to enhance safety. In this study, we investigated whether IgE might additionally function to amplify nascent antibody and Th2 responses to ingested proteins and if manipulation of IgE signaling could be exploited to modify sensitization.

Methods: Using *Il4raF709* mice, which harbor an activated form of the IL-4 receptor, we developed a novel adjuvant-free model of peanut allergy. Gavage feeding of *Il4raF709* mice with peanut butter induced elevations in serum IgE by ELISA, and increased intracellular staining for IL-4 in CD4+ T cells by flow cytometry. Enteral challenge with peanut provoked anaphylaxis, as measured by loss of core body temperature. We compared peanut sensitization in IgE- or mast cell-deficient *Il4raF709* mice, or in *Il4raF709* mice treated with anti-IgE.

Results: Mast cells and IgE were required for induction of antibody and Th2 responses as well as anaphylactic sensitivity in peanut-fed *Il4raF709* mice, and they impaired the induction of peanut-specific Foxp3+ Treg. Antibody-mediated neutralization of IgE also prevented immune sensitization to peanut. In mice with established allergy, anti-IgE facilitated desensitization, reduced Th2 responses and induced Tregs.

Conclusions: Our findings identify novel functions for IgE antibodies and mast cells in the regulation of immune responses in the intestine. Our results predict that IgE blockers, both available and in the pipeline, will serve not only to make food desensitization safer but will also enhance effectiveness of tolerance induction.

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Dissecting cellular targets of IL-10 in allergen specific tolerance induction

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Background: Human studies suggest that allergen immunotherapy leads to regulatory immune responses that suppress the development of allergic inflammation. Interleukin (IL)-10 production by allergen-specific T cells has been suggested as one of the regulatory mechanisms. This was supported by *in vivo* studies in mice in which treatment with IL-10 receptor (IL-10R) blocking antibody abrogated the beneficial effects of immunotherapy.

Objective & Methods: In the present study we address the cellular source of IL-10 during tolerance induction using transcriptional reporter mice and the cellular targets of IL-10 by using mice with a cell type specific inactivation of the IL-10R gene generated by Cre/loxP-mediated recombination. Ovalbumin (ova) sensitized mice were treated with three s.c. ova injections on alternate days for tolerance induction. After challenge by ova inhalation allergen specific antibody and cytokine responses as well as allergen induced airway inflammation were analyzed.

Results: Upon s.c. tolerance induction increased IL-10 signal was observed in draining LN T cells, B cells and dendritic cells. Tolerance induction was effective in the suppression of airway inflammation in wildtype mice but not in IL-10R null mutants (IL-10R$^{FL/FL}$ Cre deleter^{+}), confirming the involvement of IL-10R in tolerance induction. In contrast, in mice that lack IL-10 signaling specifically in T cells (IL-10R$^{FL/FL}$ CD4-Cre^{+}) the degree of tolerance induction was comparable to that of Cre negative littermate controls both displaying strongly reduced eosinophilic infiltration into the bronchoalveolar space and reduced Th2 responses to allergen specific restimulation. Effective tolerance induction was also observed in mice with a B cell specific (IL-10R$^{FL/FL}$ CD19-Cre^{+}) as well as in mice with a neutrophil/monocyte specific (IL-10R$^{FL/FL}$ LysM-Cre^{+}) deletion of the IL-10R.

Conclusion: In the murine model of allergen induced airway inflammation direct effects of IL-10 on T cells, B cells or neutrophils/monocytes are not critical for tolerance induction. Thus different cellular targets of IL-10 are likely to be involved in the beneficial effects of allergen specific tolerance induction.

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Bacterial modulation of epithelial innate immune responses to human rhinovirus infection

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Background: Respiratory viral and bacterial pathogens have been linked to acute exacerbations in several respiratory diseases, including chronic obstructive pulmonary disease (COPD), asthma and cystic fibrosis (CF). Human rhinovirus (HRV) is responsible for the majority of viral-associated exacerbations in lower airway diseases. The lower airways of patients with these diseases, particularly COPD and CF, are often chronically colonized with bacteria, with non-typeable *Haemophilus influenzae* (NTHI) being one of the most common bacteria detected. In such chronically colonized patients, infection with HRV leads to severe disease exacerbations. It is our hypothesis that the presence of bacteria alters airway epithelial cell proinflammatory and host defense responses to HRV to lead to worse clinical outcomes.

Methods: To examine this hypothesis we used primary cultures of human airway epithelial cells exposed to medium, NTHI alone, purified HRV-16 alone, or HRV-16 in combination with NTHI. Gene expression was examined by gene array and validated using quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR). Protein production was measured by ELISA.

Results: Initial gene array experiments identified a number of genes that were at least additively, and most often synergistically, induced upon exposure to a combination of HRV-16 and NTHI, relative to each stimulus alone. These genes fell into several groupings, including neutrophil chemoattractant chemokines (CXCL1, CXCL2, CXCL3, CXCL5 and CXCL8), molecules linked to dendritic cell recruitment and activation (CCL20, IL-32) and host defense molecules (human b-defensin (HBD)-2 and IL-17C). RT-PCR and/or ELISA assays were used to further validate induction of selected genes. Secretion of CCL20, CXCL8 and IL-17C proteins were all synergistically induced by the combination of HRV-16 and NTHI compared to the sum of production using either stimuli alone, while induction of CXCL1 by the combination of these stimuli was additive. Synergistic expression of IL-32 mRNA by the combination of stimuli was shown by RT-PCR. Current studies are examining the mechanisms underlying synergistic expression of chemokines and the functional consequences of these responses on dendritic cell function.

Conclusions: We conclude that simultaneous exposure of epithelial cells to bacteria and virus leads to synergistic induction of a number of molecules that could affect clinical outcomes.

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A critical role for IgE in pulmonary vascular leak during an anti-viral immune response

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Background: Severe respiratory viral infections early in life have been associated with a greatly increased risk of developing asthma. Using a mouse model, we have defined a mechanistic pathway that translates a severe paramyxoviral respiratory infection into post-viral atopic disease. Critical to this pathway is the production of IgE against the virus (Sendai virus, SeV), which binds to FcεRI on conventional dendritic cells (cDC) in the lung. The presence of virus allows the IgE to be crosslinked leading to production of CCL28, a Th2 chemoattractant, by the cDC. The recruited Th2 cells then produce IL-13, which drives the post-viral atopic disease. We and others have further demonstrated that humans make IgE against respiratory viruses, and at least one human clinical study suggested that removal of IgE markedly blunts viral exacerbations of asthma (*N Engl J Med* 2011;364:1005). The question remains as to what is the functional role of viral-specific IgE in the antiviral immune response beyond driving a pro-atopic response.

Methods: We hypothesized that anti-viral IgE is important in generating vascular leak in the lung, which could be important for optimal cellular recruitment during the anti-viral immune response. To test this hypothesis, we intranasally inoculated wild-type (WT), IgE deficient, or FcεRI deficient mice (all on C57BL6 background) with $2x10^5$ pfu SeV and injected Evans Blue Dye (EBD) intravenously one hour prior to sacrifice at various days post inoculation (PI) SeV. EBD is an azo dye that tightly binds albumin and can be used to quantify vascular leak. Once the mouse was euthanized, the pulmonary circuit was flushed with PBS and the lungs removed, minced, and the quantity of EBD determined spectrophotometrically. Using a standard curve, the resulting values were reported as fold increase in EBD concentration in the lung tissue of SeV infected mice compared to uninfected strain-matched controls.

Results: In WT mice, SeV infection led to an increase in vascular leak that paralleled production of anti-viral IgE (day 4 PI 1.74±0.41, p=0.15; day 6 PI 2.54±0.33, p=0.015; day 8 PI 3.24±0.17, p=0.0001; day 10 PI 2.89±0.43, p=0.016; mean±SEM fold EBD concentration, p value versus uninfected; n=2-6 mice per time-point). IgE deficient mice failed to demonstrate increased vascular leak at days 4 or 6 PI SeV (1.31±0.40 and 0.97±0.20, respectively; n=3). Compared to WT mice, the level of vascular leak at day 6 PI SeV was significantly reduced in IgE deficient mice (p=0.0037) with a trend for reduction in FcεRI deficient animals (1.32±0.38, p=0.058; n=3).

Conclusions: Development of pulmonary vascular leak during a severe paramyxoviral respiratory infection requires IgE and, possibly to a lesser extent, FcεRI. To our knowledge this is the first demonstration of a role for IgE during the acute anti-viral immune response. Further studies will examine the functional significance of impaired vascular leak in this model, as well as identifying the cells responsible for the leak and the specificity of the IgE response.

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Rapid antigen desensitization through the IgE receptor on wild type and humanized FcεRIα murine bone marrow-derived mast cells

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Background: Rapid antigen desensitization is being increasingly used to treat patients with IgE-mediated allergies to drug, airborne, insect and food allergens. However, a clear understanding of the key parameters underlying safe and effective rapid desensitization protocols is still lacking.

Methods: Two in vitro models of rapid desensitization were established. Bone marrow-derived mast cells (BMMCs) from wild type (WT) Balb/c mice were sensitized with anti-DNP IgE and challenged with DNP-HSA through single (activation) or sequential increasing doses (desensitization) and BMMCs from humanized FcεRIα Balb/c mice were sensitized with human serum from either dust mite or peanut allergic subjects and activated or desensitized to Dermatophagoides *pteronyssinus* (Dp) or peanut.

Results: In WT BMMCs, DNP-HSA desensitization starting at a dose of 1pg that was increased 1.5- to 2-fold every 10 minutes to reach 1ng led to a reduction of mediator release of 43%, as measured by β-hexosaminidase, and reduced by 73% the surface expression of LAMP1 as compared to activation. In humanized FcεRIα BMMCs, the antigen starting concentration, the rate of increase per step and the time interval between steps had a profound impact on the outcome of rapid desensitization. Modifying the fold increase per step from 2- to 10-fold reduced the inhibition of β-hexosaminidase release from 81% to 19% (P=0.006) in cells treated with Dp. Starting concentrations of Dp above the threshold for activation progressively reduced the inhibition of β-hexosaminidase release induced by desensitization (P=0.0002). Reducing the time between steps to 1 minute reduced the inhibition of β-hexosaminidase to 15% (P<0.0001) but prolonging it to 15 minutes did not improve the effectiveness of desensitization beyond that of 10-minutes intervals (P=0.36). Desensitization to peanut completely abolished β-hexosaminidase release from cells sensitized with serum from a peanut allergic subject when using a starting concentration below the threshold for activation, 2-fold increases per step and 10-minutes intervals.

Conclusions: The effectiveness of rapid desensitization protocols in WT and humanized FcεRIα murine mast cells is optimal when antigen starting concentrations are below the activation threshold and when ≤2-fold increases per step and 10-minutes intervals between steps are used. Further studies should help correlate these models with rapid desensitizations in humans.

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Lactobacillus strains differentially activate immunomodulatory and neuromodulatory pathways to attenuate symptoms of food allergy

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Background: There is considerable interest in the potential use of specific bacteria, particularly *Lactobacilli* and *Bifidobacteria*, in the prevention or treatment of allergies. Studies of the regulatory roles of bacteria in allergic responses have identified bacterial strain specific suppressive or immunomodulatory effects including altered lymphocyte proliferation, cytokine release, and antigen specific immunoglobulin production. However the mechanisms underlying the bacteria strain specific nature of allergy suppression remain poorly understood. Here we compare the effects and potential mechanisms of action of two distinct lactobacillus strains in a mouse model of food allergy.

Method: Male Balb/c Mice were sensitized with 50µg of ovalbumin (OVA) in alum (i.p.) on days 0 and 14. Treatment with *Lactobacillus reuteri, Lactobacillus rhamnosus* or vehicle control was administered daily, via oral gavage, from day 27 to day 49. Mice were challenged orally with 50 µg of OVA every 3 days from day 28 to day 49. Severity of diarrhea and anaphylaxis were assessed for one hour following each antigen challenge. Following the final antigen challenge, serum, spleen lymphoid and intestinal tissue samples were collected for the assessment of a range of immune parameters.

Results: Both *L.reuteri* and *L.rhamnosus* strains significantly reduced the severity of diarrhea and hypothermia following oral antigen challenge. Neither *L.reuteri* nor *L.rhamnousus* treatment lead to changes in total, or antigen specific, IgE levels. The attenuation of the allergic response in *L.rhamnosus* treated animals was associated with significantly decreased serum levels of mast cell protease-1, increased Foxp3+ T regulatory cells in Peyer's patches and increased IL-10 but decreased TNF production by antigen stimulated splenocytes. None of the tested immune parameters were altered in the *L.reuteri* treated animals. However, it was identified that *L.reuteri* but not *L.rhamnosus* acts in the intestine to inhibit activation of transient receptor potential cation channel vanilloid subtype 1 (TRPV1), a key component in the neurogenic axis of allergic disease.

Conclusion: We suggest that while *L.rhamonus* attenuates the symptomatology of food allergy through the induction of a tolerogenic or suppressive immune environment the benifical effects of *L.reuteri* are largely mediated through modulation of the neurogenic component of the allergic response.

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Vitamin D inhibits human innate inflammatory cytokine responses following bacterial (TLR4) but not TLR7/8 or respiratory virus stimulation

Hayglass

Rationale: Vitamin D plays multiple roles in regulation of protective and maladaptive immunity. While epidemiologic studies suggest linkages between poor vitamin D status and increased viral respiratory infections, we know little of how vitamin D affects viral PRR-driven cytokine production in humans or the benefits of supplementation on anti-viral responses.

Objective: Determine the effects of vitamin D on pro- and anti-inflammatory innate responses stimulated by bacterial vs viral PRR-ligands in asymptomatic humans.

Results: Fresh PBMC or isolated CD14+ monocytes were stimulated with bacterial (TLR4) or viral (TLR7/8) PRR ligands, or infectious RSV ± active Vitamin D, 1,25(OH)2D3 . TLR4-driven pro-inflammatory cytokine/chemokine responses were inhibited by vitamin D as were a broad panel of anti-inflammatory responses. In marked contrast to bacterial PRR stimulation, neither pro- nor anti-inflammatory cytokine production evoked by viral PRR ligand, or culture with infectious RSV, were inhibited despite 1,25(OH)2D3 at physiologic through to pharmacologic levels. Seeking the mechanism of differential regulation in bacterial vs viral CD14+ monocyte derived cytokine responses, we found that (i) TLR7/8, but not TLR4, stimulation markedly inhibits VDR expression, selectively reducing the sensitivity of viral PRR responses to vitamin D's activity and (ii) while Vitamin D substantially enhances IkBa expression (a negative regulator of NF-kB and cytokine production) in TLR4 stimulated monocytes, it consistently failed to do so during TLR7/8 stimulation. MKP-1, a control negative regulator of NF-kB was unaffected following either stimulation.

Conclusions: Vitamin D potently inhibits pro-inflammatory innate cytokine responses elicited by bacterial PRR ligand stimulation. Our data reveal that a broad panel of bacterial PRR-stimulated anti-inflammatory responses are similarly inhibited. Secondly, the proposed health benefits of vitamin D, prominently discussed for enhanced prevention and control of viral infection, are not associated with modification of either TLR7/8 or RSV driven cytokine production. The findings implicate differential impact of bacterial vs viral PRR stimulation on VDR expression and vitamin D's capacity to selectively increase cytoplasmic inhibitor IkBa during TLR4 but not TLR7/8 stimulation. Collectively, the data raise questions about posited benefits of extensively practiced vitamin D supplementation among healthy humans. Funding: CIHR, CRC Canada.

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Involvement of the bioactive peptide prokineticin 2 in autoimmune demyelinating disease of the central nervous system

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Background: Multiple sclerosis (MS) is a chronic inflammatory diseases of the CNS often accompanied by neuronal loss. The small molecule prokineticin 2 (PK2), also known as Bv8, is involved in multiple biological functions including neurogenesis and immune modulation. PK2 and its two known receptors PK-R1 and PK-R2 are expressed in immune and neuronal cells. In this study we investigated a possible role for PK2 in MS and its animal model experimental autoimmune encephalomyelitis (EAE).

Methods: We evaluated the expression of PK2 and its receptors in mice with myelin oligodendrocyte glycoprotein (MOG) 35-55-induced chronic EAE and in patients with relapsing-remitting MS. We blocked the effects of PK2 with a PK receptors (PK-Rs) antagonist during EAE, and evaluated the effects of this treatment on the clinical expression of the disease and on autoreactive T cell response against myelin.

Results: PK2 mRNA expression was upregulated in lymph node cells (LNCs) and spinal cord of mice with chronic EAE compared with naïve mice, and PK2 concentrations were increased in sera of mice during disease. In relapsing-remitting MS patients, transcripts for PK2 were significantly increased in PBMC compared to healthy subjects, and PK2 serum concentrations were significantly higher. PK-Rs antagonist prevented or treated established disease in both chronic and relapsing EAE, and significantly reduced CNS inflammation and demyelination. Antigen-stimulated LNCs from mice treated with PK receptors antagonist produced significantly lower amount of IFN-γ and increased amount of suppressor cytokine IL-10.

Conclusions: Our results identify for the first time a role for PK2 as a mediator of CNS autoimmunity. PK2 and its receptors might represent a novel target of treatment in CNS autoimmunity.

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The *N*-formyl peptide receptors (FPRs) expression and functions in systemic sclerosis

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Systemic sclerosis (SSc) is a disease characterized by chronic inflammation and fibrosis. Over the last decade, considerable attention has been paid to the origin of myofibroblasts and to the endothelial-mesenchymal transition (EndoMT) process, as responsible of fibrosis. One of the most important upstream regulators of the signal transduction pathways involved in EndoMT is the urokinase type plasminogen activator (uPA)/uPA receptor (uPAR) system. Several functions mediated by the uPA/uPAR system occur through uPAR interaction with the members of the fMLF receptor family (FPRs). We have examined (RT-PCR and Western blot analysis) FPR1, FPR2, and FPR3 expression in skin fibroblasts from ten normal subjects and from ten SSc patients. Both normal and SSc fibroblasts express FPRs at mRNA and protein level. SSc fibroblasts show an overexpression of FPRs mRNA compared to normal fibroblasts (fold increase SSc *vs* normal fibroblasts: FPR1 2.6; FPR2 5.16, FPR3 6.08) and of protein level (fold increase SSc *vs* normal fibroblasts: FPR1 4.07; FPR2 1.03, FPR3 1.45). Stimulation of fibroblasts with FPRs agonists (uPA-ATF, $uPAR_{88-92}$, and WKYKYVm peptide) induces a myofibroblast phenotype characterized by an increased expression of α-smooth muscle actin (α-SMA). SSc fibroblasts show an increased expression, as compared to normal fibroblasts, of a cleaved form of uPAR (cuPAR) containing the SRSRY sequence that binds FPRs and is involved in fibrosis and EndoMT (OD: 7.44 *vs* 1.58).

We also evaluated FPRs ability to regulate fibroblast proliferation and migration *in vitro*. Stimulation of SSc fibroblasts with specific FPRs ligands, showed lower proliferation and migration rates, compared to normal fibroblasts.

Taken together, our results indicate that FPRs are overexpressed in SSc fibroblasts and that a cross-talk between the uPA/uPAR system and FPRs could be involved in the pathogenesis of fibrosis and in fibroblast-to-myofibroblast transition.

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Rule of different memory cells in diagnosis of common variable immunodeficiency and specific antibody deficiency

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Background: Specific antibody deficiency (SAD) diagnosis relies on abnormal response to pneumococcal vaccine. The purpose of our study is to find a flow cytometric method for the early detection of SAD, before vaccine responses are evaluated.

Method: A total of 361 subjects (205 adults and 156 children) were included in study between 2010 and 2013. Patients with known immunodeficiency other than SAD and Common Variable Immune Deficiency were excluded. Subjects were divided into 3 groups (control, SAD, CVID) based on their immunoglobulin levels and their response to pneumococcal vaccines.

Results: We found that SAD and CVID have significant lower number of memory B-cells. Furthermore, the percentage of memory B-cell start out the same and increases with age in control group (correlation coefficient= 0.4, P value 0.0001) but not in CVID or SAD groups (Figure 1A). However, Memory T-helper cell increases with age in all three groups (correlation coefficient 0.71-0.78, P value 0.0001) (Figure 1B). In the control group, there was direct correlation between memory B-cell and CD4+ memory T-cell (correlation coefficient 0.39 with P value <0.0001), and this correlation was inversed in SAD (correlation coefficient -0.45 with P value of 0.007). Using CD4+ memory T-cell to memory B-cell ratio of 3.5 as a marker for SAD had specificity of 93% (Figure 2).

Conclusions: Using the ratio of CD4+ memory T-cell to memory B-cell can help identifying distinct cluster of SAD patients and future research is need to evaluate its prognostic value.

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The signal molecule, Regulator of Calcineurin 1 (RCAN1), is a potential regulator of sensitivity to anaphylaxis in humans

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Purpose: Anaphylaxis is considered the most severe syndrome of allergic diseases characterized as a reaction with sudden onset that may cause death. Many organs are involved, but vascular permeability and drop in the blood pressure seem to be a key feature. While animals model have been widely used, their relevance to humans have been questioned. Our aims were to study permeability changes and the relative importance of anaphylactogenic mediators by in vitro human models. Moreover, since Regulator of Calcineurin 1 (RCAN1) has been incriminated in inflammatory and vascular diseases, we studied its role in modulating permeability.

Methods: The direct effect exerted by immune effectors cells (mast cells (MC) and basophils) and their released molecules on the vascular wall (endothelial cells (EC) and smooth muscle cells (SMC)) were the main focus of the study: A wide range of ECs and SMCs cultures were cultured from great human saphenous veins (from surgical patients) or from microvasculature of dermal and hypodermal tissues (human abdominoplasty). Human MC were developed during 7 week cultures of CD34+ precursors isolated from PBMC of adult donors.

Functional permeability assays using FITC as a probe, molecular biology techniques and the use of murine models (including an RCAN1-/- strain) were applied to study the underlying mechanisms in vascular cells.

Results: Culture systems from macro-or micro-vasculature demonstrated important differences in the histamine-induced vascular permeability. Histamine and the neuropeptide CGRP affected mRNA and protein RCAN1 expression in EC and SMC. Subcutaneous permeability studies in RCAN1-/- mice showed a significant increase in the histamine-induced leakage. In addition, lentiviral transductions of vascular cells with constructs that modify the expression of RCAN1 were shown to decrease the vascular permeability in over-expressed RCAN1 vascular cells.

Conclusions: The cellular source of vascular cells plays an important role in different histamine-induced permeability patterns. Moreover, we have demonstrated RCAN1 modulation in vascular cells by histamine and CGRP, which may be a functional regulator of the vascular permeability processes. It is tempting to speculate that the vasculature may be conditioned to make some patients hyperreactive to mediators, but also to search for a pharmacological normalization to prevent anaphylaxis.

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Association of serum zinc concentration with allergic disorders in korean national health and nutrition examination survey

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Background: Zinc (Zn) is a dietary elements which has antioxidant, antiapoptotic, and anti-inflammatory effects. The association of Zn deficiency with allergic disease has not been fully understood. Recent observational studies suggested that lower serum levels of Zn were associated with more presence of asthma.

Objective: To evaluate the relationship between serum Zn concentration and the presence of allergic disorders.

Methods: We carried out comparison of the prevalence of asthma, atopic dermatitis and allergic sensitization against quartiles in serum Zn Levels based on data of the Korean National Health and Nutrition survey year 2010.

Results: The mean concentration of Zn was 135.54 μg/dL. Serum Zn concentration was negatively associated with self-reported history of asthma. After adjusting of age, sex, body mass index and smoking status, the subjects in bottom quartile of Zn level had higher prevalence of asthma than those at the top quartile (odd ratio, 2.81; 95% confidence interval, 1.33-5.93; P = 0.007). Interestingly, there was no significant association of Zn with asthma in the female subjects. Furthermore, the presence of atopic dermatitis as well as allergic sensitization gave insignificant difference upon serum Zn level.

Conclusion: In general population of Korea, serum Zn levels was associated with the history of asthma. These findings suggest that Zn could have an essential role in the pathogenesis of asthma.

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Human Rhinovirus-induced airway smooth muscle chemotaxis: a potential mechanism for airway remodeling in asthma

Richard Leigh and Sami Shariff

Background: Studies confirm that children with human rhinovirus (HRV)-associated wheezing episodes are at increased risk of developing subsequent asthma. Airway remodeling is a characteristic feature of asthma and can be observed in children before a diagnosis of asthma is established. This has led to the hypothesis that HRV infections may play a role in the pathogenesis of airway remodeling, and we have established that HRV infections up-regulate airway epithelial cell production of important mediators involved in remodeling processes. A feature of airway

remodeling is increased airway smooth muscle (ASM) mass with a greater proximity of the ASM to the subepithelial region, and we now interrogated the hypothesis that HRV-induced alterations of airway epithelial cell biology might regulate ASM chemotaxis.

Methods: Primary cultures of human bronchial epithelial (HBE) cells were exposed to purified HRV, UV-inactivated HRV or medium control for 24 hours. Supernatants were collected and used as potential ASM chemoattractants in a Boyden chamber and in an xCELLigence® migration system (Roche). ASM cells were also pretreated individually with pertussis toxin (PTX), an inhibitor of Gαi, the β2-receptor antagonist formoterol, the β-receptor antagonist ICI 118551, the forskolin analogue NKH-477, and the cell-permeable cyclic AMP (cAMP) analog, 8-Bromo-cAMP, to establish the role of cAMP signaling in HRV-induced ASM chemotaxis. Finally, multiplex assays were done to identify potential chemoattractants in the HRV-infected HBE cell supernatants.

Results: Supernatants from HRV-infected HBE cells resulted in greater ASM chemotaxis compared to control supernatants in both Boyden chamber ($p<0.01$) and xCELLigence® experiments ($p<0.001$). This chemotaxis was concentration- and time-dependent ($p<0.05$), with maximal migration occurring at 4 hours. Additionally, ASM chemotaxis was attenuated by elimination of the chemoattractant gradient ($p<0.001$), pre-treatment of the ASM cells with PTX ($p<0.05$), formoterol ($p<0.05$) NKH-477 ($p<0.01$) or 8-Bromo-cAMP ($p<0.001$). Data from the multiplex assays identified a number of HRV-induced chemokines released from the HBE cells that could act as ASM chemoattractants.

Conclusions: These data provide robust evidence that HRV infection of HBE cells produces chemoattractants that cause directional ASM migration, and provide further support for a role of HRV infection in the pathogenesis of airway remodeling in asthma.

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Diverse allergens require MD2-mediated neutrophil recruitment to mount allergic airway inflammation

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Background: Pollen exposure exacerbates allergic disorders of the airways like allergic rhinitis and asthma through direct activation of the innate responses in addition to IgE mediated Fcε crosslinking. However, the innate receptor(s) that mediate the pollen response(s) have not yet been identified.

Methods: The signaling and cytokine and oxidative stress responses induced by variety of allergenic-extracts was determined in cell lines that either lack or possess surface TLR4 with or without MD2 were determined. The lung inflammation induced by ragweed pollen extract or cat dander was quantified in wild-type, TLR4-null mice, and wild-type mice treated in vivo with siRNA to TLR4 or MD2. Human nasal challenge were performed with RWPE

Results: Here we demonstrate that Toll-like receptor 4 (TLR4) is essential for selected pollen allergenic extracts to induce rapid reactive oxygen species (ROS), activate NF-κB, and induce CXCR signaling-linked neutrophil recruitment, sustained ROS, epithelial mucin secretion and recruitment of eosinophils. We show that myeloid differentiation protein-2 (MD2) is essential for TLR4 to mount this innate response, whereas CD14 is dispensable. In the presence of MD2, pollen-activated TLR4 recruits TRAF6 to the signaling complex, induces CXCL chemokine synthesis, and facilitates neutrophilic inflammation. Cat allergen extract also requires MD2 to mount TLR4-linked neutrophil recruitment to the lungs of mice.

Conclusions: These observations indicate that diverse allergens require MD2 to initiate innate signaling and mount allergic inflammation. Inhibition of this innate signaling pathway may be a general strategy to prevent allergic inflammation.

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T cell induced-bronchoconstriction in vitro and in vivo

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Background: IgE-independent, T cell-dependent bronchoconstriction was investigated using an *in vitro* contraction assay. In the mice, T cell-transfer model was analyzed for immediate and late phase asthmatic responses upon antigen challenge.

Methods: Peripheral blood mononuclear cells (PBMC) obtained from nonatopic asthmatics were cultured with secreted aspartic proteinase 2 (SAP2), a purified protein derived from *Candida albicans.* Cytokine production was measured by specific ELISAs. A contraction assay using collagen gels embedded with cultured human bronchial smooth muscle cells (BSMCs) was employed to analyze the contractile responses induced by the culture supernatants. Ovalbumin (OVA) specific Th clones were derived from either the regional lymphnodes of Balb/c mice immunized with OVA/CFA or splenocytes of DO11.10 transgenic mice expressing T cell receptor specific for OVA/H-2^d. Th clones were adoptively transferred into unprimed mice. Upon antigen challenge, airway resistance was continuously monitored by either unrestrained whole body plethysmography (BUXCO) or resistance/compliance analyzer under anesthetized condition. Supernatants of stimulated Th clones were analyzed for contractile activity using collagen gels embedded with murine primary BSMCs.

Results: PBMC obtained from nonatopic asthmatics produced significant amount of IL-5, IL-13, and IFN-gamma upon incubation with SAP2 (responders). Upon bronchial challenge with SAP2,

late but not immediate bronchial response was induced for the responders. Neither IAR nor LAR was detectable for the control asthmatics (nonresponders). IgE-dependent mechanism was ruled out by negative RAST, histamine releasing test, or immediate skin reaction. Anti-SAP2 IgG antibody (precipitin) was not detectable. The supernatants of PBMC cultured with SAP2 induced a significant contraction of the BSMC gels. When unprimed mice were transferred with Th clones, T5-1, T6-2, T6-4, and T6-7, Penh values were significantly increased 6 hr after OVA or OVA323-339, a T cell epitope peptide, challenge. Airflow limitation was confirmed by a direct measurement of airway resistance under anesthetized, restrained, and intubated conditions. Contractile activity was detected in the supernatants of T6-2 stimulated with immobilized anti-CD3 antibody.

Conclusions: Activation of Th cells resulted in an airflow limitation in addition to eosinophilic inflammation, bronchial hyperresponsiveness, and mucous hyperplasia. T cell-derived bronchoconstrictor might be a target for treatment-resistant asthma.

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Reduced anti-viral innate immunity in severe asthma is associated with neutrophilic inflammation and high dose inhaled steroids

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Background: People with asthma are prone to viral respiratory infections and this has been linked to immune dysfunction. Though phenotypic heterogeneity is increasingly recognised in asthma, it is not clear whether impaired anti-viral immunity is present in all asthmatics, or only a subset. This study examined the hypothesis that immune dysfunction in asthma varies across different inflammatory phenotypes.

Methods: Innate immune responses to human rhinoviruses were examined in 85 adults with poorly controlled asthma (mean age 59 years, 63% female, mean Asthma Control Questionnaire (ACQ)-6 score = 1.7 ± 0.8). Blood mononuclear cells were cultured with two strains of HRV and cytokines measured by ELISA. Inflammatory phenotypes were characterized within induced sputum as eosinophilic (eosinophils >3%), neutrophilic (neutrophils >61%), paucigranulocytic (eosinophils <3% and neutrophils <61%) and mixed granulocytic (eosinophils >3% and neutrophils >61%).

Results: Human rhinovirus stimulated IFNα release (median (IQR) pg/ml; P value) at 24h was significantly lower in those with neutrophilic asthma (n=12; 55.2 (22.4, 265) pg/ml), than in those with either eosinophilic (n=35; 606 (281,1114) pg/ml ;P<0.01) or pauci-granulocytic asthma (n=35; 437 (212,1000) pg/ml; P<0.01) and not different to those with mixed granulocytic asthma (n=4; 61.4 (36.0,84.0) pg/ml; P=0.804). HRV-stimulated IL-1β, IL-6 and IL-8 synthesis did not vary across asthma phenotypes. Basal (unstimulated) release of IL-1β and IL-8 was associated with ACQ scores across all patients. Multivariate analysis demonstrated HRV stimulated IFNα release was independently associated with sputum neutrophil proportion and inhaled corticosteroid dose but not age, gender, smoking status, FEV1% predicted or ACQ-6.

Conclusions: In severe asthma, anti-viral innate immune dysfunction is most evident in those with neutrophilic airway inflammation. The extent to which this predicts subsequent risk of viral infections and asthma exacerbations remains to be determined.

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Regulation of proteinase activated receptor-2 (par-2) on airway epithelium

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Introduction: Aeroallergens are major triggers of asthma. Many of these aeroallergens can activate PAR-2 receptors on the airway epithelium. We have shown that PAR-2 activation participates in allergic sensitization and allergic airway inflammation in animal models of asthma. Moreover, PAR-2 is upregulated on the airway epithelium of asthmatics, but the mechanisms and factors responsible are unknown. The asthmatic airways are under constant influence of inflammatory mediators and oxidative metabolism products. Furthermore inflammation causes tissue hypoxia and nutrient deficiency. Thus we hypothesized that the PAR-2 expression on the airways in asthma is regulated by cellular stress.

Methods: To study the effect of growth factor deprivation on PAR-2 expression, Normal Human Bronchial Epithelial (NHBE) cells were deprived of media supplements (growth factors), all of them or individual factors separately, for 24-48hrs and PAR-2 mRNA levels were studied by qPCR. Functional effects of PAR-3 expression changes were studied by activating cells with PAR-2 activating peptides and measuring intracellular Ca responses and IL-8 release.

Results: Growth factor deprivation-induced stress upregulated PAR-2 mRNA (2.25 +/- 0.2 fold, n=6) in NHBE. Stress-induced PAR-2 upregulation was reversible after re-addition of growth factors and was not due to alterations of mRNA stability. Growth factor deprivation also increased expression of PAR-1. Cells deprived of growth factors for 24hrs showed upregulated PAR-2-mediated IL-8 release (2.1 +/- 0.2 fold, n=6) compared to control cells. Hypoxia and oxidative stress did not modulate PAR-2 expression.

Conclusions: Growth factor deprivation could be the driving force for PAR-2 upregulation in asthmatic airways. Activation of this upregulated PAR-2 can perpetuate inflammation by releasing higher levels of inflammatory mediators. Understanding PAR-

2 regulation will allow us to device approaches to decrease PAR-2 expression and prevent exacerbation of allergic airway inflammation.

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Interleukin 33 exacerbates allergic airway inflammation and bronchoconstriction via modulation of mast cell responses

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Background: Asthma is a chronic disease of the airways characterized by hyperresponsiveness, inflammation and remodeling. Polymorphisms in the genes for interleukin 33 (IL-33), it's receptor IL1RL1, or other proteins in the downstream signaling of the IL-33 pathway have recently been associated with the development of asthma and disease severity. In this study, we provide data indicating that IL-33 has the property to exert strong effects together with antigen on airway hyperresponsiveness (AHR), bronchonstriction and inflammation.

Methods: We have studied the effect of IL-33 on airway inflammation, AHR and early allergic reaction (EAR) in mice sensitized to OVA. The effects of IL-33 have been investigated in vivo where AHR and EAR were measured using flexiVent; ex vivo on isolated airways and in vitro on mast cells.

Results: Our data demonstrates that IL-33, acting through its receptor ST2, increases mast cell synthesis, storage and secretion of serotonin, which aggravates allergic airway smooth muscle contractions. We also found that IL-33 strongly exaggerates the allergen induced AHR and inflammation.

Conclusions: We have identified a new mechanism which may have important implications for airway obstruction in asthma and IL-33 as a potential target for therapeutic intervention for asthma sufferers.

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A role for mast cell tryptase in histone modification

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The current paradigm is that secretory granule compounds are destined for extracellular functions following cellular degranulation. Here we challenge this notion by showing that tryptase, a serglycin proteoglycan-dependent granule-localized mast cell protease, also can influence cell-intrinsic processes. In addition to its granular localization, we show that tryptase is found in the nucleus of viable cells where it cleaves off N-terminal tails of core histones, thereby removing sites for epigenetic posttranslational modifications and affecting chromatin condensation. During mast cell apoptosis, extensive tryptase-dependent core histone degradation was seen, the absence of tryptase leading to histone accumulation in the cytosol and histone release. Together, these findings introduce a novel concept of core histone modification, mediated by a secretory granule-derived axis.

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The formyl peptide receptor 1 exerts a tumor suppressor role in human gastric cancer by suppressing angiogenesis

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Background & Aims: *N*-formyl peptide receptors (FPR1, FPR2, and FPR3) are involved in innate immunity, inflammation, and cancer. A strong association between FPR1 polymorphisms and stomach cancer has recently been described. We previously showed that FPRs are expressed on gastric epithelium and are required for wound repair and restitution of barrier integrity. We have assessed the role of FPRs in gastric cancer (GC).

Methods: We have characterized the functions of FPRs in GC epithelial cells in culture (MKN28 and AGS) by assessing migration (Boyden chamber, and wound healing assay), proliferation (BrdU incorporation), resistance to apoptosis (TUNEL assay), and activation of the epithelial-to-mesenchymal transition (PCR and Western blot). We have also defined the tumorigenicity of GC epithelial cells silenced for FPRs by xenotransplant in immunocompromised (athymic and SCID) mice.

Results: Activation of each FPR induced the epithelial-to-mesenchymal transition, proliferation, resistance to apoptosis, and migration of GC cells in culture. Specific antagonist or RNA interference of each FPR reverted these effects. shFPR1 GC cell xenografts in both athymic and SCID mice exhibited increased tumorigenicity, decreased necrotic areas, and increased microvessel density compared with shCTR, shFPR2 and shFPR3 cells. HIF-1α and VEGF mRNA levels were higher in shFPR1 xenografts than in controls. Consistently, the production of proangiogenic factors in response to an FPR2/3 agonist or the proinflammatory mediator (IL-1α) was higher in shFPR1 GC cells than in shCTR, shFPR2 and shFPR3 cells, suggesting that FPR1 inhibits GC angiogenesis.

Conclusions: Our data indicate that FPR1 acts as a suppressor of GC angiogenesis. FPR1 stimulation may represent a novel therapeutic approach to counteract tumor angiogenesis.

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Evolution of antibody responses to allergenic molecules in childhood and implications for immunological intervention

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Objective: To investigate the role of route and dose of exposure in the evolution of IgG and IgE responses to recombinant PR.10 molecules.

Methods: The German Multicentre-Allergy-Study examined a birth cohort born in 1990. Blood samples were collected at age 1,2,3,5,6,7,10,13 years. Participants were included in the present analysis if they had 1)

at least 1 serum sample at each of the 4 age periods or time points: 1y-3y, 5y-7y, 10y, and 13y and 2) IgE responses to birch (birch-atopics) or no IgE response at all to nine common aero- and food-allergens (nonatopics). Therefore, serum IgE antibodies to a panel of four airborne and five foodborne extracts as well as to Bet v1 were measured in singleplex, while IgG and IgE antibodies to a panel of 3 airborne PR.10 molecules

(rBet v 1, rAln g 1, rCor a 1.0101) and 8 foodborne PR.10 molecules (rCor a 1.0401, rMal d 1, rPru p 1, rGly m 4, rAra h 8, rApi g 1, rDau c 1) were tested by multiplex microarray.

Results: In the present analyses, we included 28 birch-atopic children and randomly selected 28 non-atopic children from the 190 fulfilling the inclusion criteria. Two different patterns of IgG responses to PR.10 molecules could be identified. Among non-atopic subjects, a "default" IgG response was directed mostly against foodborne PR.10, started often before age 2y, stayed weak and mostly transient. Among all atopic subjects, the "default" IgG response at age 1y was overwhelmed after age 2y by an "atopic" IgG response, which started together or shortly before the IgE response, being intense and persistent. This "atopic" IgG response, as well as the IgE response, involved progressively most foodborne PR.10 with a frequency and levels related to their homology with Bet v 1.

Conclusions: The results suggest that children develop a "default" antibody response to PR.10 molecules which is early, weak, transient, not involving IgE and initiated by foodborne PR.10. By contrast, an "atopic" antibody response to PR.10 molecules is delayed, strong, persistent, involving both IgG and IgE, and initiated by airborne PR.10 molecules.

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In vivo effects of silica crystals on airway inflammation in mice

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Background: Silica crystals (Silica) are the main mineral component of volcanic ash and desert dust. Exposure to silica-containing dust increases the risk of exacerbation in patients with chronic respiratory diseases such as asthma and COPD. We previously reported that silica induced cell injury to normal human bronchial epithelial cells *in vitro* only when cells were simultaneously exposed to poly I:C, but not to LPS or TNF-α.

Objective: We investigated the *in vivo* effects of silica inhalation in mice.

Methods: Silica (median particle size: 5 μm; 500 μg/day) and/or poly I:C (20 μg/day) were intranasally administered to C57BL/6 mice for 3 consecutive days (once per day), and histopathological changes and airway inflammation were evaluated on day 4.

Results: Histopathological analysis revealed that Silica or poly I:C alone induced marginal airway inflammation, whereas their combined administration significantly enhanced both neutrophilic infiltration and epithelial damage in the airway, without IL-1β or IL-17 release into the BALF. The combined administration synergistically enhanced the levels of such inflammatory cytokines as IL-6, TNF-α, IFN-γ, HMGB-1 and a neutrophil chemokine, CXCL1/KC, in the BALF. However, neither IL-1β nor activated caspase-1 proteins were detected in the lung tissues by Western blotting.

Conclusions: Our results suggest that inhalation of silica-containing dust may cause neutrophilic airway inflammation, presumably by damaging the epithelial barrier, especially at the time of viral infection, but not through inflammasome activation. These responses may also be involved in acute lung injury induced by inhalation of silica-containing dust.

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Effects of a new potent PARP-1/PARP-2 inhibitor in an *in vivo* murine model of bleomycin-induced lung fibrosis

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Background: Pulmonary fibrosis is a progressive and lethal illness characterized by inflammation, excessive deposition of collagen and abnormal remodeling of lung parenchyma. This results in a progressive airway stiffening and thickening of the air-blood membrane, which make breathing difficult and eventually lead to respiratory failure. Pulmonary fibrosis is the end stage of a wide range of lung inflammatory conditions. Among the fibrotic disease of the lungs, idiopathic pulmonary fibrosis is the most common one. There is no effective therapy available, therefore, novel therapeutic strategies are urgently required, including molecular targeting of specific signaling pathways activated during fibrotic processes [1].

Poly(ADP-ribose)polymerases (PARPs) are a family of enzymes that catalyzes the covalent attachment of poly(ADP-ribose) from NAD to target proteins. PARP-1, the most abundant enzyme located in cell nuclei, modulates the expression of inflammatory genes.

Method: We investigated the effects of a selective PARP-1/PARP-2 inhibitor, HYDAMTIQ, in an *in vivo* murine model of bleomycin-induced lung fibrosis. C57BL/6 mice were treated intra-tracheally with bleomycin (0.05 IU). Animals received HYDAMTIQ (3 and 10

mg/kg b.wt) or saline i.p. for 21 days. Airway resistance to inflation (PAO) was assayed and lung tissue was removed and processed for histology and for evaluating the production of oxidative stress (8-OHdG), pro-inflammatory and pro-fibrotic cytokines (IL-1β, TNF-α and TGF-β) production, as well as for collagen deposition, percentage of positive goblet cells and thickness of smooth-muscle layer in small bronchi.

Results: Our results indicate that HYDAMTIQ exerts an anti-inflammatory and anti-fibrotic effect, as shown by the significant decrease of inflammatory cytokine (TNFα, IL-1β) production. Moreover, HYDAMTIQ reduces goblet cell relative number, collagen deposition and pro-fibrotic cytokine (TGF-β). These effects are accompanied by a decrease of PAO, an index of lung stiffness. Furthermore, it decreases the oxidative stress, reducing 8-OHdG, a marker of DNA oxidation.

Conclusions: These results suggest that PARP inhibitors could be a promising approach to evaluate the possible anti-fibrotic potential of these molecules. We hypothesize that new potent and selective PARP-1 inhibitors (such as HYDAMTIQ, a derivative of TIQA) can reduce the signs and symptoms of lung fibrosis and control post-inflammatory bronchial remodeling and hyper-responsiveness.

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Interleukin-32 attenuates collagen production in fibroblasts via modulation of focal adhesion kinase signaling

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Background: Airway fibrosis is critical pathogenic process in development of severe asthma with fixed airway obstruction. However, not much is understood about the pathogenesis of airway fibrosis and its prevention is yet to be solved. The objective of this study was to investigate the role of IL-32γ in regulation of peribronchial fibrosis.

Method: For in vitro studies, human lung fibroblasts (MRC-5) and airway epithelial cells (BEAS-2B) were used. To generate chronic asthma model, C57BL6 mice were immunized with ovalbumin and fungal protease twice a week for 4 weeks via nasal route. To generate lung fibrosis model, bleomycin (1U/kg) was treated via tracheal route in human IL-32γ transgenic mice.

Results: Treatment of human recombinant IL-32γ reduced peribronchial collagen deposition in chronic asthma model. Moreover, Human IL-32γ overexpression prevented collagen deposition in bleomycin-induced lung fibrosis model. IL-32γ also attenuated production of fibronectin, collagen, and α-smooth muscle actin in TGFβ1 stimulated lung fibroblasts in vitro. IL-32γ regulated expression of extracellular matrix proteins in lung fibroblasts independent of both TNF-α signaling and SMAD pathway. Interestingly, IL-32γ attenuated phosphorylation of focal adhesion kinase (FAK) paxillin, which otherwise were significantly increased in TGFβ1 stimulated fibroblasts.

Conclusions: IL-32γ attenuated collagen production in lung fibroblasts partly via modulation of FAK signaling. Il-32γ could be a treatment target for prevention of airway fibrosis in asthma.

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"Auto-anti-IgE": naturally occurring IgG anti-IgE antibodies may inhibit allergen-induced basophil activation

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Background: Naturally occurring IgE-specific IgG autoantibodies have been identified in patients with asthma and other diseases, but their spectrum of functions is poorly understood.
Objective: Address the hypothesis that: (i) IgG anti-IgE autoantibodies are detectable in the serum of all subjects but elevated in asthmatics regardless of atopic status as compared with controls; (ii) some activate IgE-sensitised basophils; (iii) some inhibit allergen-induced basophil activation.

Methods: IgE-specific IgG autoantibodies were detected and quantified in sera using ELISA. Sera were examined for their ability to activate IgE-sensitised human blood basophils in the presence and absence of allergen using a basophil activation test, and to inhibit binding allergen to specific IgE on a rat basophilic cell line stably expressing human FcεRI.

Results: IgG autoantibodies binding to both free and FcεRI bound IgE were detected in patients with atopic and non-atopic asthma as well as controls. While some were able to activate IgE-sensitised basophils, others inhibited allergen-induced basophil activation, at least partly by inhibiting binding of IgE to specific allergen.

Conclusion: Naturally occurring IgG anti-IgE autoantibodies may inhibit, as well as induce basophil activation. They act in a manner distinct from therapeutic IgG anti-IgE antibodies such as omalizumab. They may at least partly explain why atopic subjects who make allergen-specific IgE never develop clinical symptoms, and why omalizumab therapy is ineffective in a subset of atopic asthmatics.

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Of mice and not men: Vitamin D3 differentially induces TSLP in mouse and human skin

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Background: Thymic stromal lymphopoietin (TSLP) plays an important role in allergic diseases and is highly expressed in keratinocytes in human lesional atopic dermatitis (AD) skin. In nonlesional AD skin TSLP expression can be induced by applying house dust mite allergen onto the skin in the atopy patch test. Several studies have demonstrated that the induction of TSLP expression in mouse skin does not only lead to AD-like inflammation of the skin, but also predisposes to severe inflammation of the airways. In mice, TSLP expression can be induced by application of the 1,25-dihydroxyvitamin D3 (VD3) analogue calcipotriol and results in the development of eczema-like lesions.

Aim of the study: To investigate the effect of VD3 (calcitriol) or calcipotriol on TSLP expression in human skin and compare this to cynomolgus monkey skin and mouse skin.

Methods: Using multiple ex vivo experimental setups, the effects of calci(po)triol on TSLP expression by normal human primary keratinocytes, normal human skin, and skin from AD patients were investigated and compared to effects of calcipotriol on mouse and cynomolgus monkey skin.

Results: No induction of TSLP expression (mRNA or protein) was observed in human keratinocytes, normal human skin, nonlesional AD skin or cynomolgus monkey skin samples after stimulation with calcipotriol or topical application of calcitriol. The biological activity of calci(po)triol in human skin samples was demonstrated by the increased expression of the VD3-responsive Cyp24 gene. TSLP expression was induced by pro-inflammatory cytokines (IL-4, IL-13 and TNFα) in skin samples from all three species. In contrast to the findings in (non-) human primates, a consistent increase in TSLP expression was confirmed in mouse skin biopsies after stimulation with calcipotriol. *In silico* analysis revealed that the architecture of the VD3 responsive elements in the human TSLP was too different from those in the mouse promoter to allow extrapolated to humans.

Conclusion: In contrast to mouse models, VD3 failed to induce expression of TSLP in human or monkey skin.

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What can we learn from East-West German reunification about causes of the allergy epidemic?

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Background: The surprising finding of lower hay fever and asthma prevalence rates in East compared to West Germany at the time of reunification in 1990 gave rise to epidemiological studies showing a steep increase of allergy prevalence in East Germany in the following ten years. This phenomenon may be considered as a model for explaining the allergy epidemic in the "Western" world. Whether common patterns of risk factors were observed in all the comparison studies performed and for various allergic manifestations has not yet been profoundly studied.

Methods: Here we investigated 14 cross-sectional studies after 1989 with parallel investigations in East and West German preschool or school children, describing prevalence rates of asthma, hay fever, eczema and allergic sensitization. The prevalence ratios for West/ East comparison were calculated and compared and the explanatory power of specific known risk factors with regard to exposure to outdoor or indoor factors, early childhood influences, nutrition as well as awareness is given.

Results: Throughout all the studies the largest West-East differences were found for hay fever and sensitization against birch pollen at the time of reunification and showing a steep increase in prevalence in East Germany in the following decade. Single room heating with fossil fuels as well as living as an only child in a family were partly explanatory factors in this pattern. Eczema showed the opposite pattern with a higher prevalence in East Germany. Early child care was associated with more eczema and explained the East/West differences.

In single studies interesting co-factors like outdoor traffic exposure or lack of pertussis vaccination were found, however, they were not consistently studied in all 14 studies.

Hay fever as the most typical atopic disease showed the most significant difference in allergy pattern between East and West Germany. The risk factors identified differ profoundly from classical risk factors for airway diseases as e.g. "single child" or "single room heating". The pattern of skin manifestation of atopy differs from airway atopic disease.

The factors identified most likely represent only proxies for some still unknown final cause for the increase of allergy prevalence in our time.

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Histamine 4 receptor stimulation modulates the differentiation process and chemokine release in human monocyte derived macrophages

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Histamine is an important mediator of biological functions and present in high amounts in inflammatory skin lesions. Such skin lesions evoke a migration of monocyte precursor cells, in part via histamine induced chemotaxis, into the inflamed tissue whereby they differentiate into macrophages. The expression and function of the histamine receptors, especially the histamine H4 receptor (H4R), has already been determined on subtypes of antigen presenting cells in our previous studies. However, the effects of histamine on macrophages which are present in allergic inflammation in many organs seems rather unclear.

The aim of this study was to assess a functional role of the histamine receptors, with focus on the histamine H4 receptor, on these professional phagocytes. First we could show that polarized M1 and M2 macrophages express the H1R, the H2R and the H4R but not the H3R on mRNA level. On M2 macrophages we observed an up-regulation of the H1R and H4R upon activation with IL-4. Interestingly we could show that the phenotype of M1 and M2 macrophages was significantly altered when H4R ligands were added continuously to the media during the period of differentiation. A significant up-regulation of the subtype specific surface marker CD68 and a down-regulation of CD163 were detected by flow cytometry in response to treatment with the H4R agonist.

Furthermore fully differentiated macrophages were stimulated and the cell free supernatants were analyzed by ELISA. When stimulated with IFN-γ and LPS in the presence of histamine or a H4R agonist, macrophages produced substantially lower amounts of the interferon-γ induced protein 10 (IP-10, CXCL10) and MIP-1ß (CCL4).

In conclusion we could show that the H4R is functionally expressed on activated macrophages. The H4R induced down-regulation of chemokines in macrophages which rather attract cells expressing TH1 associated cytokines may polarize the cytokine milieu towards TH2 in situ. This may also have implications for the treatment of allergic diseases.

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Histamine and histamine receptor expression in mucosal inflammatory diseases

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Background: Histamine is a key immunoregulatory mediator in both immediate type hypersensitivity reactions and chronic inflammatory responses. Toll-like receptors (TLR) recognize bacterial ligands and histamine alters TLR signaling cascades, in particular via histamine receptor 2 (H2R). However, the influence of histamine on TLR responses in cells from inflamed patients has been poorly described.

The aim of this project is to investigate the effects of histamine on TLR stimulated PBMCs isolated from patients suffering from inflammatory bowel disease (IBD) and asthma.

Method: Forty eight patients diagnosed with IBD (24 with Crohn's disease and 23 with ulcerative colitis), ten asthma patients and twenty three healthy volunteers were included in the study. Expression of histamine receptors (H1, H2, H4R), histamine-related enzymes (HDC, HNMT and DAO) as well as TLR-1, TLR-2, TLR-4, TLR-5 and TLR-9 was investigated using REAL-TIME PCR. Cytokine levels were determined in culture supernatants after 24-hour Pam3-Cys (TLR-2 ligand) or LPS (TLR-4 ligand) stimulation in the presence or absence of histamine or famotidine (H2R antagonist). T cell, B cell and monocyte expression of histamine receptors was evaluated by flow cytometry.

Results: Significant differences in H1R, H4R, TLR-6, TLR-9 and DAO gene expression were observed in patients with IBD compared to healthy volunteers, while HDC and H1R expression was altered in PBMCs from asthma patients. Histamine altered TLR-induced IL-12, TNF-α, G-CSF, IFN-γ, MCP-1, IL-1β, IL-6 and GM-CSF secretion from healthy volunteers, while similar histamine effects on TLR-induced cytokine responses in IBD patients was only observed for TNF-α and G-CSF. Histamine mediated these effects primarily through the H2R as famotidine negated the differential response. The percentage of H2R+ monocytes was significantly reduced for IBD patients, compared to healthy volunteers.

Conclusions: Patients with IBD and asthma display dysregulated expression of TLRs and histamine receptors. The anti-inflammatory influence of H2R signaling on TLR-2 innate immune response is abrogated in IBD patients, suggesting that deliberate manipulation of H2R-signalling may provide beneficial effects to patients with IBD.

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The effect of oral challenge with wheat protein in mice systemically or percutaneously sensitized with hydrolyzed wheat protein

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Introduction: Recently, a hydrolyzed wheat protein (HWP) is reported to induce IgE-mediated hypersensitivity by percutaneous sensitization and/or food ingestion of wheat protein, although it has not yet been examined whether wheat or HWP can induce allergic responses after intraperitoneal or percutaneous sensitization with HWP.

Objective: To address this issue, we attempted to establish a mouse model of food allergy.

Methods: Female BALB/c mice were sensitized by intraperitoneal injection or percutaneous exposure to HWP, glupearl 19S, in the back skin for 3 days per week for 4 weeks. Ten days after second injection or 4 days after the epicutaneous sensitization, mice were challenged orally with gluten 3 times per week for 3 weeks. In some groups, mice were given orally with aspirin 1 hour before oral challenge with gluten for accelerating gluten absorption.

Results: A decrease in rectal temperature or anaphylactic death was not observed in the group challenged orally with gluten. In contrast, oral administration of gluten with aspirin induced a significant decrease in rectal temperature and led to some cases of anaphylactic shock within 24 hours after the oral challenge.

Conclusion and implication: These findings demonstrate that percutaneous sensitization with a hydrolyzed wheat protein and oral challenge with gluten and aspirin induces anaphylactic response, and suggest that the protein has high antigenicity by acidification and hydrolysis with heat during the manufacturing process.

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Advertisements impact the physiological efficacy of a branded drug

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We conducted randomized clinical trials to examine the impact of direct-to-consumer advertisements on the efficacy of a branded drug. We compared the objectively measured, physiological effect of Claritin (Merck & Co.), a leading antihistamine medication, across subjects randomized to watch a movie spliced with advertisements for Claritin or advertisements for Zyrtec (McNeil), a competitor antihistamine. Among subjects who test negative for common allergies, exposure to Claritin advertisements rather than Zyrtec advertisements increases the efficacy of Claritin. We conclude that branded drugs can interact with exposure to television advertisements.

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Hyperexpression of Mas-related gene X2 (MrgX2) in skin mast cells of severe chronic urticaria patients; MrgX2 is a receptor for basic eosinophil granule proteins

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Background: Chronic urticaria (CU) is defined as the occurrence of systemic daily wheals for at least 6 weeks. CU patients reportedly exhibit enhanced wheal reactions, compared with healthy control subjects, when some neuropeptides such as substance P (SP) and vasoactive intestinal peptide (VIP) are intradermally injected. Recently, MrgX2 was found to be a receptor for basic peptides including SP and VIP, in human cord blood-derived cultured mast cells (MCs). In CU patients, eosinophil infiltration is commonly observed; the marked deposition of major basic protein (MBP) and eosinophil cationic protein in CU lesions have provided evidence that eosinophil granule proteins are released and could contribute to local inflammatory processes in patients with CU either directly or indirectly via other inflammatory cells.

Aims: We sought to compare the frequency of MrgX2 expression in skin MCs from CU patients and non-atopic control (NC) subjects and to identify the receptor for basic eosinophil granule proteins on human skin MCs.

Methods: Severe CU was defined as a UAS7 score of greater than 30. MrgX2 expression was investigated using immunofluorescence in skin tissues from NC subjects (n = 13) and severe CU patients (n = 9) and on skin-derived cultured MCs. MrgX2 expression in human MCs was reduced using a lentiviral shRNA silencing technique. Ca^{2+} influx was measured in CHO cells transfected with MrgX2 in response to eosinophil granule proteins. Histamine and prostaglandin D2 were measured using enzyme immunoassays.

Results: The number of $MrgX2^+$ MCs was significantly higher in skin tissues from patients with severe CU (116 ± 19.0/mm^2) than in those from NC subjects (68.5 ± 51.1/mm^2, $P < 0.001$). The percentage of $MrgX2^+$ MCs in all the MCs in the severe CU patients (47.0% ± 6.9%) was significantly higher than that in the NC subjects (21.6% ± 7.8%, $P < 0.001$). Eosinophil infiltration in the urticaria region was observed in 7 out of 9 severe CU patients. The colocalization of MCs and eosinophils was observed in the urticaria region in the CU patients. Substance P, MBP and eosinophil peroxidase, but not eosinophil derived neurotoxin, induced histamine release from skin-derived MCs via MrgX2.

Conclusion: MrgX2 may be a new target molecule for the treatment of wheal reactions in severe CU patients.

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Enhanced expression of vasoactive mediators (CGRP, VEGF and IL-25) and increased vascularity in weals from skin biopsies in chronic spontaneous urticaria

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Background: The mechanisms of vascular leakage ("wealing") and the role of angiogenesis in chronic spontaneous ("idiopathic") urticaria (CSU) are incompletely understood. Several vasoactive mediators including CGRP (a potent vasodilator), VEGF (a leakage and angiogenic factor) and IL-25 (involved in local angiogenesis and vascular remodelling) have been identified in allergic inflammation.

Objective: To study new blood vessel formation and the expression of CGRP, VEGF and IL-25 in weals from skin biopsies from patients with CSU.

Methods: Eight paired biopsies (one from 4- 8 hour spontaneous weals and one from uninvolved skin) were taken from eight patients with CSU and nine control subjects and studied by immunohistochemistry and confocal microscopy.

Results: There were small but significant increases in CD31+ endothelial cells (p=0.04: approximate 50% increase) and CD31+ blood vessels (p=0.02; approximate 50% increase) when lesional was compared with non-lesional skin.There were also significant increases in cells positive for CGRP (p=0.02: approximate 3 fold increase) and VEGF (p=0.01: approximate 30% increase) when lesional was compared to non-lesional CSU skin. No significant differences were observed in CGRP and VEGF expression between non-lesional skin and controls. In lesional skin, VEGF and CGRP co-localised to UEA-1+ blood vessels. CGRP was also expressed by neutrophils and eosinophils and to a lesser extent by CD90+ fibroblasts, mast cells, CD3+ and CD68+ cells. IL-25+ cells were significantly elevated in the dermis of lesional, as compared to both non-lesional, skin (p=0.011) and control (p=0.001) biopsies and co-localised principally to CD31+ epithelial cells, MBP+ eosinophils and tryptase+ mast cells.

Conclusions: Several vasoactive mediators, including CGRP, VEGF and IL-25, may contribute to the wealing and increased vascularity in CSU lesions of several hours duration and are therefore potential therapeutic targets.

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Expression of IL-33 in lesional skin in chronic spontaneous urticaria

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Background: Chronic spontaneous ("idiopathic") urticaria (CSU) is a common disease associated with recurrent weals and/or angioedema resulting from persistent activation of skin mast cells by unknown mechanisms. We previously demonstrated expression of Th2 (IL-4+, IL-5+) cells in skin biopsies from patients with CSU (Ying et al, JACI, 2004). Interleukin (IL)-33, an alarmin, is released largely through innate immune mechanisms and induces both Th2 cell production and mast cell activation.

Objective: To determine whether IL-33 is expressed in CSU skin and to compare weals with uninvolved skin and a control group.

Methods: Eight paired biopsies (one from 4- 8 hour spontaneous weals and one from uninvolved skin) were taken from eight patients with CSU and nine control subjects and studied by immunohistochemistry and confocal microscopy.

Results: We confirmed that Th2 (IL-4+, IL-5+ and IL-13+) cells were expressed in CSU, but not in controls. There were no quantitative differences between involved and uninvolved skin. In contrast IL-33+ cells were significantly elevated in the dermis of lesional as compared to both non-lesional skin (p=0.002) and control (p=0.001) biopsies. In the dermis IL-33 co-localised principally to CD31+ epithelial cells, CD90+ fibroblasts, CD68+ macrophages and tryptase+ mast cells. In the epidermis IL-33 was constitutively expressed both in control skin and biopsies from CSU. However, there was significantly less immunofluorescence in all layers (stratum basale (p=0.04), spinosum (p=0.003) and granulosum (p=0.001) when lesional and non-lesional skin were compared with controls.

Conclusions: IL-33+ cells are localised in the dermis of weals from CSU skin and may account, at least in part, for mast cell activation. In the epidermis involved and uninvolved skin both contained less IL-33 than controls. These results raise the possibly that in CSU stimulation of innate pathways may lead to IL-33 expression and subsequent mast cell activation.

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Aspirin-exacerbated cutaneous disease: A subphenotype of chronic urticarial

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Background: A subset of patients suffering chronic urticaria (CU) experience disease exacerbations after receiving nonsteroidal anti-inflammatory drugs (NSAIDs). This condition has been designated as Aspirin-Exacerbated Cutaneous Disease (AECD).

Objectives: The purpose of this study was twofold: 1. Investigate the demographic and clinical features of patients affected by AECD. 2. To compare those characteristics between patients with AECD and NSAID-tolerant subjects with chronic urticaria.

Methods: Patients with AECD and a group of unselected patients with CU tolerant to NSAIDs were studied. Demographic and clinical data were obtained by direct questioning and physical examination done by experienced allergists. Laboratory

investigations and immediate-type skin tests with allergens were performed only in selected patients, as guided by the medical history.

Results: Out of 423 patients with CU admitted in the clinics, 52 (12.2 %) had AECD. Compared with NSAID-tolerant CU, AECD patients had significantly longer disease duration (57.7±118.4 versus 24.4±42.6 months, p<0.05), a higher prevalence of angioedema (72.7 % vs 30.9 %, p<0.05) and atopy (83.8 % vs 58.4 %, p<0.05), and more frequent involvement of the face and upper respiratory tract (54.5 % vs 29.6 %, p<0.05).

Conclusions: AECD is a distinct phenotype that should be considered for inclusion as a separate subtype of chronic urticaria.

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Management of hypersensitivity reactions from intravenous iron

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Background: Intravenous iron products (IVIP) are increasingly used, and hypersensitivity reactions have become an issue. Previously high molecular weight dextran iron products elicited immune complex-mediated anaphylaxis due to anti-dextran IgG. Current IVIP are colloids that consist of spheroidal iron-carbohydrate nanoparticles. The core is surrounded by a carbohydrate shell, that stabilizes the iron oxyhydroxide, maintains the particles in suspension, controlling the release of iron. Due to instability of the carbohydrate complex free iron may lead to adverse reactions. IVIP may cause hypersensitivity reactions ranging from local pruritus to anaphylaxis. Apart from immune complex mediated anaphylaxis the pathophysiology is not elucidated, an immunological mechanism has not been convincingly demonstrated. Direct toxicity from free iron, complement involvement and other mechanisms resulting in non-specific activation of mast cells and basophils have to be considered.

Patients and methods: Patients with hypersensitivity reaction from IVIP were retrospectively analyzed. Iron carboxymaltose (Ferinject®) or iron sucrose (Venofer®) were implicated. Workup included skin tests with respiratory allergens, in some with IVIP, and in two basophil activation tests. If indicated controlled reexposure was done.

Results: 15 patients (14 females), 18 to 72 years (mean: 39) were investigated. The eliciting compound was Iron carboxymaltose (n = 8) and iron sucrose (n = 7). In 7, previous use of iron products resulted in a reaction. Onset of symptoms was few minutes to several hours. Severity grades were local (n = 1), grade I (n = 5), grade II (n = 2), grade III (n = 5), grade IV (n = 1), and lethal outcome (n = 1). Skin prick tests with the culprit IVIC were done in 8, with an alternative in 6 patients, with negative results. Intradermal tests were done in 1 patient with equivocal result. Basophil activation was negative in two. Reexposure was done with IVIC in 8 patients (2 with premedication), with oral iron in 2 without symptoms.

Conclusions: The mechanism of hypersensitivity reactions from IVIP remains unclear. Skin and in vitro tests are not established. If indicated, controlled reexposure can be performed in a majority of patients. Risk factors have not been identified.

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Long-term safety of replication-defective smallpox vaccine (MVA-BN) in atopic eczema and allergic rhinitis

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Background: Availability of a safe smallpox vaccine may be necessary under certain circumstances. Use of the old life virus vaccine was associated with serious adverse events, particularly in the setting of atopic eczema and immunodeficiency. MVA(modified virus Ankara)-BN, a highly attenuated strain of vaccinia virus, was developed for vaccination with improved safety profile.

Methods: A phase 1 study was conducted in 60 subjects without history of smallpox vaccination to gain experience with smallpox vaccination using this strain in healthy and atopic subjects. Healthy subjects, subjects with a history of atopic eczema (AE), subjects with mild active AE and subjects with mild allergic rhinitis without AE were equally allocated in 4 groups. MVA-BN was injected s.c. in a dose of 10^8 TCID50 twice in a four weeks interval.

Results: No serious or unexpected adverse reactions were reported. All subjects experienced mild to moderate pain and redness at the injection site. Dermatologic examinations did not reveal any unfavourable reactions to the study medication, particularly no sign or exacerbation of eczema for as long as 196 days. All subjects seroconverted after 2 vaccinations and no significant difference in antibody titers between the four different groups was observed.

Conclusions: A good safety profile of the MVA-BN vaccine was shown. The absence of adverse events in subjects with atopic disorders appears promising for the development of a safe smallpox vaccine for patients with AE or other atopic diseases.

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A randomized, controlled intervention trial of early emollient use in prevention of atopic dermatitis and allergic sensitization during infancy

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Background: Recent genetic and epidemiological studies suggest that epidermal barrier dysfunction may leads to the development of atopic dermatitis (AD) and other allergic diseases such as food allergy.

Objective: We hypothesized and examined whether the use of a moisturizer from neonatal period to protect the skin barrier may prevent the development of AD (32 weeks of age) and allergic sensitization.

Methods: We used a milky lotion-based moisturizer for the intervention group. This study was randomized, controlled, single center, investigator-blinded trial, involved 116 participants with high risk for allergic family history. The participants within the first week after birth were subdivided randomly into the two groups (n=51 for intervention: n=48 for control). The infants of intervention group were treated by a milky lotion-based moisturizer at least once daily. The onset of AD (infantile eczema) was judged by a dermatology specialist, based on the modified Hanifin and Rajka criteria. The primary outcome was the incidence of AD or infantile eczema until 32 weeks from birth (Kaplan-Meier method). One of the secondary outcomes, allergen sensitization was evaluated by the serum levels of IgE antibodies against egg white, milk and other allergens by using a diamond-like carbon-coated chip device.

Results: The intervention with a milky lotion-based moisturizer was found to significantly lower (approximately at 30%) the risk of AD plus infantile eczema, compared to controls(p=0.0042; log-rank test) until 32 weeks from birth. As a result of secondary outcomes, the two groups produced similar rates of allergen sensitization at least until 32 weeks. However, the rate of allergic sensitization (based on the levels of anti-egg white IgE antibody) of infants with any skin lesions including eczema was significantly higher than that of infants without lesions where the cut off level was set at 0.34 UA/ml CAP-RAST equivalent value (Odds Ratio, 3.18; 95%CI, 1.19-8.46).

Conclusion: Daily use of a moisturizer in early life reduced the risk of infantile eczema or atopic dermatitis. Regarding allergic sensitization, it was affected by the presence of AD, infantile eczema or skin rash but not by the use of a moisturizer.

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Classification of elderly asthma phenotypes and its implementation

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Background: Thus far, there has been no attempt to classify asthma phenotypes in the elderly population. It is essential to clearly identify clinical phenotypes to achieve an optimal treatment of elderly asthmatics. In this study, we classified elderly asthmatics by cluster analysis and developed a way to utilize the resulting cluster in practice.

Methods: We applied k-means cluster to 872 elderly asthmatics aged 65 years or older in a prospective, observational, and multi-centered cohort. Acute asthma exacerbation data collected over the prospective follow-up of two-year was used to evaluate clinical trajectories of these clusters. Subsequently, a decision-tree algorithm was developed to facilitate implementation of these classifications.

Results: Four clusters of elderly asthmatics were identified: (i) long symptom duration and marked airway obstruction; (ii) female dominance and normal lung function; (iii) smoking male dominance and reduced lung function; and (iv) obese and borderline lung function. Cluster grouping was strongly predictive of time to first acute asthma exacerbation (log-rank P = 0.0105). The developed decision-tree algorithm included two variables (FEV1 pred.% and smoking pack years), and its efficiency of proper classification was confirmed in the secondary cohort of elderly asthmatics.

Conclusions: We defined four elderly asthma phenotypic clusters with distinct probabilities of future acute exacerbation of asthma.

Our simplified decision-tree algorithm can be easily administered in practice to better understand elderly asthma and to identify an exacerbation-prone subgroup of elderly asthmatics.

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Late-onset asthma in the elderly: Roles of upper airway diseases and staphylococcal enterotoxin sensitization

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Background: Adult asthma is considered as a heterogeneous disorder. However, the pathophysiology of the late-onset adult asthma is still largely unknown, particularly for severe disease in the elderly (aged ≥ 65 years old). The present study aimed to investigate risk factors for late-onset elderly asthma and its severity.

Methods: Case-control analyses were performed for 229 late-onset elderly asthmatics and 50 non-asthmatic elderly controls. Late-onset asthma was defined as having asthma onset ≥ 40 years old. Baseline investigations included spirometry, inhalant allergen skin prick test, induced sputum, rhinoscopy, and structured questionnaires for comorbidity. Sera were measured for total IgE and staphylococcal enterotoxin specific IgE (SE IgE) levels by ImmunoCAP. Asthma severity was prospectively assessed by the clinical courses of asthma during one-year follow-up period. The definition of severe asthma followed the international criteria.

Results: At baseline, several factors were significantly related to late-onset elderly asthma (onset age: mean 65 years old), such as body mass index (BMI; 24.6±3.3 vs. 23.3±3.6 kg/m^2, P=0.014), atopy (43.1% vs. 14.0%, P<0.001), rhinosinusitis (60.7% vs. 16.0%, P<0.001), and serum SE IgE levels (median 0.13 [interquartile range 0.04-0.52] vs. 0.07 [0.00-0.15] kU/L, P<0.001). In multivariate analyses, rhinosinusitis and atopy were independent risk factors for asthma.

Severity assessment has found 66 severe asthma and 163 non-severe asthma; severe asthma had more rhinosinusitis (87.9% vs. 49.7%, P<0.001), gastroesophageal reflux disease (GERD; 29.2% vs. 8.7%, P<0.001), and higher levels of serum SE IgE (0.52 [0.14-0.98] vs. 0.11 [0.03-0.30], P<0.001), compared to non-severe asthma. No difference was found for BMI or atopy. In multivariate logistic regression analyses, rhinosinusitis, GERD, and SE IgE sensitization (≥ 0.35 kU/L) were significantly associated with severe asthma. Interestingly, SE IgE sensitization was closely related to both of rhinosinusitis and severe asthma; however, the association of SE IgE sensitization with severe asthma was independent from the presence of rhinosinusitis. In multivariate linear regression, serum SE IgE levels showed positive correlations with the variability of spirometric parameters during one-year follow-up, independently from the presence of GERD or rhinosinusitis.

Conclusion: The present study found several risk factors for late-onset elderly asthma and its severity. The associations of rhinosinusitis indicate close inter-relationships between upper and lower airway diseases in the elderly. Independent correlations of SE IgE sensitization with asthma severity markers may suggest its additional roles in the pathogenesis of severe asthma.

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Serial oral provocation tests in ASA-Intolerance Syndrome (M. Samter) demonstrate a high variance of results

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Intolerance reactions to acetylsalicylic acid (ASA) are common findings in patients with chronic airway diseases. The full clinical picture of the ASA-intolerance syndrome reveals a classic triad of symptoms (Samters triad): aspirin induced bronchial asthma, aspirinsensitivity and chronic rhinosinusitis with nasal polyps. Clear evidence has been pointed out that ASA-intolerance is related to an abnormal metabolism of arachidonic acid leading towards excessive leukotriene (LTs) production. The resulting eicosanoid dysbalance between leukotriens and prostaglandines might be the crucial pathophysiologic keypoint of the disease.

Challenge tests with ASA are performed as the diagnostic tool of choice and are described and standardized in a position paper of the European Academy of Allergy and Clinical Immunology (EAACI).

We examined 97 patients with nasal polyps and a typical history of ASA-intolerance and/or asthma. All patients were provoked with aspirine in an oral challenge procedure according to the EAACI recommendations (0, 25, 50, 100, 300, 500 mg oral aspirine in 1.5 hour intervals). All patients had to have a positive response in the first challenge test to be included into the study. Therafter, the patients were serially re-challenged 2 times in intervals of 3 months with the identical procedure. All patients taking medications that might have interfered with the oral challenge procedure (e.g. oral steroids, leukotriene-antagonists or antihistamines) were excluded from the trial. Only n=51/ 97 patients (52%) showed a reproducible positive test result at all 3 challenge dates, 27 patients (28%) were positive at 2 challenges and 19 patients (20%) had negative test results at both following challenges after the initial test procedure.

We conclude, that the oral provocation test as the current standard method for the diagnosis of ASA-intolerance has a high variance in serial re-provocations. Improvements of the diagnostic work-up of these patients are recommended.

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Asthma susceptibility genes: Which genetic variants are important?

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Background: Genome-wide association studies (GWASs) have identified multiple asthma susceptibility genes. However, multiple single nucleotide polymorphisms (SNPs) have been identified per gene (usually noncoding) and for some chromosomal regions, multiple genes are identified due to correlation between variants in a given chromosomal region (Linkage Disequilibrium). Therefore, it is not clear which variants are casual and need to be studied further.

Objective: Perform expression quantitative trait loci (eQTLs) analyses for previously identified asthma susceptibility genes to provide insight into functional genes/SNPs.

Methods: *Cis*-eQTL analyses of 30 asthma susceptibility genes were performed using expression data from cells from human bronchial epithelial biopsy (BEC, n =107) and from bronchial alveolar lavage (BAL, n=94) using investigative bronchoscopy in well phenotyped subjects with mild to severe asthma.

Results: In general, eQTLs were observed for either BEC or BAL but not both for the same gene. eQTLs were observed for the following genes with p values <10-4: 1) *TSLP:* rs3806932 (G allele protective against eosinophilic esophagitis) and rs2416257 (A allele associated with lower eosinophil counts and protective against asthma) were correlated with decreased expression of *TSLP* in BAL ($p=7.9x10^{-11}$ and $5.4x10^{-4}$, respectively). However, rs1837253 (consistently associated with asthma susceptibility) showed no correlation with *TSLP* expression levels. 2)*ORMDL3-GSDMB* region: rs8067378 (G allele protective against asthma) was correlated with decreased expression of *GSDMB* ($p=1.3x10^{-4}$) but not *ORMDL3* in BEC. 3) *IL33*: promoter SNP rs992969 (A allele associated with higher eosinophil counts and risk for asthma) was correlated with increased expression of *IL33* in BEC ($p=1.3x10^{-6}$).

Conclusions: Cell specific regulation of the expression of asthma susceptibility was observed and SNPs in multiple asthma susceptibility genes including *TSLP*, *IL33* and *GSDMB* affect asthma risk through *cis*-regulation of its gene expression.

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Association of rare variants in β2-Adrenergic receptor pathway genes with asthma severity in African Americans

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Introduction: We have previously shown an association of rare β2-adrenergic receptor gene (*ADRB2*) variants with hospitalization and loss of asthma control on asthma subjects being treated with a LABA (Ortega et al, Lancet Respiratory 2014). Two variants were identified: $Thr^{164}Ile$ in individuals of European white descent and a 25 base-pair promoter polynucleotide insertion in individuals of African descent. These results may explain rare but serious events that have been associated with LABA therapy in asthma (FDA boxed warning and current surveillance trial). The β2-adrenergic receptor (β2AR) regulates a G protein-coupled signaling pathway and, therefore, a pathway approach may identify additional variants associated with measures of asthma severity.

Methods: Analysis of exome sequencing data of 46 β2AR pathway-related genes was performed on 191 African American asthmatics from NHLBI Severe Asthma Research Program through the GO Exome Sequencing Project (ESP). Rare variants (frequency<0.05) in β2AR pathway-related genes were analyzed by testing all variants in a given gene (collapsing statistical method) for association with lung function and healthcare utilization. Asthmatics with and without rare variants were compared using generalized linear models and fisher exact testing.

Results: 303 functional rare variants in 41 β2AR pathway-related genes resulting in an amino acid coding change or a polynucleotide insertion-deletion were identified. Asthma subjects with rare variants in 3 β2AR pathway-related genes related to Protein Kinase A (PKA) and its regulation (*AKAP2, AKAP5,* and *PRKACA*) had a lower baseline FEV1 percentage of predicted when compared to those without rare variants (69% versus 88%, p=0.005). This relationship was only observed in asthmatics on a LABA. Asthmatics with rare variants within 6 β2AR pathway-related genes related to downstream kinase signaling (*CREB1*, *LAMTOR3, MAP2K1, MAP2K2, MAPK3, RAF1*, and *RPS6KA1*) had a lower FEV1/FVC ratio compared to those without rare variants (65% vs 72%, p=0.02). In addition, these downstream signaling-related rare variants were associated with an increased risk for hospitalization over the past year (OR=4.1, 95%CI=1.4-12, p=0.04) and a lifetime history of mechanical ventilation (OR=3.5, 95%CI=1.2-10, p=0.02) for an exacerbation.

Conclusions: These preliminary findings provide evidence that there are multiple rare variants in β2AR pathway genes that are associated with decreased lung function and increased health care utilization in African American asthmatics. In addition, a subset of these variants is associated with more severe asthma or loss of asthma control when these individuals are treated with a LABA. Further studies of this pathway may identify a small subset of asthmatics for which LABA therapy is less ineffective or may increase the risk for loss of asthma control.

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Epigenetic mechanisms for the regulation of barrier integrity and bronchial epithelial tight junctions in asthma

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Background: Tight junctions (TJ) form homodimeric cell-cell contacts and control cellular integrity and paracellular flow of molecules and cells, and represent an essential component of epithelial cell barrier. Defects in bronchial epithelial TJs may leads to loss of barrier function and play a role in initiation and chronicity of asthma and may be controlled by epigenetic mechanisms.

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

Results: Asthmatic bronchial epithelial cells showed an inherited low TJ integrity compared to control epithelial cells, which was consistent even after several passages of the cells. Th2 cells co-cultured with air-liquid interface cultures of human primary bronchial epithelium significantly decreased transepithelial resistance and increased paracellular flux particularly mediated by IL-4 and IL-13, but not TSLP, IL-25 and IL-33. Bronchial epithelial cell stratifications suggesting opening of TJs was observed in immunoflurescence staining of the adaptor protein ZO-1 and occluding in human biopsies and mouse model of asthma. We observed also weaker expression of occludin in asthmatic epithelium compared to non-asthmatic controls. To investigate, whether epigenetic mechanisms responsible for the differences between healthy and asthmatic bronchial epithelial cells, we incubated the cells with a histon deacetylase inhibitor JNJ-264. We observed a recovery of barrier integrity with increased transepithelial resistance and upregulation of the tight junction mRNAs, as well as increased expression of junctional molecules occluding and ZO-1 in immunofluorescence staining. The analysis of all list of epigenetic regulators such as histone acetytransferases, histone deacetylase inhibitors and methylation analysis of regulated junctional molecules is going on.

Conclusion: Our data demonstrate that Th2 cells and their cytokines downregulate the integrity of bronchial epithelial cells. Inhibition of endogenous histone deacetylase activity corrected the defective barrier by upregulation of the expression of tight junction molecules.

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Epigenetic regulation as an important mechanism in the origin and early development of allergy and asthma

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The (early) development of allergy and asthma is the result of complex gene x environment interactions. Epigenetic mechanism such as DNAmethylation and histone acetylation are important tools to regulate gene expression by environmental and lifestyle factors leading to the TH1 and TH2 dysbalance. We have shown recently that the exposure to a microbial-rich environment (traditional farming as a model situation) leads to profound alterations in gene expression resulting in a neonatal TH2 phenotype. Further analysis of DNAmethylation and H3/H4 acetylation of immuno-regulatory genes revealed that a TH2 phenotype is the result of marked alterations in the genes GATA3, IL-13 and IL-9 as well as (with opposite effects) on IFN-γ. High maternal folate exposure is further augmenting this effect. We then went on to analyse the underlying mechanism of epigenetic regulation early in life by employing animal model systems of experimental asthma. Mice exposed to the prototypic asthma-protective bacterium *Acinetobacter lwoffii* protected experimental asthma. This effect depends on mucosal activation of pro-inflammatory pathways which is an important mechanism to regulate mucosal homeosthasis. Important mediators transmitting the asthma-protective effect include IL-6 and TNF-α. IL-6 KO-mice are not able to mount this asthma-protective effect. Furthermore, IL-6 plays a critical role in epigenetic gene regulation of naïve T cells which determine TH1 and TH2 development. IL-6 (and partly TNFα) trigger a program leading to changes in DNA methylation and histone acetylation of inflammatory genes. Therefore, we could discover a novel mechanism of asthma protection which depends on pro-inflammatory signals derived from mucosal cells.

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Loss of normal peripheral airway bronchial epithelial gene profile in severe asthma

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Background and methods: Impulse oscillometry (Jaeger MasterscreenTM) measures were made in patients with severe asthma (n=121) as part of the Wessex Severe Asthma Cohort study and the findings compared to those in health (n=80) and

mild (n=35) and moderate (n=35) asthma. Peripheral airways resistance (R5- R20) was significantly greater in severe asthma than in the other groups both before and after bronchodilator therapy (p<0.001). As post-mortem studies in asthma have identified structural and inflammatory changes affecting the small peripheral airways (those less than 2mm diameter) and epithelial cell activation and mesenchymal signalling have relevance to these pathological findings, we undertook a gene array analysis (Affymetrix HG U133 plus 2.0 beadchips [Affymetrix, Santa Clara, California, USA]) of bronchial epithelial cell brushing samples obtained from both the central and peripheral airways at bronchoscopy. Measures were made from 18 severe asthmatics (GINA stage 4 or 5) with inadequate disease control and 23 healthy non-asthmatic controls.

Results: In healthy controls, 2615 genes were identified as differentially expressed (false discovery rate (FDR) corrected p-value < 0.05) between peripheral and central airways, indicating a large biological distinction between the two airways. However, in severe asthmatics, only 225 genes were identified as differentially expressed (FDR corrected p-value < 0.05) between the two airways. PCA analysis further revealed a large distinction between central and peripheral airways in healthy controls, which disappeared in severe asthmatics. This indicates that the two airways go from having large biological differences in healthy to being in a more similar state of distress in severe asthma.

178 of the 225 differentially expressed genes in severe asthma were also evident in health, with a similar pattern of expression suggestive of their importance in maintaining the distinct compartmentalisations of peripheral and central airways even after disease progression. The remaining 47 genes that were uniquely differentially expressed between peripheral and central airways in severe asthma included those relevant to airway inflammatory and immune responses.

Conclusions: These studies identify that the peripheral airways are abnormal in severe asthma and that the bronchial epithelium at this site represents an important focus for therapeutic targeting.

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Sialylated glycans carried by the airway mucin Muc5b are endogenous ligands for Siglec-F and mediate killing of mouse eosinophils in vitro and in vivo

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Background: Siglec-F is a glycan binding protein expressed on mouse eosinophils and its engagement induces eosinophil apoptosis, suggesting a pathway for controlling eosinophil-associated diseases. Siglec-F recognizes specific a2,3-linked, sialylated, sulfated glycans in vitro, but the identities of endogenous glycoprotein ligands in vivo are unknown.

Methods: Lungs from normal and mucin-deficient mice, as well as cultured tracheal epithelial cells from mice (mTEC) were examined. Western blotting and immunocytochemistry was completed looking for Siglec-F-Fc binding glycoproteins. Liquid chromatography-tandem mass spectrometry analysis of Siglec-F-Fc binding glycoproteins was performed, and mouse eosinophil mucin binding and cell death assays were done using flow cytometry.

Results: We characterized glycoproteins isolated from mTEC and mouse lung tissue homogenates that bind to Siglec-F-Fc in a sialic acid dependent manner. Binding of Siglec-F-Fc to mTEC was sialidase-sensitive and was increased after treatment with IL-4 or IL-13. Sialidase-sensitive, PNGaseF-resistant binding of Siglec-F-Fc to glycoproteins of apparent MW ≈500 kDa and 200 kDa was detected by western blotting of mTEC lysates and culture supernatants, indicating the importance of sialylated O-linked glycoprotein glycans for Siglec-F binding. Binding was also resistant to solvolysis, suggesting that these endogenous ligands do not require sulfation for Siglec-F recognition. The expression of these glycoprotein ligands was increased during mouse allergic airways inflammation. Liquid chromatography-tandem mass spectrometry-based proteomics, cross-immunoprecipitation, and histochemical analysis of lungs validated the identity of the glycoproteins as Muc5b, and also possibly Muc4. Muc5b null mice, but not Muc5ac null mice, had diminished Siglec-F airway ligands as determined by western blotting and immunohistochemistry. Purified mTEC mucins carried sialylated glycans, bound to eosinophils and induced their death in vitro. In vivo, BAL eosinophil numbers were increased, and apoptosis was decreased, in Muc5b conditional null mice after intratracheal administration of IL-13 compared to wild type mice.

Summary: These studies demonstrate that sialylated glycans displayed by the airway mucins Muc5b and Muc4 are endogenous ligands for Siglec-F and that Muc5b mediates Siglec-F dependent killing of mouse eosinophils in vitro and in vivo.

Conclusion: These data identify a previously unrecognized endogenous anti-inflammatory property of specific mucins by which their sialylated glycans can control lung eosinophilia through Siglec-F engagement.

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Type 1 and Type 2 immunity in severe asthma: Convergence at the airway epithelium

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Background: Asthma is increasingly recognized as a heterogeneous disease. Studies support a Type-2 immune processes in ~50% of patients, with targeted biologic therapies confirming the importance of these pathways. However, evidence

for Type-2 immunity is present over a range of asthma severity from mild to severe and is not likely to fully explain these severity differences. Certain severe asthma phenotypes in particular, may be associated with more complex immunity, explaining the persistence of inflammation and associated symptoms despite high dose corticosteroids. We hypothesized that in addition to Type-2 immunity, some patients with severe asthma would manifest evidence for concomitant Type-1 immunity, leading to enhanced nitrative stress in the airway epithelium.

Methods: To address this hypothesis, asthmatic and healthy controls from the Severe Asthma Research Program (SARP) at the University of Pittsburgh underwent bronchoscopy with airway brushing and bronchoalveolar lavage (BAL). BAL cells were evaluated by quantitative PCR for interferon (IFN)γ while epithelial brushings were evaluated for the Type-2 biomarker CCL26/ eotaxin-3 to determine the mix of Type-1 and Type-2 immune processes. Primary human airway epithelial cells (HAECs) were cultured at an air-liquid interface (ALI) and stimulated with IFNγ (10-100 ng/ml) and IL-13 (1 ng/ml) alone and in combination and evaluated for oxidative and nitrative pathway responses.

Results: Although BAL cell IL-13 mRNA levels were extremely low, BAL cell IFNγ and epithelial CCL26 were significantly increased in severe as compared to milder asthma and healthy controls. Both were associated with higher epithelial inducible nitric oxide synthase (iNOS) mRNA. When IFNγ was added to low dose IL-13 in HAECs in culture, a synergistic increase in iNOS mRNA, protein and nitrite levels, as well as synergistic increases in dual oxidase-2 (DUOX2) and H2O2 levels occurred. This increase was accompanied by increases in epithelial cell nitrotyrosine levels and could be explained by the generation of NO2 radical, rather than superoxide. These in vitro findings were recapitulated in vivo in fresh epithelial cells.

Conclusions: These results suggest certain phenotypes of severe asthma are associated with mixed Type-2 and Type 1 immune processes, which converge on the epithelium to enhance levels of nitro-oxidative stress.

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RabGEF1, a negative regulator of thymic stromal lymphopoietin production and skin inflammation

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RabGEF1 (also called Rabex-5) is an effector of Rab5, a member of a family of small GTPases important for endosome fusion during receptor internalization and endocytic vesicular trafficking. We cloned the mouse ortholog of *Rabgef1* and showed that RabGEF1 can down-regulate FceRI- and c-kit-dependent signaling and function in mast cells. Mice with ubiquitous deletion of RabGEF1 (*Rabgef1-/-*) exhibit skin and systemic findings similar to those in human atopic dermatitis, including epidermal hyperkeratosis, spongiosis and hyperplasia, as well as increased serum IgE and prominent dermal infiltrates consisting of mast cells, eosinophils, lymphocytes, monocytes/macrophages, and neutrophils. *Rabgef1-/-* mice also express in the skin, serum, and spleen abundant amounts of thymic stromal lymphopoietin (TSLP), a pro-allergic cytokine strongly associated with human atopic dermatitis and other allergic diseases. Interference of TSLP receptor signaling in *Rabgef1-/-* mice substantially diminishes granulocyte infiltration in the dermis, but fails to protect the mice from developing skin disease. To investigate whether TSLP overproduction and skin inflammation displayed by *Rabgef1-/-* mice reflect cell autonomous function of keratinocytes, we used a Cre-Lox recombination approach to specifically delete RabGEF1 expression in keratinocytes. We generated transgenic C57BL/6 mice in which *Rabgef1* exon 2 was flanked by two loxP sites (*Rabgef1fl/fl*) and then crossed them with mice expressing the Cre recombinase under the control of keratinocyte-specific K14 promoter to create *K14-Cre*; *Rabgef1fl/fl* mice. The efficiency and specificity of *Rabgef1* deletion in these mice were assessed by single-cell sorting followed by genomic PCR analysis. *K14-Cre; Rabgef1*$^{fl/fl}$ mice appeared to be normal at birth but developed morbidity and skin inflammation by 2-3 days after birth and died between 1 and 8 weeks of age. *K14-Cre; Rabgef1fl*$^{/fl}$ mice expressed increased TSLP in the skin, and developed dermatitis and systemic inflammation similar to those observed in *Rabgef1-/-* mice. In summary, our findings show that RabGEF1 can negatively regulate TSLP production *in vivo* and that excessive production of TSLP contributes to some of the phenotypic abnormalities in *Rabgef1-/-* mice. However, specific deletion of *Rabgef1* in keratinocytes is sufficient to drive severe skin inflammation, suggesting a key role for keratinocyte-associated RabGEF1 in maintaining skin homeostasis *in vivo*.

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Distinct mechanisms for allergic sensitization to protease antigen between skin and airway

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Cysteine protease activity of papain, a plant-derived occupational allergen homologous to mite major protease allergens Der p 1 and Der f 1, is essential to serum IgE/IgG1 production [1] and lung eosinophilia [1, 2] induced by intranasal administration of papain in mice, and IL-33 contributes to these responses. In the present study, we investigated skin responses and antibody production induced by subcutaneous papain administration and responses induced by subsequent airway challenge with papain as a model of "atopic march" after the sensitization through the skin.

Subcutaneous papain injection into ear lobes induced chronic swelling associated with increased epidermal thickness, dermal inflammation, serum IgE/IgG1 responses, and Th2 cytokine production in draining lymph node cells restimulated in vitro.

These responses were absent or markedly less upon subcutaneous administration of protease inhibitor-treated papain. Results obtained by using mast cell-deficient mice and basophil-depleting antibody suggested the contribution of mast cells and basophils to papain-specific antibody responses in the subcutaneous but not intranasal sensitization. Interestingly, the ear swelling and antibody responses were equivalent between wild-type and IL-33$^{-/-}$ mice. After the subsequent airway challenge with papain, the subcutaneously presensitized wild-type mice showed more severe lung eosinophilia than those without the presensitization. The presensitized IL-33$^{-/-}$ mice showed modest eosinophilic inflammation, which was absent without the presensitization, but its severity and IgE boost by the airway challenge were markedly less than the presensitized wild-type mice.

These results suggest that mammals can sense environmental proteases as innate danger signals leading to allergic sensitization regardless to the route of sensitization but via distinct pathways according to the sensitization route and that cooperation of initial sensitization via skin and re-exposure via airway to protease allergens maximizes the magnitude of the transition from skin inflammation to asthma in the atopic march, natural history of progression of allergic diseases.

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The BET family of epigenetic readers contributes to chronic airways inflammation

Cara M.M. Williams, Hannah Jones, Michael Jesson, Michael Primiano, Agustin Casimiro Garcia, Paul Bonin, Julia Shin, Sarah Soucy, Jeffrey Pelker, Zhiyong Yang, John Kubera, Cedric Hubeau, Andrea Bree, Christelle Perros Huguet, Bruce Lefker

Background: The bromodomain and extraterminal domain (BET) family readers recognize specific N-acetylated lysines on histone tails and regulate transcription of a number of pro-inflammatory genes that are thought to contribute to numerous respiratory diseases including asthma and COPD.

Methods: We have utilized cells from healthy donors and from COPD patients to determine whether or not BET inhibition can attenuate inflammatory mediators that may drive disease pathology. Similarly, we have assessed the impact of BET inhibition in a cigarette smoke exposure model in the mouse.

Results: We demonstrate that BET inhibition suppresses IL-6 release after LPS challenge in human whole blood, human PBMC's, and rat PBMC's as well as in TLR3 stimulated human COPD patient epithelial cells. Likewise, BET inhibition resulted in potent inhibition of human pulmonary artery smooth muscle cell proliferation and the expression of VEGF protein in these cells. In vivo studies also confirmed anti-inflammatory activity following BET inhibition. Of note, in a murine 10 day cigarette smoke/TLR3 airway challenge model that we have previously shown to be insensitive to the anti-inflammatory activity of steroids, we can demonstrate a 50% reduction in bronchoalveolar lavage fluid neutrophils with a small molecule BET inhibitor (15 mg/kg BID). Similarly, plasma IP-10 levels were significantly inhibited at this dose. Histological assessment of tissue inflammation also revealed suppression of inflammation at the 15mg/kg dose BID. Likewise, lung tissue mRNA expression of various genes that are known to contribute to COPD/asthma pathophysiology were suppressed in animals that were treated with the BET inhibitor tool compound.

Conclusions: Taken together, these data support the notion that the BET family of epigenetic readers may contribute to gene induction and the associated steroid insensitive chronic inflammation that characterizes both severe COPD and severe asthma.

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The heterogeneous molecular signature of eczema subtypes

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Affecting more than 10% of the population, eczema is one of the most common diseases worldwide. Apart from causing immense health costs it often reduces quality of life significantly. It is an unresolved riddle why within the heterogeneous group of eczemas some entities are self-limited while others are chronic and relapse. Therapy for all subtypes is mainly limited to unspecific immunosuppressive approaches. The lack of personalized specific therapies is mainly due to incomplete knowledge of the molecular mechanisms underlying the different eczema subtypes.

In this pilot study, we compared the molecular signature of allergic contact dermatitis (ACD) to nickel (n=10), nummular or dishydrotic eczema (nAE, n=8), and atopic eczema (AE, n=6) by performing whole genome expression analysis of lesional as compared to autologous non-involved skin.

We found the transcriptional variability in the ACD group to be smaller compared to the one in chronic eczemas (nAE and AE), which was reflected by the fact that 172 genes were exclusively regulated in ACD but not in chronic eczemas, and only 28 genes were exclusively regulated in chronic eczemas. 33 genes were regulated in common, among them many epithelial antimicrobial peptides. Interestingly, a mutual antagonistic picture was observed

regarding genes of the epidermal differentiation: while genes of the LCE3 family were strongly up-regulated in nAE and AE, they were unchanged in ACD as compared to autologous non-involved skin. Inversely, LCE1 and LCE2 genes were down-regulated exclusively in ACD. Regarding the immune system, a similar pattern was observed in all eczema forms, with a general trend of stronger regulation in ACD. Notably, ACD reactions to nickel were accompanied by activation of the inflammasome, extracellular matrix and cell-cell adhesion molecules/cytotoxicity.

In summary, the epithelial signature seems to discriminate self-limited from chronic eczema forms. Based on this study, further investigation with larger cohorts individually characterized in clinical, histological and transcriptional aspects needs to be performed to evaluate identified key players for target-oriented therapies.

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Th17 cells and tissue remodeling in atopic and contact dermatitis

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Background: Eczematous skin lesions of atopic dermatitis (AD) as well as allergic and irritant contact dermatitis (ACD, ICD) are characterized by the same typical clinical signs, although due to different causes. In both AD and ACD, the presence of T helper 17 cells which play an important role in host defense, has been reported. Furthermore, IL-17 is involved in tissue repair and remodeling. This study aimed to investigate IL-17 expression in acute eczematous skin lesions and correlate it with markers of remodeling in AD, ACD and ICD.

Methods: Skin specimens were taken from positive patch test reactions to aeroallergens, contact allergens and irritants at days 2, 3 and 4. Inflammatory cells as well as the expression of cytokines and extracellular matrix proteins were evaluated by immunofluorescence staining and confocal microscopy.

Results: ACD and ICD were characterized by IFN-g expression, whereas in AD lesions, IL-13 expression and high numbers of eosinophils were the prominent phenotype. Expression of IL-17, but also IL-21 and IL-22, was observed in all eczema subtypes. The number of IL-22+ T cells correlated with the number of eosinophils. Markers of remodeling such as MMP-9, procollagen-3 and tenascin C were observed in all acute eczematous lesions, while a correlation of IL-17+ T cell numbers with tenascin C-expressing cells and MMP-9+ eosinophils was apparent.

Conclusion: The expression of IL-17 and related cytokines, such as IL-22, was demonstrated in acute eczematous lesions independent of their pathogenesis. Our results suggest a potential role for IL-17 in remodeling of the skin.

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Autoimmunity ("autoallergy") – a driving mechanism in the perpetuation of atopic dermatitis?

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that is characterized by a T cell-dominated skin infiltrate. 80% of the patients show IgE sensitization to exogenous allergens and exposure to these allergens can exacerbate the disease. A subgroup of AD patients is sensitized to autoantigens, a phenomenon called autoallergy. One of these autoallergens is α-NAC (Hom s 2), a multi-faceted protein that acts as chaperon, allergen and alarmin.

Since most data exists on autoreactive IgE, we isolated α-NAC reactive T cells from blood and lesional skin of AD patients by T cell cloning in order to investigate the specific T cell response. Interestingly, T helper as well as cytotoxic T cells could be generated from both compartments. While T helper cell clones were not found to be restricted to the Th2-profile, but could also be assigned to the Th1 or Th17 subset, cytotoxic T cells were analyzed further on by MHC-tetramer staining. Here, we detected higher frequencies of terminally differentiated α-NAC-specific cells in a direct-ex-vivo-approach in the blood of AD patients. These cells are regarded as the main source of perforin and granzyme B, but also of IFN-γ and TNF-α.

Furthermore, α-NAC proved to be a strong activator of innate immune cells, such as monocytes and dendritic cells, which secreted IL-12, IL-6 and other cytokines after stimulation with α-NAC. In turn, these cytokines might promote pro-inflammatory T cell responses and accordingly we could observe secretion of IFNg, IL-17, IL-22 and IL-13 in α-NAC-stimulated PBMCs. When we compared AD patients sensitized to α-NAC and control donors, most cytokines were efficiently induced in both groups. However, the anti-inflammatory IL-10 was strongly reduced in supernatants from immune cells of the sensitized AD patients.

Since alpha-NAC might be released from damaged keratinocytes in inflamed skin lesions, the combined effect of specific T cell responses and activation of innate immune cells may contribute to perpetuation and exacerbation of atopic skin inflammation.

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The story goes on: Auto-IgE represents a natural response that is not necessarily associated with pathology in childhood

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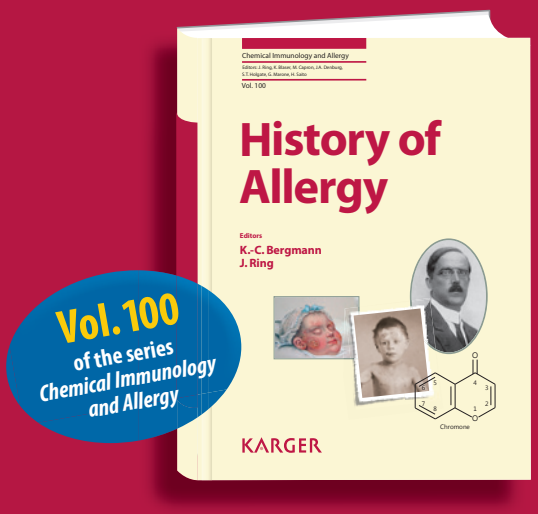
Th2-mediated allergies are characterized by specific responses and cumulate in humoral IgE responses. The observation of autoreactive IgE (auto-IgE) against skin-derived proteins in atopic eczema (AE) but also against protein of bronchial epithelial cells (CIA meeting 2012) was interpreted as excessive tissue inflammation secondarily to allergen responses. Because auto-IgE was mainly reported in adults with severe and chronic disease, a role for IgE-mediated autoimmunity was assumed in chronic AE, even in the absence of causative exogenous allergens.

Until today, it has never been addressed whether auto-IgE is a pathogenic driver for disease chronicity or just an immunological epiphenomenon. Moreover, it remains unclear whether auto-IgE targets the skin exclusively and is present only in chronic adult AE or whether it indicates the loss of immune-tolerance already in childhood AE. We already reported on the last CIA meeting that auto-IgE is more frequent in younger patients, however the prevalence in healthy individuals and on population level is yet unknown. Using a high-throughput immunoassay, we analyzed IgE-autoreactivity against protein from keratinocytes (HaCaT cells) and primary bronchial epithelial cells (NHBE) in 2861 10-years-old children from the GINI and LISA birth cohort. The screening verified occurrence of auto-IgE against proteins derived from keratinocytes and airway epithelium in 50.5% of healthy children. Furthermore we demonstrated an association between the occurrence of auto-IgE and the sensitization-status of the children: (i.) children with food sensitization were characterized by auto-IgE towards skin- and gut-derived proteins (OR 2.14; $p< 0.001$), whereas children sensitized towards inhalants had auto-IgE towards lung proteins exclusively (OR 1.24; $p< 0.05$). In contrast to previous studies in adult patients, clinical relevant atopy did not correlate with auto-IgE in children.

The current study shows for the first time that auto-IgE in children represents a natural Immunoglobulin that occurs in healthy children, which is in sharp contrast to the findings in adults. Moreover auto-IgE in children is organ-specifically associated with sensitization patterns towards food and aeroallergens, which might indicate a clinical relevance in adolescence or adulthood.

Speaker and Chair Index

Speaker and Chair Index



A treasure trove of fascinating and richly illustrated information

History of Allergy

Editors
Karl-Christian Bergmann
Johannes Ring

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

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