Allergy Across the Life Course - From Origins Towards Prevention

COLLEGIUM INTERNATIONALE ALLERGOLOGICUM

CIA

32nd Symposium of the Collegium Internationale Allergologium

30 SEPT - 5 OCT **2018**

Mallorca SPAIN

Final PROGRAM

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COLLEGIUM INTERNATIONALE ALLERGOLOGICUM

CIA

30 SEPTEMBER - 5 OCTOBER 2018 Mallorca SPAIN

32nd Symposium of the Collegium Internationale Allergologicum

555 E. Wells St., Suite 1100
Milwaukee, WI 53202 USA
www.ciaweb.org

Dear Friends,

On behalf of the Council, Marianna Castells and I welcome you to the 32nd Symposium of the Collegium Internationale Allergologicum, entitled Allergy Across the Life Course - From Origins Towards Prevention, will be held in Palma, Mallorca on the 30 September - 5 October 2018.

From its inception in 1954, the Collegium has been a very special international group of friends pursuing research excellence across the full spectrum of allergic diseases and allied disorders, in a spirit of open intellectual exchange and debate at meetings held in interesting and stimulating locations across the Globe. We will be revisiting this vision, this time in the charming city of Palma, capital of Mallorca (Majorca). As its name implies, Majorca is the largest island in the Spanish Balearic Islands archipelago in the beautiful Mediterranean.

Through oral and poster presentations, this Symposium will highlight the significant scientific progress being made by our discipline worldwide, including some of the latest advances in allergy, asthma and immunology. With the genetic and environmental factors initiating allergy and the immunological and other factors driving the evolution of its different organ manifestations becoming ever clearer, there has never been a better opportunity to bring together researchers in these fields to explore mechanisms, prevention and novel therapeutic approaches.

Palma, Mallorca stands out as a jewel in the Mediterranean Sea with its rich and unique Spanish culture and cuisine, including the famous paella. While we are on the island we will be visiting different parts to sample these as well as our traditional boat trip. We have chosen the end of September for the 2018 meeting because of the lovely climate at this time of the year.

From the Council and the local organizers we thank you for attending the 32nd Symposium, and look forward to seeing you over the next week in Palma de Mallorca!

Kindest Regards,

Stephen T. Holgate, MD,
DSc FMedSci
President

Mariana Castells, MD
Organizer, 32nd Symposium

GENERAL INFORMATION

The 32nd Symposium of the *Collegium* will be held in Palma, Mallorca, Spain. Founded in 124 BC, Palma is a resort city and capital of the Spanish island of Mallorca (Majorca), in the western Mediterranean. The massive Santa María Cathedral, a Gothic landmark begun in the 13th century, overlooks the Bay of Palma. The adjacent Almudaina is a Moorish-style Arab fortress converted to a royal residence. West of the city, hilltop Bellver Castle is a medieval fortress with a distinctive circular shape.

Currency

The currency used in Spain is the Euro (EUR). There are ATMs widely available for cash withdrawal. Credit cards are also accepted at most hotels, restaurants and shops. The standard electrical voltage in Spain is 220 Volts and 50 Hertz.

Language

The official language of the 32nd Symposium is English.

Social Events

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

Registration Desk Hours

Sunday 30 September	16:00 - 20:00
Monday 1 October	7:00 - 13:30, 16:00 - 19:00
Tuesday 2 October	7:30 - 13:00
Wednesday 3 October	7:30 - 13:00, 17:00 - 19:00
Thursday 4 October	7:30 - 13:30, 17:00 - 19:00
Friday 5 October	7:30 - 13:00

Welcome Reception

Sunday, 30 September 2018, 18:30 – 21:30

The Welcome Reception will be held at the Melia Palma Marina with refreshments and an assortment of hors d'oeuvres.

Informal Dinner

Monday, 1 October 2018, 20:00 – 23:00

The Informal Dinner will take place at the Finca Son Termes restaurant.

Boat Ride

Tuesday, 2 October 2018, 13:30 – 19:00

Following in the tradition of past Collegium meetings, a boat ride will take place on the third day of the meeting. The group will depart from the Gran Melia Hotel and Melia Palma Marina Hotel at 13:30 to depart on the boat at 13:45

Gala Dinner

Thursday, 4 October 2018, 20:00 - 23:00

An elegant dinner will be held on the last evening of the Symposium at Moli de's Comte restaurant.

Time Zone

Palma, Mallorca is in the Western European Time Zone (UTC), which is one hour ahead of the Coordinated Universal Time (UTC).

Tipping

Restaurants in Palma generally do not expect a tip on orders. If you feel that the service warranted an extra amount you are free to but the general assumption is that tipping is not a requirement.

Travel Arrangements and Airport Transfers

The Palma International Airport (PMI) is the closest airport to the hotel options.

CIA will not provide airport transportation from the Mallorca Airport to the hotels but there are local options that will be available for you to choose from.

To find more details on the transportation options please see the Mallorca Airport website, http://www.palma-airport.info/transportation.html.

Venue

The 32nd Symposium of the *Collegium* will be held at the Melia Palma Marina Hotel.

Weather

The average temperature in October is between 75°F/24°C and 62°F/17°C.

2018 TOUR CHOICES

Cathedral Walking Tour

Monday, 1 October, 9:00 – 13:00
Half Day Morning (4 hours)

Walking tour in the old town of Palma including Cathedral visit

Price: €45.00

Excursion to La Granja

Monday, 1 October, 9:00 – 13:00
Half Day Morning (4 hours)

Visit the beautiful Majorcan country estate "La Granja" near by Esporales. The 17th century mansion is surrounded by lush vegetation, magnificent gardens and natural springs. Visitors will have an insight into the crafts and life of past centuries on a typical Majorcan farm. Some of these handicrafts are still in operation today! The group will get the possibility to taste typical liqueurs and pastries from the region.

Price: €50.00

Palma Bike Tour

Tuesday, 2 October, 9:00 – 11:30
Quarter Day Morning (2.5 hours)

Get to see beautiful Palma from a different angle – bike tour

Price: €35.00

Cuevas del Drach in Porto Cristo

Tuesday, 2 October, 8:30 – 12:30
Half Day Morning (4 hours)

The Cuevas del Drach are impressive dripstone caves located on the east coast of the island south of Porto Cristo. Mallorcans know of their existence for over 3,000 years but they only opened to the public in 1935. The Cuevas del Drach consist of 4 different caves and the biggest underground lake in Europe. The light spectacular is breath taking and a must see in Mallorca.

Price: €60.00

Miró Museum

Wednesday, 3 October, 9:00 – 13:00
Half Day Morning (4 hours)

Visit the Fundació Pilar i Joan Miró a Mallorca, an exclusive museum dedicated to the work of artist Joan Miró. Miró had strong ties to Mallorca as his mother and wife were born there. The main building of the museum exhibits the work donated by the artist. In addition guests can have a look at the library, a sculpture garden, the studio Sert as well as the Finca Son Boter which Miró used as atelier.

Price: €50.00

Valdermossa

Wednesday, 3 October, 9:00 – 18:00
Full Day (9 hours)

Valldemossa is a very small town in the mountains of Mallorca. It is known for being the birth place of the only Majorcan saint, Santa Catalina Tomàs. The kings of Mallorca valued the pleasant climate so much that they built their summer domiciles in this village and the Carthusian monks from Tarragona built their monastry here. The town became famous, however only after the visit of Frédéric Chopin and George Sand who spent their winter 1838/1839 in the monastry. Visit the original Monastry Valldemossa including the Chopin cell and the Palace King Sancho.

Price: €75.00

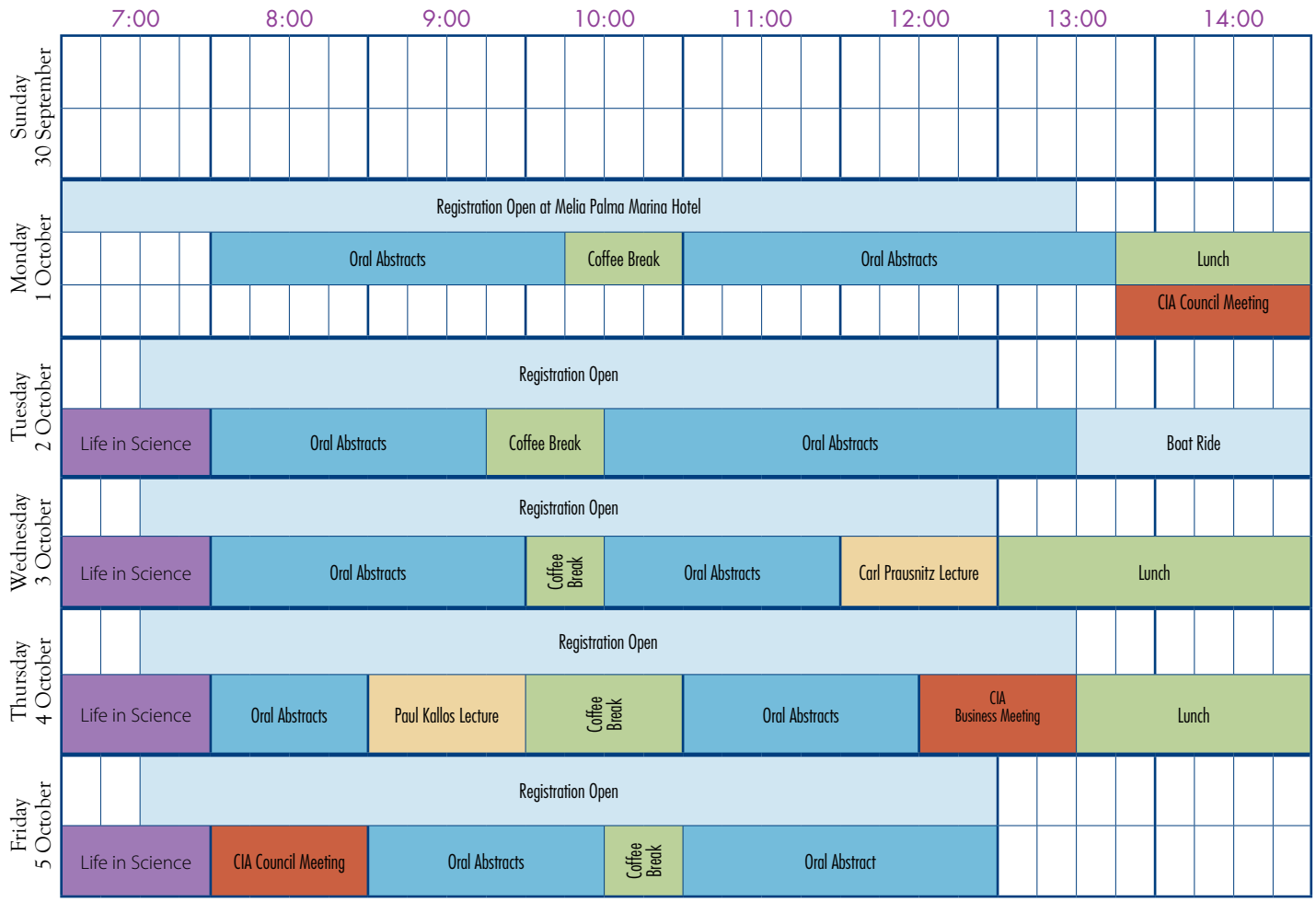
Excursion to Sóller and Sa Calobra

Thursday, 4 October, 9:00 – 18:00
Full Day (9 hours)

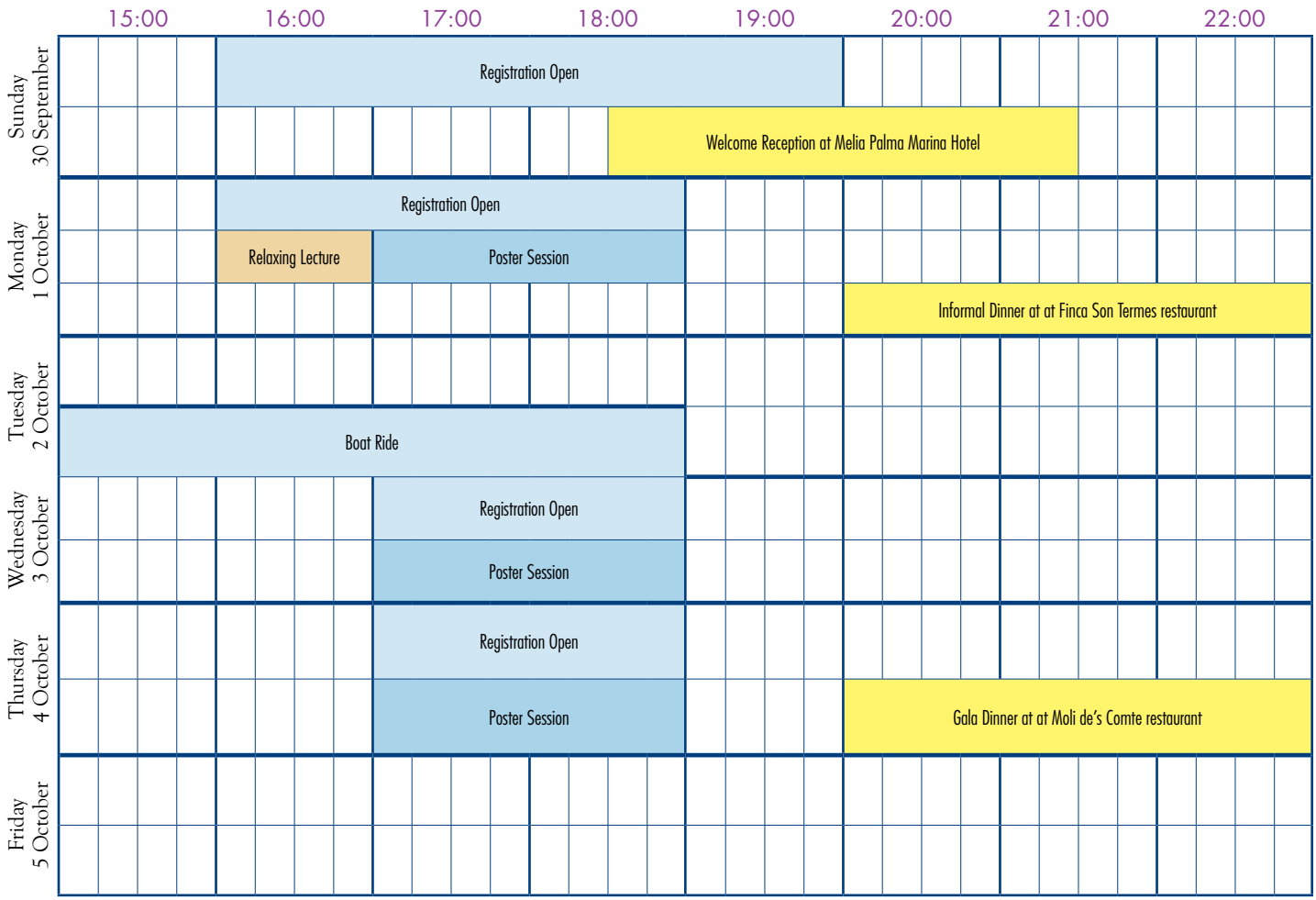
Sóller is a small village in the heart of the Tramuntana Mountains which in 2011 received the UNESCO world heritage status. The high mountain ranges separated the valley over centuries from the rest of the island, helping in that way to unfold its unique charm. The group will take the traditional and most nostalgic railway of the island "Tren de Sóller" from Palma to Sóller city. After a stroll in the city, the group can take the historic trolley to Port Sóller. Here a boat will depart to Sa Calobra, one of the most famous Majorcan beaches and bays.

Price: €90.00

2018 PROGRAM-AT-A-GLANCE



2018 PROGRAM-AT-A-GLANCE



*Schedule subject to change.

ALAIN L. DE WECK TRAVEL GRANT RECIPIENTS

For the fifth 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:

Rana Salah Abadalkareem, United Kingdom
Loubna Akhabir, Canada
Mohammad Alzahrani, United Kingdom
Lora Bankova, United States
Moshe Ben-Shoshan, Canada
Katherine Niederer Cahill, United States
Pei-Chi Chen, Taiwan
Sandra Milena Coronado Rios, Columbia
Aarif Eifan, United Kingdom
Hans Michael Haitchi, United Kingdom
Miao-Hsi Hsieh, Taiwan
Hideaki Morita, Japan
Natalia Mykhaylova, Canada
Anne Marie Singh, United States
Ursula Smole, Austria
Emily Swindle, United Kingdom
Hock Tay, Australia
Gilda Varicchi, Italy
Marieke Wandel, United Kingdom
Jeffrey Wilson, United States

Travel Grant Recipients will be awarded with a certificate during the Gala Dinner on 4 October 2018.

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

SCHEDULE OF EVENTS

The 32nd Symposium of the Collegium Internationale Allergologicum

Allergy Across the Life Course - From Origins Towards Prevention • Palma, Mallorca 30th September – 5th October 2018.

Sunday, 30 September

18:30 – 21:30 **Welcome Reception**
Location: Melia Palma Marina Hotel Terrace

Monday, 1 October

Oral Session 1:

Genomics, Environmental Factors and Precision Medicine

Moderators: Moderators: Marianne van Hage, Sweden; Stephen Holgate, United Kingdom
Time: 8:00 – 10:15
Location: Melia Palma Marina - Room 7

8:00	1	***Genetic analysis of lung function genes for asthma and chronic obstructive pulmonary disease (COPD)*** Deborah A. Meyers, United States
8:15	2	**Genome-wide association study of asthma in The Consortium on Asthma Among African-ancestry Populations in the Americas (CAAPA) recapitulates asthma risk loci in non-African populations** Kathleen C. Barnes, United States
8:30	3	***Transplant of Amish but not Hutterite gut microbiota is sufficient to protect germ-free mice from experimental asthma*** Donata Vercelli, United States
8:45	4	***Infant immune response and severity of respiratory syncytial virus infection is associated with future respiratory morbidity in early childhood*** Tina Hartert, United States
9:00	5	***Fish oil supplementation in pregnancy reduces risk of childhood asthma partly through the bacteriome and virome*** Hans Bisgaard, Denmark
9:15	6	***Sputum metagenomic profiling reveals airway dysbiosis in severe asthma linked to neutrophilic inflammation*** Peter H. Howarth, United Kingdom
9:30	7	***Integrated genome-wide expression of lung immuno-inflammatory, injury and repair pathways identifies molecular phenotypes of asthma: Possible magnification by high dose ß2 agonist use*** Brian Modena, United States
9:45	8	***Regulation of Syk activity in the FcεRI signaling pathway by antiviral innate immune adaptor MAVS*** Yuko Kawakami, United States
10:00	9	***RNA degradation acts as an anti-viral mechanism deficient in the asthmatic airway epithelium*** Rocio Teresa Martinez-Nunez, United Kingdom
10:15 – 11:00		**Coffee break**

Oral Session 2:

Detector and Effector Cells: Dendritic cells, monocytes/macrophages, neutrophils, eosinophils, and epithelial cells

Moderators: Moderators: Lisa Beck, United States; Kenji Matsumoto, Japan
Time: 11:00 – 13:45
Location: Melia Palma Marina - Room 7

11:00	10	***Women and men differ boys and girls, not so much: constitutive innate immunoregulatory homeostasis in vivo*** Kent T. HayGlass, Canada
11:15	11	***Simultaneous induction of tolerance to multiple allergens by regulatory dendritic cells from asthmatic and peanut allergic subjects*** John Robert Gordon, Canada
11:30	12	***Impaired germinal center reaction in STAT3-HIES (Hyperimmunoglobulin E syndromes) patients favors development of IgE-producing B cells and plasma cells*** Willem Van de Veen, Switzerland
11:45	13	***Combining immunofluorescent imaging and micro-CT to visualize cells and 3D networks in human lung tissue*** Jane Warner, United Kingdom
12:00	14	***Neutrophil extracellular trap formation requires OPA1-regulated glycolytic ATP production*** Hans-Uwe Simon, Switzerland

12:15 **15** ***IL-13 mediated neutrophilic airway inflammation and hyperresponsiveness is corticosteroid-resistant***
Hans Michael Haitchi, United Kingdom

12:30 **16** ***Neutrophils promote allergic inflammation by presenting allergen to specific CD4⁺ T-cells***
Barbara Bohle, Austria

12:45 **17** ***Evidence of mast cell activation in Zika virus infection***
Adrian Piliponsky, United States

13:00 **18** ***New insights into the intracellular regulation of human basophil responses***
Bernhard F. Gibbs, Germany

13:15 **19** ***Skin barrier released sphingosine-1 phosphate promotes antimicrobial activity in mast cells***
Anna Di Nardo, United States

13:30 **102** ***Genetic restriction of antigen-presentation dictates allergic sensitization and disease in humanized mice***
Winfried F. Pickl, Austria

13:45 **Lunch** (located at the Melia Palma Marina)

Relaxing Lecture

"The great Majoricans: Llull, the computer science founder, Serra; a saint founder of the Californians; and the internet of things ... that matter"

Dr Andreu Veá, Digital Champion for Spain at the European Commission

Date: Monday, 1 October
Time: 16:00 – 17:00
Location: Melia Palma Marina - Room 7

Andreu Veá is the founder and current President of the Internet Society (ISOC-ES) and is the only European to be selected to serve on the advisory board of the Internet Hall of Fame.

After his doctoral dissertation thesis on the technology, history, and social structure of the Internet (which for 8 years was one of the top 25 most downloaded, 260,000 copies), he was invited by Vint Cerf (one of the "fathers of the Internet") to continue his original research at Stanford University (California, USA), from which he launched the international research program WiWiW.org.

Mr. Veá is known as "The Internet biographer" after his book "*How we created the internet*" and in recognition of his support for the internet, this year, Mr. Veá has received a lifetime achievement award by The Spanish Internet society.

Dr. Veá has a very interesting and unique profile, being a seasoned Spanish engineer from Barcelona, turned into a historian of science and technology (at Stanford) who is passioned by the origins of our local scientists, innovators, and pioneers such as Ramon Rull, Fray Junipero Serra and others, whose under-reported importance in history will be combined with the newest technologies that are changing our lives nowadays, within an inspirational live-changing keynote that shall not leave any of us indifferent.

Poster Session 1:

Date: Monday, 1 October
Time: 17:00 – 19:00
Location: Melia Palma Marina - Room 2-6

Genetic and Environmental Factors, Effector and Immunoregulatory Cells in Allergy and Asthma, Dendritic Cells, Mast Cells, Monocytes and Granulocytes, Lymphocytes and Mediators of Immunoregulation

Moderators: Kathleen Barnes, United States; Eugene Bleecker, United States; Joshua Boyce, United States; Mitchell Grayson, United States; Bettina Jensen, Denmark; Sally Wenzel, United States

Genetic and Environmental Factors, Effector and Immunoregulatory Cells in Allergy and Asthma

20 ***A polymorphism in thymic stromal lymphopoietin (TSLP) is associated with chronic rhinosinusitis with nasal polyps and aspirin-exacerbated respiratory disease in the Japanese population***
Tomomitsu Hirota, Japan

21 ***Allergen-nanoparticle conjugates at the lung epithelial barrier – insights into particle uptake and immune responses***
Albert Duschl, Austria

22 ***Post-transcriptional gene dysregulation as a novel mechanism underlying severe asthma corticosteroid non-responsiveness***
Jennifer Rynne, United Kingdom

24 ***Low-cost sensor array devices as a method for reliable assessment of exposure to traffic-related air pollution on development of allergy and asthma***
Natalia Mykhaylova, Canada

25 *Gastrointestinal Staphylococcus aureus, immune responses to its enterotoxins and regulatory T cell dysfunction in childhood food allergy*
Anne Marie Singh, United States

26 *Interleukin 23; a glimpse into the understanding of nonallergic eosinophilic asthma*
Heung-Woo Park, United States

27 *Environmental factors controlling the peripheral differentiation of ILC2-cells*
Shigeo Koyasu, Japan

28 *HLA I shield tumor skin T lymphocytes from NK-cell-mediated elimination*
Emmanuella Guenova, Switzerland

Dendritic Cells, Mast Cells, Monocytes and Granulocytes

29 *Galectin-3 activates human IgE-bearing cells to secrete pro-inflammatory cytokines*
John T. Schroeder, United States

30 *High fat diet exacerbates skin inflammation independent of obesity: Saturated fatty acids as key players*
Jan C. Simon, Germany

31 *Innate immune signals induce the accumulation of lung mast cells during influenza infection in mice*
Jenny Hallgren Martinsson, Sweden

32 *In vivo mast cell activation by the MRGPRX2 receptor ligands in CSU and healthy subjects*
Sarbjit S. Saini, United States

33 *Inhibiting protein isoprenylation suppresses IgE- and IL-33-induced mast cell function*
John J. Ryan, United States

34 *Cockroach extract down-regulates interleukin-13-induced CCL26 expression in human airway epithelial cells*
Harissios Vliagoftis, Canada

35 *Structural alterations of allergens at the surface of engineered nanoparticles can modify their biological impact on phagocytic cells*
Martin Himly, Austria

36 *No difference in human mast cells derived from peanut allergic versus non-allergic subjects*
Bettina M. Jensen, Denmark

Pathophysiology of Allergic Disorders and Inflammation

37 *Novel mimotopes bind to IgE and block peanut allergen-induced activation of sensitized RBL SX-38 cells*
Stephen C. Dreskin, United States

38 *Surfactant protein D alleviate ozone and cigarette-induced lung inflammation and emphysema in murine model of COPD*
Miao-Hsi Hsieh, Taiwan

39 *Role of epithelial cells in aspirin-exacerbated respiratory disease*
Hae-Sim Park, Korea

40 *Phenotypes of allergen-specific T-cells in peanut and tree nut allergic patients, asymptomatically sensitized and non-sensitized tolerant subjects*
Lars K. Poulsen, Denmark

41 *Immune cell phenotype and functional defects in Netherton syndrome*
Annamari Ranki, Finland

42 *Analysis of microRNA expression in the lung and bone marrow of IL-33 challenged mice*
Madeleine Rådinger, Sweden

43 *Cutaneous immune responses to external stimuli in terms of inducible skin-associated lymphoid tissue (iSALT)*
Kenji Kabashima, Japan

44 *Role of histaminergic H4 receptors in anti-inflammatory and lung anti-fibrotic activity of glucocorticoids*
Emanuela Masini, Italy

20:00 – 23:00 **Informal Dinner**
Buses will leave from the Gran Melia Hotel at 19:30

SCHEDULE OF EVENTS

Tuesday, 2 October

Life in Science Breakfast Discussion

with Dr. Thomas Bieber
Time: 7:00 – 8:00
Location: Melia Palma Marina - Studio 3

Oral Session 3:

Lymphocytes and Mediators of Immunoregulation

Moderators: Barbara Bohle, Austria; Kelly McNagny, Canada
Time: 8:00 – 10:00
Location: Melia Palma Marina - Room 7

8:00	**45**	***Retinoic acid controls lung homeostasis by converting group 2 innate lymphoid cells to a regulatory phenotype*** Hideaki Morita, Switzerland
8:15	**46**	***The role of RORα and Innate lymphoid cells in mucosal inflammatory disease*** Kelly M. McNagny, Canada
8:30	**67**	***Genetic variation in surfactant protein-A2 alters responses to IL-13 in Asthma*** Monica Kraft, United States
8:45	**48**	***Pathogenic Th2 (Tpath2) cells in airway inflammation: Fibrosis inducing Tpath2 cells*** Toshinori Nakayama, Japan
9:00	**49**	***Crucial roles for basophils in Th2 and non-Th2 immune responses*** Hajime Karasuyama, Japan
9:15	**50**	***Generation of a novel DerP1-specific CD8⁺ T cell receptor transgenic mouse*** David M. Kemeny, United Kingdom
9:30	**51**	***Thymic stromal lymphopoietin (TSLP) confers steroid resistance to airway lymphoid cells in asthma by engaging a novel MEK-ERK kinase 2 (MEK2)-chromobox 7 (CBX7) signaling pathway*** Rafeul Alam, United States
9:45	**52**	***Use of classical and non-classical initiation codons for translation of human thymic stromal lymphopoietin (TSLP) determines its secretory pathway*** Donna E. Davies, United Kingdom
10:00 – 10:30		**Coffee break**

Oral Session 4: Food and Drug Allergies, Gastrointestinal Disorders and Autoimmunity

Moderators: Madeleine Rådinger, Sweden; Scott Boyd, United States
Time: 10:30 – 13:15
Location: Melia Palma Marina - Room 7

10:30	**53**	***Lack of gut lactobacilli in early life associates with a Th2 skewed cytokine/chemokine profile in plasma, elevated FeNO levels as well as allergy at 1-10 years of age*** Eva Sverremark-Ekström, Sweden
10:45	**54**	***A potential role for soluble Toll-like receptor 2 in the regulation of oral tolerance development*** Jean S. Marshall, Canada
11:00	**55**	***Antibody repertoires in the gastrointestinal tract of peanut allergic individuals*** Scott D. Boyd, United States
11:15	**56**	***Evidence of an abnormal epithelial barrier in active, untreated and corticosteroid-treated eosinophilic esophagitis*** Dagmar Simon, Switzerland
11:30	**57**	***Specific IgG4 and IgE antibodies to cow's milk proteins in pediatric eosinophilic esophagitis*** Thomas Platts-Mills, United States
11:45	**58**	***Immunoprofile of alpha-Gal- and B-antigen-specific responses differentiate red meat allergic patients from healthy individuals*** Marianne van Hage, Sweden
12:00	**59**	***IgE to the mammalian oligosaccharide alpha-Gal is associated with coronary atheroma volume and plaques with unstable characteristics*** Jeffrey M. Wilson, United States
12:15	**60**	***Holo-beta-lactoglobulin prevents allergic sensitization and promotes arylhydrocarbon receptor activation*** Franziska Roth-Walter, Austria

12:30 **61** *Two oral daily doses of ibrutinib, an FDA-approved irreversible inhibitor of Bruton's tyrosine kinase (BTK), markedly inhibits or eliminates skin test responses and IgE-mediated basophil activation ex vivo in adults with food allergy*
Bruce Bochner, United States

12:45 **62** *Tissue-specific stem cell origin of allergic and autoimmune diseases*
Toshiaki Kawakami, United States

13:00 **63** *Identification of 2 distinct early life eczema and non-eczema phenotypes with high risk for asthma development in a prospective birth cohort: redefining the atopic march*
Gurjit K. Khurana Hershey, United States

13:30 – 19:00 **Boat Ride – Lunch provided on Boat**
Meet in lobby of Gran Melia Hotel or Melia Palma Marina Hotel to walk to boat dock

Wednesday, 3 October

Life in Science Breakfast Discussion

with Prof. Wayne Thomas
Time: 7:00 – 8:00
Location: Melia Palma Marina - Room 9

Oral Session 5:

Asthma, Rhinitis and Atopic Dermatitis

Moderators: Glenis Scadding, United Kingdom; Kent HayGlass, Canada
Time: 8:00 – 9:45
Location: Melia Palma Marina - Room 7

8:00 **64** *Diminished IL-6 responses during picornavirus-induced asthma exacerbations are associated with chronic airway obstruction*
Fernando Martinez, United States

8:15 **65** *Atopic dermatitis subjects colonized with Staphylococcus aureus have a distinct phenotype and endotype*
Lisa A. Beck, United States

8:30 **66** *Lipid-mediator governed molecular phenotyping of asthma sub-phenotypes*
Sven-Erik K. Dahlén, Sweden

8:45 **47** *IL-33 promotes the egress of group 2 innate lymphoid cells from the bone marrow*
R. Stokes Peebles Jr., United States

9:00 **68** *Circulating miRNAs and airways responsiveness in asthma*
Kelan Tantisira, United States

9:15 **69** *Antisense Technologies – An emerging field for the development of new therapeutic molecules: Anti-GATA3 DNAzyme as a prototypic example*
Harald Renz, Germany

9:30 **70** *The functional implications of airway epithelial cell remodeling*
Nora A. Barrett, United States

9:45 – 10:30 **Coffee break**

Oral Session 6:

Urticaria, Angioedema, Anaphylaxis, Mastocytosis and Mast Cell Activation Disorders

Moderators: Mariana Castells, United States; Sarbjit Saini, United States
Time: 10:30 – 11:45
Location: Melia Palma Marina - Room 7

10:30 **71** *Mechanisms of allergen-induced activation of "Itch Nerves" terminating in mouse skin*
Bradley J. Undem, United States

10:45 **72** *The role and relevance of IgE, FcεRI, and mast cells in the pathogenesis and treatment of chronic urticaria*
Marcus Maurer, Germany

11:00 **73** *Ceramide-CD300f interaction in mast cells inhibits IgE-dependent and independent anaphylactic responses*
Jiro Kitaura, Japan

11:15 **74** *Antigen rapid IgE desensitization is driven by differential phosphorylation of SHIP1, Lyn, Syk and p38 MAPK at suboptimal antigen doses*
Mariana C. Castells, United States

11:30 **75** *The efficacy and safety of venom immunotherapy in patients with clonal mast cell disorders and hymenoptera venom anaphylaxis*
Theo Gulen, Sweden

SCHEDULE OF EVENTS

Carl Prausnitz Lecture

"The five ages of asthma: a simple concept but a complex disease"

Stephen T. Holgate FMedSci, Medical Research Council Clinical Professor, University of Southampton, UK

Date: Wednesday, 3 October
Time: 12:00 – 13:00
Location: Melia Palma Marina - Room 7

Stephen is MRC Clinical Professor of Immunopharmacology at the University of Southampton. After qualifying in Medicine in London, he pursued a research career on the mechanisms of asthma and allergy involving a wide range of different approaches. He has a particular interest in the toxicology of air pollutants and the roles of viruses and allergens as drivers of airway inflammation and remodelling. His work has resulted in over 1000 peer reviewed publications and an h index of 162. He has been President of the British Society for Allergy and Clinical Immunology, the British Thoracic Society and is currently President of the British Association for Lung Research and the Collegium Internationale Allergologicum. He has been Chair of MRC Population and Systems Medicine Board, the MRC Translational Research Group, Member of MRC and NERC Strategy Boards and Chaired the Clinical, Health and Biological Sciences Main Panel of the UK Research Excellence Framework (REF) 2014. He is a Trustee of the British Lung Foundation, Cancer Research UK and the Kennedy Trust. In 2003 he cofounded Synairgen, a drug discovery company for respiratory disease and was Chair of the UK Government Hazardous Substances Advisory Committee. He is a Member of the Governing Council of the Nuffield Council of Bioethics and the Natural Environment Research Council. His contributions have been recognised by a number of awards including The King Faisal International Prize in Medicine and the J Allyn Taylor International Prize in Medicine. He was a Founder Member of the Academy of Medical Sciences, served on its Council and founded the Clinical and Veterinary Section of the *Academie Europea*. In 2011 Stephen was awarded Commander of the Order of the British Empire (CBE) by the Queen in her Birthday Honours in recognition of his contributions to clinical science.

13:00 **Lunch** (located at the Gran Melia Hotel)

Poster Session 2:

Date: Wednesday 3 October
Time: 17:00 – 19:00
Location: Melia Palma Marina - Room 2-6

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

Moderators: Stephen Durham, United Kingdom; Stephen Dreskin, United States; Ronald van Ree, Netherlands; Jane Warner, United Kingdom

Allergens, Biomarkers and Diagnosis of Allergy

76 ***Alterations in cord blood hemopoietic progenitor cell surface receptor expression precede atopy: a 1 year follow up in a longitudinal birth cohort study***
Loubna Akhabir, Canada

77 ***Time to challenge food allergy diagnosis?***
Magnus P. Borres, Sweden

78 ***Comparative analysis of specific allergen levels in baked milk challenge materials***
James P. Hindley, United Kingdom

79 ***A new set of cockroach allergens reveals new major components and different patterns of B and T cell immunodominance in a US population of cockroach allergic patients***
Anna Pomés, United States

80 ***Development of a potential allergic reaction predictor tool for peanut challenges***
Sharon Chinthrajah, United States

81 ***Robust marker selection and cost reduction for basophil activation test usage with high specificity and sensitivity***
Uta Jappe, Germany

82 ***Raster-scan optoacoustic mesoscopy for precision assessment in allergy patch testing of the skin***
Ulf G. Darsow, Germany

83 ***Mugwort pollen is the main vector for outdoor endotoxin***
Jeroen Buters, Germany

84 ***Calcium binding protein, spermatid-associated 1: A biomarker of stress with an anti-inflammatory motif***
Dean Befus, Canada

85 ***Periostin in allergic bronchopulmonary aspergillosis: serum levels and its expression in the lungs***
Koichiro Asano, Japan

Allergen Specific Immunotherapy

86 ***Grass pollen immunotherapy: relationships among clinical response to nasal allergen challenge, seasonal symptoms, nasal tissue eosinophils and the impact of treatment compliance***
Aarif Eifan, United Kingdom

87 ***Diverse and highly cross-reactive T cell responses in ragweed allergic patients from different geographical regions***
Peter A. Würtzen, Denmark

88 ***Hypoallergenic properties of a hybrid protein from Dermatophagoides pteronyssinus allergens***
Leonardo Puerta, Colombia

89 ***Immunological biomarkers of successful immunotherapy***
Pawel Gajdanowicz, Poland

90 ***The immunomodulation effect of secreted peptide, LGp40, from Lactobacillus gasseri LGP40 in allergic diseases***
Pei-Chi Chen, Taiwan

91 ***Knowledge of pulmonologists, allergists, ENTs and paediatricians related to maintenance treatment of asthma***
Désirée E. Larenas Linnemann, Mexico

23 ***Reduced bitter taste perception of intranasal azelastine in chronic rhinosinusitis without nasal polyps (CRSsNP)***
Glenis K. Scadding, United Kingdom

143 ***Group 2 innate lymphoid cells (ILC2) and surfactant protein D (SP-D) in asthma and air pollution-induced airway inflammation***
Angela Haczku, United States

Thursday, 4 October

Life in Science Breakfast Discussion

with Prof. Ruby Pawankar
Time: 7:00 – 8:00
Location: Melia Palma Marina - Room 9

Oral Session 7: Allergens and diagnosis of allergy

Moderators: Anna Pomes, United States; Adnan Custovic, United Kingdom
Time: 8:00 – 9:00
Location: Melia Palma Marina - Room 7

8:00 **92** ***Cyclophilin – a novel cross-reactive determinant in peanuts***
Jonas Lidholm, Sweden

8:15 **93** ***Multidimensional endotypes in patients with allergic reactions revealed by topological data analysis of clinical and immunological parameters: Associations between mast cell mediator release, atopy and reaction severity***
Rana Abadalkareem, United Kingdom

8:30 **94** ***Interaction patterns between component-specific IgE antibodies in component-resolved diagnostics and prediction of asthma: Machine learning approach***
Adnan Custovic, United Kingdom

8:45 **95** ***Basophil activation as determined by basogranulin release, and altered expression of membrane-bound and intracellular basogranulin stores: Sensitive means for diagnosing allergic sensitivity***
Mohammad Alzahrani, United Kingdom

SCHEDULE OF EVENTS

Paul Kallos Lecture

"Gut reactions: Immune pathways in the intestine in health and disease"

Fiona Powrie FRS FMedSci, Director of the Kennedy Institute of Rheumatology, University of Oxford, UK
Date: Thursday, 4 October
Time: 9:00 – 10:00

Fiona Powrie is Director of the Kennedy Institute of Rheumatology, a basic and translational inflammatory sciences centre at the University of Oxford. She gained a PhD in immunology from the University of Oxford and then moved to the DNAX Research Institute in Paulo Alto. She returned to the University of Oxford in 1996 as a Wellcome Trust Senior Research Associate and she was the Sidney Truelove Professor of Gastroenterology and Head of the Translational Gastroenterology Unit from 2009-2014. Fiona's research has identified the functional role of regulatory T cells in intestinal homeostasis and established the cytokine IL-23 as a therapeutic target in chronic intestinal inflammation. Her current interests include characterisation of the interaction between the intestinal microbiome and the host immune system and how this mutualistic relationship breaks down in inflammatory bowel disease, arthritis and cancer. Fiona received the Ita Askonas Award from the European Federation of Immunological Societies for her contribution to immunology in Europe and the Louis Jeantet Prize for Medicine in 2012. She was elected a Fellow of the Royal Society in 2011, EMBO in 2013 and the Academy of Medical Sciences in 2014 and is a Governor of the Wellcome Trust.

10:00 -10:30 **Coffee break**

Oral Session 8: Pathophysiology of allergic disorders and inflammation

Moderators: Dagmar Simon, Switzerland; Paul Foster, Australia
Time: 10:30 – 13:00
Location: Melia Palma Marina - Room 7

10:30 **96** ***Epithelial barrier dysfunction in asthma: new role for protein kinase D***
Steve N. Georas, United States

10:45 **97** ***IL-24 causes epidermal barrier dysfunction downstream of the IL-13/periostin pathway in allergic skin inflammation***
Kenji Izuhara, Japan

11:00 **98** ***Novel role for the acute phase protein serum amyloid A in the initiation of type 2 immunity***
Ursula Smole, Austria

11:15 **99** ***The leukotriene E4 receptor CysLT3R regulates airway brush cell function and Type 2 inflammation***
Lora Bankova, United States

11:30 **100** ***Cysteinyl leukotriene receptor 2 drives lung immunopathology through a platelet and high mobility box 1-dependent mechanism***
Joshua A. Boyce, United States

11:45 **101** ***Differential prostaglandin E homeostasis explains sex differences in aspirin-exacerbated respiratory disease***
Katherine Cahill, United States

12:00 **103** ***Genomic and transcriptomic analyses of clara cell secretory protein (CC16) as a biomarker for asthma and chronic obstructive pulmonary disease (COPD)***
Eugene R. Bleecker, United States

12:15 **104** ***Adam33 null mice do not exhibit post-natal airway hyperresponsiveness as a consequence of maternal allergy***
Marieke Wandel, United Kingdom

12:30 **105** ***House dust mite sensitization and challenge protects against a lethal paramyxoviral respiratory infection***
Mitchell H. Grayson, United States

12:45 – 13:30 **CIA Business Meeting**

13:30 **Lunch** (located at the Gran Melia Hotel)

Poster Session 3:

Date: Thursday, 4 October
Time: 17:00 – 19:00
Location: Melia Palma Marina - Rooms 2-6

Treatment of Immune Disorders, Pathophysiology of Allergic Disorders and Inflammation, Urticaria and Angioedema, Clinical Aspects of Allergic Disorders

Moderators: Adnan Custovic, United Kingdom; Marta Ferrer Puga, Spain; Marcus Maurer, Germany; Thomas Platts-Mills, United States; Claudia Traidl-Hoffmann, Germany

Treatment of Immune Disorders

107 ***Neutralizing natural anti-IL-17F autoantibodies may protect from asthma***
Annamari Ranki, Finland

108 *Ascaris lumbricoides cystatin has strong anti-inflammatory effects on mice respiratory allergic inflammation*
Sandra Coronado Rios, Colombia

109 *Modulation of human T cell responses to peanut allergen through treatment of dendritic cells with STAT6-inhibitory peptide. A management approach for food allergies*
Christine T. McCusker, Canada

110 *Development of trans-cultural National Asthma Guidelines, broadly accepted by local physicians, using the ADAPTE approach*
Désirée E. Larenas Linnemann, Mexico

111 *Distinct immune cell phenotypes are associated with response to therapy in rheumatoid arthritis*
Thomas Issekutz, Canada

Urticaria, Anaphylaxis and Angioedema

112 *Anaphylaxis to monoclonal antibodies: new classification of symptoms and desensitization approach*
Mariana C. Castells, United States

113 *Elevated plasma S1P levels are associated with protection from anaphylaxis during food challenges in food allergic pediatric patients*
Eva Untersmayr-Elsenhuber, Austria

114 *A subset of patients with mast cell activation syndrome (MCAS) associated with postural orthostatic tachycardia syndrome (POTS) and Ehlers-Danlos Syndrome (EDS) have classic clinical MCAS features*
Mariana C. Castells, United States

115 *The effect of omalizumab in mastocytosis patients. Prospective double-blind placebo-controlled multicentre study*
Peter Schmid-Grendelmeier, Swizterland

116 *Children with limited cutaneous mastocytosis often present with systemic symptoms*
Mariana C. Castells, United Statess

117 *Management of pediatric cases of anaphylaxis at school and day-care*
Moshe Ben Shoshan, Canada

118 *Risk factors for severe systemic sting reactions in wasp and honeybee venom allergic patients*
Peter Schmid-Grendelmeier, Swizterland

Clinical Aspects of Allergic Disorders

119 *Alcohol hyper-responsiveness in chronic rhinosinusitis with nasal polyps*
Philippe Gevaert, Belgium

120 *Burden of disease and individual suffering in adults with severe atopic eczema (AE)*
Johannes Ring, Germany

121 *Chronic pruritus in skin diseases more common and more severe than we thought*
Marcus Maurer, Germany

122 *Hypersensitivity to NSAIDs: results of an Austrian cohort study*
Wolfram Hoetzenecker, Austria

123 *Patient education for adults and children with atopic dermatitis in Switzerland*
Martin Glatz, Switzerland

124 *A case of type-1 allergy to polyethylene glycols in vaginal suppository and cream*
Jan C. Simon, Germany

125 *A novel handheld digital device for objective assessment of skin lesions in atopic dermatitis and urticaria*
Maja A. Hofmann, Germany

126 *Duration of eczema from its onset and its severity are risk factors of food allergy at 2 years of age*
Yukihiro Ohya, Japan

127 *Early Detection of Gastroduodenal Disorders in Patients with Common Variable Immunodeficiency*
Gilda Varricchi, Italy

20:00 – 23:00 **Gala Dinner Event**
Location: Moli d'es Comte
Buses will leave from the Gran Melia Hotel Lobby at 19:30

SCHEDULE OF EVENTS

Friday, 5 October

Life in Science Breakfast Discussion
with Dr. Eugene Bleecker
Time: 7:00 – 8:00
Location: Melia Palma Marina - Room 9

Oral Session 9: Mast Cells and Basophils

Moderators: Emily Swindle, United Kingdom; Ruby Pawankar, Japan
Time: 9:00 – 10:30
Location: Melia Palma Marina - Room 7

9:00 **128** ***Autoantibodies to IgE and FceRI and the natural variability of SYK expression in basophils in the general population***
Donald W. MacGlashan, United States

9:15 **129** ***Specific expression profiles of microRNA in human mast cell-derived exosomes in innate and acquired immunity***
Yoshimichi Okayama, Japan

9:30 **130** ***Interleukin-33 induces histidine decarboxylase transcription and histamine accumulation in skin-derived mast cells by a mechanism involving p38 mitogen-activated protein kinase, but not c-Jun N-terminal kinase***
Torsten Zuberbier, Germany

9:45 **131** ***Interleukin-33 enhances the permissiveness of mast cells for rhinovirus replication via increased ICAM1 expression***
Emily J. Swindle, United Kingdom

10:00 **132** ***Divergent effects of acute or prolonged Interleukin-33 exposure on mast cell IgE-mediated function***
Gunnar Nilsson, Sweden

10:15 **133** ***Exosome-mediated uptake of mast cell tryptase into the nucleus of melanoma cells: a novel mechanistic axis regulating proliferation and gene expression in tumor cells***
Gunnar Pejler, Sweden

10.30 – 11.00 **Coffee break**

Oral Session 10: Allergen specific immunotherapy

Moderators: Mohamed H. Shamji, United Kingdom; Peter Creticos, United States
Date: Friday, 5 October
Time: 11:00 – 13:30
Location: Melia Palma Marina - Room 7

11:00 **134** ***Pollen exposure weakens Innate defense against respiratory viruses***
Claudia Traidl-Hoffmann, Germany

11:15 **135** ***Transcriptomic and proteomic signature of allergen-specific $CD4^+$ T and regulatory T cells during allergen-specific immunotherapy***
Milena Sokolowska, Switzerland

11:30 **136** ***Epigenetic changes in SATB1 gene in $FoxP3^+$ regulatory T cells reflect Immune tolerance status during grass pollen subcutaneous and sublingual immunotherapy***
Madison Burki, United Kingdom

11:45 **137** ***Sublingual grass pollen tablet immunotherapy: local and systemic allergen-specific IgA represents a distinct mechanism of long-term tolerance***
Stephen R. Durham, United Kingdom

12:00 **138** ***Nanoparticles as an effective adjuvant for oral peanut immunotherapy***
Marta Ferrer Puga, Spain

12:15 **139** ***Efficacy and safety of AR101: Results of the Phase 3 Peanut Allergy Oral Immunotherapy Study for Desensitization (PALISADE) trial***
Andrea Vereda, United Kingdom

12:30 **140** ***A randomized clinical trial of passive immunotherapy with single-dose anti-Fel monoclonal antibodies R1908-1909 in cat-induced rhinoconjunctivitis: Clinical efficacy endpoints and biomarkers***
Mohamed H. Shamji, United Kingdom

12:45 **141** ***Impact of allergen-specific immunotherapy on cells of the lower airways***
Ulrich M. Zissler, Germany

13:00 **142** ***Predictors of severe adverse events during venom immunotherapy (VIT), and the use of omalizumab as adjunct to VIT in patients with systemic adverse events***
Peter Korosec, Slovenia

13:15 **Closing remarks**

1

Genetic analysis of lung function genes for asthma and chronic obstructive pulmonary disease (COPD)

DA Meyers, ER Bleecker, P Howarth, X Li, SARP and SPIROMICS NHLBI investigators. University of Arizona, US and University of Southampton, UK

Background: Genome-wide association studies (GWAS) of lung function in general populations has identified more than 100 SNPs. The aim of this study is to identify genetic loci associated with FEV1, and develop a multi-gene predictive model for lung function in asthma and COPD.

Methods: Candidate SNPs in HHIP, FAM13A, GSTCD, RARB, and ZNF323 identified in general populations and Th1 genes (IL12A, IL12RB1, STAT4, and IRF2) identified in asthmatics (Li, JACI, 2013) were tested for association with FEV1 in non-Hispanic White European descents from SARP, CSGA, ACRN, and TERNOR cohorts (n=1,544) and replicated in Southampton cohort (n=584). GWAS of FEV1 was performed in 1,645 non-Hispanic White European descent smokers in SPIROMICS COPD cohort. Joint analysis of genetic scores (the number of risk alleles presented in the candidate SNPs) of 10 confirmed candidate SNPs (HHIP, FAM13A1, AGER, PID1, HDAC4, RARB, CHRNA3, RIN3, MMP12, and SERPINA1) was performed in SPIROMICS.

Results: In asthma, SNPs in HHIP, FAM13A, GSTCD, RARB, and ZNF323 were associated with FEV1 in asthmatics ($p<0.05$). Four Th1 SNPs were jointly associated with FEV1 in SARP, CSGA, ACRN, and TERNOR cohorts ($p=3.3x10^{-11}$) and replicated in Southampton cohort ($p=0.03$). In COPD, a functional rare variant in SERPINA1 (rs28929474: Glu342Lys) was significantly associated with FEV1 ($p=2.1x10^{-9}$). In addition, this variant was associated with COPD (OR=2.3; $p=7.8x10^{-4}$) and severity (OR=4.1; $p=0.0036$). Heterozygous subjects (CT genotype) had significantly lower lung function and higher percentage of COPD and more severe COPD than subjects with the CC genotype. 8.6% of the variance of post-bronchodilator FEV1/FVC can be explained by SNPs in 10 genes with age, sex, and pack-years of cigarette smoking ($P<2.2x10^{-16}$).

Conclusion: Candidate genes are associated with lung function in general asthmatic subjects. Th1 pathway genes are associated with lung function specifically in asthmatics. This study is the first to show genome-wide significant association of rs28929474 in SERPINA1 with lung function in smokers. Of clinical importance, heterozygotes of rs28929474 (4.7% of subjects) have significantly reduced pulmonary function, demonstrating a major impact in smokers. The multi-gene model is significantly associated with CT-based emphysema and clinical outcome measures of severity.

2

Genome-wide association study of asthma in The Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA) recapitulates asthma risk loci in non-African populations

Kathleen C. Barnes, Michelle Daya, Nicholas Rafaels, Sameer Chavan, Henry Richard Johnston, Aniket Shetty, Christopher R. Gignoux, Meher Preethi Boorgula, Monica Campbell, Pissamai Maul, Trevor Maul, Candelaria Vergara, Albert M. Levin, Genevieve Wojcik, Dara G. Torgerson, Victor E. Ortega, Ayo Doumatey, Maria Ilma Araujo, Pedro C. Avila, Eugene Bleecker, Carlos Bustamante, Luis Caraballo, Georgia M. Dunston, Mezbah U. Faruque, Trevor S. Ferguson, Camila Figueiredo, Jean G. Ford, Pierre-Antoine Gourraud, Nadia N. Hansel, Ryan D. Hernandez, Edwin Francisco Herrera-Paz, Eimear E. Kenny, Jennifer Knight-Madden, Rajesh Kumar, Leslie A. Lange, Ethan M. Lange, Antoine Lizee, Alvaro Mayorga, Deborah Meyers, Dan L. Nicolae, Timothy D. O'Connor, Ricardo Riccio Oliveira, Christopher O. Olopade, Olufunmilayo Olopade, Zhaohui S. Qin, Charles Rotimi, Harold Watson, Rainford J. Wilks, L. Keoki Willia, James G. Wilson, Carole Ober, Esteban G. Burchard, Terri H. Beaty, Margaret A. Taub, Ingo Ruczinski, Rasika Ann Mathias, the CAAPA Consortium.

Background: Asthma is a complex disease with striking disparities across racial and ethnic groups, but despite its relatively high burden of disease, representation of African ancestry populations in asthma genome-wide association studies (GWAS) has been limited. **Methods:** We performed a genome-wide association study (GWAS) meta-analysis of 7,009 asthma cases and 7,645 controls, the largest GWAS to date in African ancestry populations. **Results:** We observe strong evidence for association of three known asthma loci, including the well-known chromosome 17q12-21 locus, and some evidence for association for seven additional loci. We also report strong evidence for association for the chr12q13 locus, a novel un-replicated asthma locus recently identified by the Trans-National Asthma Genetic Consortium (TAGC), the largest asthma GWAS to date. We describe two novel associations on chromosome 8p23 and 8q24 that may be specific to asthma risk in African ancestry populations. **Conclusions:** In this the most definitive GWAS on asthma among populations of African ancestry, we replicate several of the strongest asthma loci not previously confirmed in African admixed populations and identify novel loci unique to populations of African ancestry.

3

Transplant of Amish but not Hutterite gut microbiota is sufficient to protect germ-free mice from experimental asthma

Justyna Gozdz[1], Linnea K. Honeker[1], Betty Theriault[2], Carole Ober[2] and Donata Vercelli[1]. [1]Asthma and Airway Disease Research Center, University of Arizona, Tucson, AZ, USA and [2]University of Chicago, Chicago, IL, USA

Novel preventive strategies are needed for childhood asthma, a disease that can currently be treated but not cured. In this respect, children raised on traditional farms are protected from asthma. The "farm effect" is largely explained by the child's early life contact with farm animals and their microbes. The asthma-protective effects of traditional farming have been well illustrated by our work (N. Engl. J. Med. 375: 411-421, 2016; N. Engl. J. Med. 375:1897-1899, 2016) in the Amish and the Hutterites, two U.S. farming populations with similar genetic ancestries and lifestyles but distinct farming practices: traditional among the Amish, and industrialized among the Hutterites. Amish and Hutterite children showed striking disparities in asthma prevalence (4-times lower in the Amish), and profound differences in the proportions, phenotypes and functions of innate immune cells. These were paralleled by profound environmental differences: median levels of home endotoxin (an index of microbial load) were 6.7-fold higher among the Amish. Most importantly, when instilled into the airways of ovalbumin (OVA)-sensitized mice, extracts of Amish but not Hutterite house dust dramatically reduced airway hyperresponsiveness (AHR), broncho-alveolar lavage (BAL) eosinophilia and OVA-specific serum IgE.

While these findings supported the asthma-protective role of environmental microbes, it remained unclear whether these microbes act directly and/or through the host's microbiome. To address this question, germ-free (GF) Balb/c mice were administered fecal microbiome transplants from healthy 3 year old Amish or Hutterite children and allowed three weeks for

microbial stabilization. AHR, BAL eosinophilia, lung cytokine mRNA and OVA-specific serum IgE were measured after sensitization and challenge with OVA. As expected, vigorous OVA-induced IgE, AHR and BAL eosinophilia were detected in unassociated GF mice. Transplant of both Amish and Hutterite fecal microbiota significantly suppressed OVA-specific IgE. In contrast, transplant of Amish but not Hutterite fecal microbiota was sufficient to abrogate AHR and suppress lung IL13 mRNA expression. Interestingly, BAL eosinophilia was not attenuated in mice associated with Amish fecal microbiota, but these cells exhibited morphological and transcriptional signatures of delayed maturation, consistent with decreased pathogenic activity. Our results suggest that the gut microbiome plays an important asthma-protective role in the Amish farm environment.

4

Infant immune response and severity of respiratory syncytial virus infection is associated with future respiratory morbidity in early childhood

Tina Hartert, Christian Rosas-Salazar, Kedir Turi, Larry J. Anderson, Jyoti Shankar, Suman Das, Stokes Peebles, and Tebeb Gebretsadik

Background: Respiratory syncytial virus (RSV) lower respiratory infections (LRIs) in infancy have been strongly and consistently associated with the development of recurrent wheeze and asthma. Whether RSV upper respiratory infections (URIs) in infancy are also associated with the development of recurrent wheeze is not known.

Methods: INSPIRE is an ongoing prospective birth cohort study designed to capture the first and any subsequent infant RSV infections and identify mechanisms of acute and chronic pathogenicity. Respiratory surveillance was conducted biweekly during RSV season during each infant's first year. A respiratory illness visit was conducted if the infant met pre-specified criteria for an illness. In infants with RSV infection confirmed by PCR, we measured the acute response to infection using multiplex bead assays for 53 immune mediators. RSV antibodies were measured in blood at one-year. RSV illness and exposures were defined as RSV LRI, RSV URI, no RSV (no RSV illness and serologically negative). All children are followed annually for the outcome of interest, recurrent wheeze.

Results: Among 1,702 infants, 791 (46%) had an RSV ARI (20% were LRI). Adjusted odds ratio (aOR, 95%CI) for recurrent wheeze among infants with RSV LRI, compared to those with RSV URI, and those with no RSV infection (reference group) were 4.8 (95%CI:3.0-7.6) and 1.7 (95%CI:1.2-2.5), respectively, with a similar exposure-dependent relationship for recurrent wheeze at ages 2- and 3-years. We also identified two distinct immune-response patterns during acute infection; the pattern characterized by high Type 2 and Type 17 inflammation and diminished anti-viral response was significantly associated with 2- and 3-year recurrent wheeze (aOR 3.34 [1.31, 8.56]; 2.64 [1.04, 6.74]).

Conclusion: RSV LRIs in infancy are strongly associated with the development of recurrent wheeze in early childhood, and RSV URIs are associated with an intermediate risk compared with uninfected infants. A distinct cytokine response pattern characterized by high Type 2 and Type 17, and low antiviral response to RSV infection is associated with subsequent increased risk of recurrent wheeze. These data support a causal relationship between RSV and recurrent wheeze, suggesting that strategies to prevent recurrent wheeze through infant RSV prevention could decrease childhood respiratory morbidity.

Acknowledgements TVH received support from NIH grants U19 AI095227, K24 AI77930, and U54 RR24975. The Vanderbilt Institute for Clinical and Translational Research (VICTR).

5.

Fish oil supplementation in pregnancy reduces risk of childhood asthma partly through the bacteriome and virome

Hans Bisgaard; Professor of Pediatrics; University Hospital of Copenhagen; Copenhagen Prospective Studies on Asthma in Childhood - COPSAC

Double-Blind Randomized Trial We performed a double-blind, randomized, controlled trial in 736 unselected pregnant women. Pregnancy supplementation with fish oil from week 24 showed a preventive effect on childhood asthma with a risk reduction of 1/3. The effect was driven by women in the lowest baseline tertile with respect to dietary intake and blood-levels of n-3 LCPUFA.

Gene – fish oil interaction FADS gene variation affects the efficiency of metabolizing fish oil. The effect of fish oil supplementation was accordingly depending on FADS gene variants. Low levels of fish oil and variant in the FADS gene identified a high-risk population of approx. 1/3 of the population, where the risk reduction o childhood asthma was more than 50%. There was no effect in the other 2/3 of the population. This allows for precision prevention.

Bacteriome mediation Fish oil effects were analyzed on microbial samples characterized by 16S gene amplicon sequencing from maternal vagina (Week 36), infant gut (1 week, 1 month and 1 year) and airways (1 week, 1 month and 3 months). Fish oil supplementation did not affect the alpha diversity in any compartment but we observed affected beta diversity and reduced relative abundances of the genera Veillonella and Gemella in the child's airway microbiota at 1 month. We observed no effects on maternal vaginal or infant gut composition. Our findings suggest that the intervention effects of fish oil on asthma risk might to a degree be explained by microbial alterations in the neonatal airways. Both airway and the gut compositions have been associated with asthma development in the children before age 6 years.

Virome mediation The human gut virome consists mainly of bacteriophages and is speculated to be important for human health either directly or through bacterial effects. We sequenced 576 viromes from infant gut (1 year). Fish oil supplementation significantly increased alpha diversity and changed beta diversity, after adjustment for bacterial composition. Whether this effect is direct or mediated through the bacteriome remains an open question.

In conclusion; Supplementation with fish oil in the last trimester of pregnancy reduces risk of childhood asthma. Measurements of FADS variants and n-3 LCPUFA blood level identifies approx. 1/3 of the pregnant women benefitting from fish oil supplementation while the other 2/3 have no effect. This is a first example of precision prevention of asthma. The preventive effect might be partly mediated through the gut bacteriome and virome.

6.

Sputum metagenomic profiling reveals airway dysbiosis in severe asthma linked to neutrophilic inflammation

Peter Howarth*, Stuart Bates, John Riley, Ratko Djukanovic, Ian Adcock, Fan Chung, Peter Sterk on behalf of U-BIOPRED
*Clinical and Experimental Sciences, Faculty of Medicine, Southampton General Hospital, Southampton SO16 6YD, GSK

Background Biologics that target aspects of type 2 inflammation have reduced the disease burden in severe asthma, as ameliorating eosinophilic inflammation reduces exacerbation risk, improves quality of life and benefits asthma control. However, these novel therapies do not target neutrophilic severe asthma and there is a need to understand the biological basis of the neutrophil airway recruitment in this severe asthma phenotype. As a previous culture independent molecular microbiological study has linked airway neutrophilic inflammation in severe asthma to the presence of potentially pathogenic bacterial (Green et al PLoS One 2014), we undertook a metagenomic analysis in severe asthma.

Methods Sputum metagenomic profiling was undertaken on bacterial 16s V4 rDNA, extracted from induced sputum samples, by MiSeq analysis and filtered DNA sequences mapped against a reference database of all proteins within the KEGG databases. Samples were analysed from four study groups, comprising Healthy controls (n=23, Cohort D), GINA step 2, steroid treated asthma (n=25, Cohort C) and two groups of GINA step4/5 severe asthma, one life-long non-smokers or < 5pk year history (n=97, Cohort A) and another containing ex (> 5pk year history) or current smokers (n= 50, Cohort B).

Results There was significantly reduced alpha diversity in severe asthma cohorts (A&B) compared to healthy controls (D) and mild asthmatics (Group D). The observed alpha diversity negatively correlated with percent neutrophils for taxa, genes and pathways.
In severe asthma, the abundance of Haemophilus influenza and Moraxella catarrhalis both positively correlated with sputum neutrophil %. Smoking status was not a main driver of microbiome differences in severe asthmatic subjects. In addition, Staphylococcal Enterotoxin genes were found in Cohort A, B, and C, but not found in D. Two other enterotoxin genes, non-hemolytic enterotoxin A, and B/C, were found only in Cohort A.

Conclusion The airway dysbiosis, particularly in relationship Haemophilus and Moraxella abundance, is linked to neutrophilic airway inflammation in severe asthma and a better understanding of the determinants of this altered colonisation may offer avenues for therapy in this severe asthma phenotype.

7.

Integrated genome-wide expression of lung immuno-inflammatory, injury and repair pathways identifies molecular phenotypes of asthma: Possible magnification by high dose β2 agonist use

Modena B, Weathington N, Whisenant T, Kaminski N, Bleecker E, Hastie A, Meyers D, Busse W, Jarjour N, Erzurum S, Tedrow J and Wenzel S.

Although asthma is a heterogeneous disease, a central defect may underlie several phenotypes. Airway epithelial cell (AEC) gene expression has enabled identification of potential underlying mechanisms. Recently, weighted gene co-expression network analysis (WGCNA) identified two gene modules, a T2 and an epithelial growth and repair (EGR) module, which associated with worsening asthma traits (Modena Am J Resp Crit Care Med 2017). Subsequently, bronchoalveolar lavage (BAL) cell gene expression was profiled in 154 asthma patients and healthy controls from the Severe Asthma Research Program. Gene expression was first related to various asthma traits, controlling for demographics and cell types using linear models (LIMMA software). Next, WGCNA was applied, identifying 49 co-expression modules. Only one module strongly but primarily inversely correlated with worsening asthma traits. This module included genes involved with cAMP signaling, the signaling mechanism for β2-agonists, including *CREM, PDE4A-B, PTGER4, CASP9*. Several epithelial growth factor receptor ligands, including amphiregulin and epiregulin, were also down-regulated with worsening asthma traits, especially increasing β2 agonist load.

100 participants had both BAL and AEC gene expression profiles from the same bronchoscopy performed on the same microarray platform. In a combinatorial analysis, 17 BAL cAMP module hub genes were combined with 8 EGR and 8 T2 hub genes from AEC modules, to generate 5 subject clusters (SCs) using K-means clustering, with high predictive accuracy according to a cross-validation and a Random Forests computation model. Two severe asthma phenotypes were molecularly defined by low BAL cAMP and AEC EGR module expression, and primarily distinguished from each other by the presence/absence of the T2 signature.

Since BAL cell gene expression indicated dysregulation of cAMP signaling in association with high β2-agonist use, a monocyte cell line (THP1), chosen since BAL cells are ~90% monocytes/macrophages, was stimulated chronically with the beta agonist isoproterenol to recapitulate ex vivo responses. 19% of the BAL cAMP genes were also differentially expressed in THP1 cells after prolonged isoproterenol, including several hub genes. Thus, combining BAL cell with AEC gene expression analysis identifies downregulation of epithelial repair pathways as integral to severe asthma phenotypes. High dose β2 agonist use may contribute to these abnormalities.

8.

Regulation of Syk activity in the FceRI signaling pathway by antiviral innate immune adaptor MAVS

Yuko Kawakami, Miho Kimura, Thea Lu, Joshua Ober, Toshiaki Kawakami

Abstract: Mast cells are the major effector cell type for allergen/IgE-mediated allergic reactions. Recent studies revealed the novel role for mast cells in orchestrating the host response to viral infections. Therefore, it is possible that, upon virus infection, mast cells and basophils may contribute to disease exacerbation in allergic patients. Here we show that influenza A virus (IAV) infection in mast cells synergizes with FceRI (high-affinity IgE receptor) stimulation to enhance the production of inflammatory cytokines. Unexpectedly, we found that the adaptor molecule MAVS in the antiviral signaling pathway inhibits the catalytic activity of Syk kinase, the key signaling molecule that is required for most, if not all, known activation events induced by FceRI triggering. Then we investigated whether FceRI signaling is controlled by MAVS. We found that the regulation of Syk by MAVS was not in the RIG-I signaling pathway-dependent fashion. Syk inhibition by MAVS seems indirect via MAVS-recruited SHP-1 phosphatase. Consistent with these results, mast cells derived from MAVS-deficient mice, unlike those from RIG-I- or IRF3-deficient mice, exhibited dramatically increased cytokine production, despite near-normal degranulation. MAVS-deficient mice showed enhanced late-phase responses in passive cutaneous anaphylaxis (PCA) experiments, which is caused by cytokines secreted from mast cells but not by histamine release, which contributes to early phase of PCA. Thus, this study demonstrates that the antiviral innate immune molecule MAVS intersects the adaptive immune FceRI signaling pathway.

9.

RNA degradation acts as an anti-viral mechanism deficient in the asthmatic airway epithelium

Taowen Wong, Peter H. Howarth, Michael Edwards, Rocio T Martinez-Nunez

Background: Common cold (rhinovirus) infections are the most frequent trigger of asthma exacerbations, the main cause of hospitalization in asthmatics that contributes to lung function decline over time. Asthma is a common chronic inflammatory disease of the airways. The mechanisms by which asthmatics exhibit increased symptoms upon respiratory infections leading to asthma exacerbations are inadequately understood. There is impaired antiviral immunity mediated via interferon-deficient responses in asthma. However, inhaled interferon therapy has failed to show the expected improvement on asthma patients in clinical trials and not all studies of cultured asthmatic cells have observed impaired interferon responses. These data suggest that other antiviral mechanisms are deficient in the airways epithelium.

Method: We applied *Frac-seq* in primary bronchial epithelial cells from asthmatics and healthy controls. *Frac-seq* (sub-cellular fractionation and RNA-sequencing) is a method to isolate and characterise mRNAs bound to polyribosomes, the cellular translational machinery. Thus, it determines which fraction of the transcriptome contributes to the proteome. RNA immunoprecipitation as well as siRNA knock-down experiments in BEAS-2B cells coupled with qPCR and protein assays were employed to determine the effects of *UPF1* depletion on rhinovirus (RV) 16 infection.

Results: *Frac-seq* data show decreased *translation* of *UPF1* (upstream frameshift 1) in asthma bronchoepithelium. *UPF1 translational* levels negatively correlate with reversibility (r=-0.7235 *P*=0.0067) and positively with FEV1 (r=0.4835 *P*=0.0485). Upf1 is the main mediator of nonsense mediated decay (NMD), an RNA surveillance pathway that degrades potentially deleterious mRNAs (e.g. mRNAs with premature termination codons) and postulated to regulate 10-30% of all mRNA transcripts. RNA immunoprecipitation showed that Upf1 directly binds RV16 RNA. Emetine treatment (a translation inhibitor that leads to upregulation of NMD targets) results in increased RV16 RNA levels. Bronchial epithelial cells transfected with siRNAs against *UPF1* showed deficient interferon (beta and lambda) induction by RV infection and decreased polyIC (a synthetic RNA that mimics viral infection)-induced signaling.

Conclusions: Together our data suggest that decreased levels of UPF1 in asthmatic bronchoepithelium may lead to ineffective rhinoviral RNA processing resulting in decreased interferon responses, contributing to their impaired antiviral response and increase in exacerbation severity.

10.

Women and Men Differ — Boys and Girls, not so much: Constitutive innate immunoregulatory homeostasis in vivo.

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Clear sex differences are evident in the prevalence and severity of many chronic inflammatory diseases. Thus, asthma is of greater impact in males during childhood but following puberty, it becomes more prevalent and severe in females. The immunologic mechanisms underlying these sex-associated differences in clinical outcomes are uncertain. Here, we examine a population of 500 asymptomatic males and females to establish if differences exist in innate immune homeostasis among healthy adults. Unexpectedly, constitutive in vivo plasma pro- and anti-inflammatory innate cytokine /chemokine levels display a pronounced bias towards increased pro-inflammatory status in healthy middle aged men vs their female spouses. Median differences ranged from 25-73% for the panel of cytokines examined ($p< 0.0001$). Conversely, in vivo expression of anti-inflammatory biomarkers (IL-1Ra, sTNF-RI, sTNF-RII) was indistinguishable between men and women, with the notable exception of IL-10. These sex specific differences were not evident prior to extra-uterine exposure to environmental stimuli (ie. at birth) in the 130 male/female babies examined. Healthy three year old children, having previously experienced a typical burden of naturally occurring URT and GI infections, also exhibited no differences in constitutive innate immune balance in vivo between males and females. Collectively the data indicate a sex-dependent bias towards increased expression of inflammatory innate immune cytokines and chemokines by healthy adult males. This differential phenotype of basal innate immune status had not developed at birth or by early childhood. Given the important roles that innate immunity plays in programming development and maintenance of chronic allergic inflammatory disorders, the data provide a baseline from which to define innate immune regulation in vivo in healthy as well as allergic individuals or those with other chronic inflammatory disorders. Support: Canada Research Chairs, CIHR, AllerGen NCE.

11.

Simultaneous induction of tolerance to multiple allergens by regulatory dendritic cells from allergic subjects

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Background. Regulatory dendritic cells (DCreg) can induce tolerance in mouse models of allergy, displacing the animal's Th2 cells with activated regulatory T cells (Treg). Human IL-10-differentiated DCreg (DC10) similarly induce asthmatic Th2 cell tolerance via induction of Treg.

Methods. Herein we explored multiple populations of human DCreg for their therapeutic potential, including cells differentiated with vitamin D/dexamethasone (DC-VitD/dex), vasoactive intestinal peptide with (DC-VIP/10) or without (DC-VIP) IL-10, or cyclosporin (DC-CsA), and used DC10 as our comparator cells. We characterized each population by FACS, ELISA and qRT-PCR for stimulatory and inhibitory marker expression, and by RNAseq and cell surface protein mass spectrometry. We also assessed the abilities of these allergen-pulsed DCreg to suppress specific allergen-presenting stimulatory DC (DCstim) activation of autologous T cells from allergic donors, their induction of Treg, and the abilities of these Treg also suppress down-stream activation of autologous Th2 cells.

Results. When titrated into DCstim/Th2 co-cultures, the specific, but not irrelevant allergen-presenting DCreg suppressed Th2 cell activation in a dose-dependent and hierarchical fashion (DC-CsA>DC-VIP/10≈DC-VitD/dex>DC-VitD>DC10). DC-CsA expressed very high levels of IL-10 mRNA relative to the other populations (≈200-fold increase vs DCstim), while DC-VIP expressed very high levels of TGFβ (≈15-fold increase). Addition of IL-10 to VIP-differentiating DCreg substantially increased their regulatory activities, as assessed by expression of HLA-DR and

immunoglobulin-like transcripts (ILT-3; 6-fold increase) and suppression of Th2 cell IL-13 (≈82% decrease) and proliferative (60% decrease) responses. These DCreg induced differentiation of $CD25^{+}CD127^{-}$ Treg, which in turn effectively suppressed DCstim activation of Th2 cells in an allergen-specific fashion. When DCreg from cat and peanut dual-allergic individuals were loaded with both cat and peanut allergen, they induced differentiation of Treg pools that could suppress autologous Th2 cell responses to either allergen, while single allergen-pulsed DCreg induced only allergen-specific Treg.

Conclusions. These data demonstrate that human DCreg can regulate Th2 cell responses, but that they carry markedly differing levels of activity. The data also provides proof-of-principle that they can be used to simultaneously target multiple allergen sensitivities among T cells of poly-allergic individuals.

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12.

Impaired germinal center reaction in STAT3-HIES patients favors development of IgE-producing B cells and plasma cells

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Background Limited data are available on the mechanisms that regulate IgE production in humans. STAT3-Hyper-IgE syndrome (STAT3-HIES) is caused by heterozygous mutations in the STAT3 gene and is associated with eczema, elevated serum-IgE, and recurrent infections.

Method To investigate the impact of STAT3 signaling on B cell responses, in particular the regulation of IgE production, we assessed lymph node and bone marrow, in vitro proliferation and antibody production, presence of B and plasma cells, and somatic hypermutation (SHM) of STAT3-HIES patients and healthy controls.

Results Lymph nodes of STAT3-HIES patients showed normal germinal center architecture with $CD138^{+}$ plasma cells residing within the paracortex. These plasma cells expressed IgE, IgG and IgM but not IgA. IgE^{+} plasma cells were abundantly present in STAT3-HIES bone marrow. Proliferation of naive B cells upon stimulation with CD40L+IL-4 was similar in patients and controls, while patient cells showed reduced proliferative responses to IL-21 and were skewed towards plasma cells differentiation. IgE, IgG1, IgG3 and IgA1 transcripts showed reduced somatic hypermutations. Elevated IgE^{+} memory B cells were found in STAT3-HIES peripheral blood, while other memory B cell frequencies with the exception of $IgG4^{+}$ cells were decreased.

Conclusions STAT3-HIES patients show signs of impaired germinal center reactions illustrated by a reduced memory B cell compartment and limited molecular maturation of select IGHV regions, supporting the need for immunoglobulin substitution therapy. IgE^{+} B cell and plasma cell development was favored in these patients, indicating that STAT3-signaling is critical for the generation of fully mature GCs that support affinity maturation, while impaired STAT3 signalling results in a weakened GC reaction, which favours differentiation to plasma cells that produce IgE.

13.

Combining immunofluorescent imaging and micro-CT to visualize cells and 3D networks in human lung tissue

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Micro-CT provides non-destructive, high resolution(5-8μM) imaging of human lung tissue and can be used to examine 3D networks of small airways and blood vessels. Reconstructing these structures manually is time consuming and individual cells such as mast cells and macrophages or fine structures such as lymphatic vessels cannot be identified using morphology alone. We have combined immunofluorescent imaging with micro-CT to obtain high quality 3D images of human peripheral lung tissue. We have segmented out the airways, vasculature and lymphatic vessels and identified individual cell types.

Human lung tissue was formalin fixed, embedded in paraffin wax and imaged in a Nikon Med-X micro-CT scanner. Images were reconstructed at 5-8μm. The tissue was sectioned and stained with antibodies to airway epithelium(cytokeratin 8/18), lymphatic vessels(LP21 staining podoplanin), mast cells(AA1) or macrophages CD68). A secondary antibody conjugated to Alexa Fluor 647 allowed us to visualize the immunostaining with minimal interference from tissue autofluorescence.

The FITC channel revealed autofluorescent collagen fibres surrounding the blood vessels and allowed us to reconstruct the peripheral vascular network. Staining with cytokeratin 8/18 santibody showed airways epithelium and combining the two with the micro-CT data set allowed us to reconstruct the 3D networks of both airways and blood vessels in a semi-automated manner. Staining with an antibody to podoplanin revealed a large number of fine lymphatic vessels throughout the tissue as well as surrounding the blood vessels and the pleural surface.

Individual cells such as mast cells and macrophages were identified by immunostaining, visualised with an Alexa Fluor 647-conjugated antibody and mapped onto the micro-CT dataset. Distance maps were created to compare the distribution of the individual cell types in relation to the different 3D networks. Mast cells were distributed throughout the tissue with evidence of some mast cells in close proximity to the airway wall. Macrophages could be seen in both the tissue and the airway lumen with clusters of cells accumulating together.

In summary, we have developed techniques to allow the rapid semi-automatic segmentation of the 3D networks in human lung tissue and plot the distribution of individual cells such as mast cells and macrophages.

14.

Neutrophil extracellular trap formation requires OPA1-regulated glycolytic ATP production

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Background: Neutrophil extracellular traps (NETs) consist of DNA and granule proteins and are able to bind and to kill extracellular pathogens. Although the functional importance of NETs is generally accepted, the origin of the DNA scaffold in NETs, as well as the mechanism of their generation, remains unclear and a matter of dispute. Optic Atrophy 1 (OPA1) is a mitochondrial inner membrane protein known for its role in mitochondrial fusion and morphology. Mutated OPA1 causes atrophy of the optic nerve leading to blindness.

Methods: We analysed reactive oxygen, NAD^+ and ATP production as well as microtubule, NET formation and bacterial killing in OPA1-deficient mouse and human neutrophils. The clearance of Pseudomonas (P.) aeruginosa was investigated in an experimental in vivo lung infection model.

Results: Lack of OPA1 reduced the activity of the mitochondrial electron transport complex I in neutrophils, resulting in ATP production through glycolysis. OPA1-regulated ATP production in these cells was required for the growth of the microtubule network and for the formation of NETs. Conditional knockout mice lacking Opa1 in neutrophils ($Opa1^{N\Delta}$) exhibited reduced antibacterial defense capability against P. aeruginosa.

Conclusion: The findings presented here provide a significant step forward in understanding OPA1 mitochondrial functions in non-neuronal cells and extends the impact of these functions to the innate immune system.

15.

IL-13 Mediated Neutrophilic Airway Inflammation and Hyperresponsiveness Is Corticosteroid-Resistant

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Background Effective treatment for severe corticosteroid refractory asthma is a significant unmet clinical need. It affects only a small percent of the asthmatic population but accounts for a disproportionate use of healthcare resources. Recent understanding of asthma heterogeneity has evolved beyond clinical characteristics, allowing definition of distinct disease phenotypes such as those defined by levels of Type 2 inflammation (Type-2 high 'eosinophilic' disease and Type-2 low 'neutrophilic' disease). A recent study using dupilumab (an antibody that blocks the common IL-4 and IL-13 receptor chain, IL-4Rα) as an add-on therapy in adults with uncontrolled persistent asthma showed efficacy irrespective of baseline eosinophil count. It suggests by blocking the receptor, dupilumab may block IL-4/IL- 13 responses not blocked downstream by corticosteroids. The aim of this work was to use a previously described IL-13 transgenic mouse to test the hypothesis that a subset of IL-13 mediated airway responses are corticosteroid-unresponsive and contribute to ongoing airways symptoms.

Method Lung specific IL-13 expression was induced using Doxycycline (DOX) in Ccsp-rtTA/Otet-Il- 13 double-transgenic (Ccsp/Il-13) mice. Littermate control mice also received DOX. Where indicated, mice received intra-peritoneal injections of 3mg/kg Dexamethasone for 3-7 days and control mice sham-treated with saline. Lung function to methacholine challenge was performed and lungs harvested for mRNA analysis and immunohistochemistry (IHC). BALF was obtained for ELISA and differential cell counts.

Results As before, Ccsp/Il-13 mice showed significant increase in bronchial hyperresponsiveness (BHR) to methacholine as well as increased bronchial smooth muscle and goblet cell metaplasia. Whilst BALF contained mixed eosinophilic and neutrophilic inflammation, neutrophils predominated. Characteristic Th2-responsive genes as well as genes characteristic of Th17 responses were elevated. Dexamethasone treatment reduced eosinophilia and the associated 'Th2' gene signature, but BHR, neutrophil numbers and the 'Th17' gene signature remained elevated.

Conclusions Although IL-13 promotes eosinophilic airways disease, it can also drive corticosteroid refractory inflammation characterized by persistent neutrophilia, Th17 cytokines and maintenance of BHR. The Ccsp/Il-13 mouse may be a useful model for dissecting the molecular pathways and mechanisms associated with predominant neutrophilic, corticosteroid refractory airway disease.

16.

Neutrophils promote allergic inflammation by presenting allergen to specific CD4+ T-cells

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Human neutrophils are abundant in allergic late-phase responses (LPR) and promote inflammation by the release of cytokines, chemokines and pathogenic compounds. We demonstrate that neutrophils may also deteriorate inflammation by direct activation of allergen-specific effector T-cells as antigen-presenting cells. The cytokines GM-CSF, IFN-γ, and IL-3, typically found in allergic LPR, enhanced the life-span, allergen uptake and expression of HLA-DM and HLA-DR of neutrophils from allergic individuals. Neutrophils exhibited a well-developed capacity for lysosomal proteolysis and presentation of allergens to HLA-DR-restricted allergen-specific T-cell clones with known epitope specificity. T-cell-activation by neutrophils involved the costimulatory molecule CD58. HLA-DR-positive neutrophils were detected in cutaneous LPR of allergic individuals induced by intradermal injections of allergen. Finally, cytokine-activated neutrophils exacerbated allergic airway responses in vivo in a human/mouse chimeric model. Our results add a compelling new immune mechanism to the pathophysiology of allergy. The antigen-presenting capacity of neutrophils contributes to the pathology of allergic diseases and may also be relevant for other T-cell-mediated inflammatory responses.

17.

Evidence of mast cell activation in Zika virus infection

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Background: The Zika virus (ZIKV) is an arbovirus (mosquito-borne), which typically causes a self-limited infection. Recent ZIKV outbreaks have been linked to congenital ZIKV syndrome, which describes a spectrum a range of central nervous system anomalies in the fetus. There is limited information on how innate immune activation may contribute to the extent of fetal injury during viral infection and pregnancy. Mast cells are resident sentinel cells that play a central role in immune surveillance. New evidence indicates that mast cells can contribute to increased disease severity in viral infections such as those caused by Dengue virus (DENV), a flavivirus related to ZIKV.

In this study, we investigated whether ZIKV induces mast cell degranulation and release of preformed mediators, and whether evidence of mast cell activation can be observed in a nonhuman primate model that mimics key features of the congenital ZIKV syndrome.

Methods and Results: Bone marrow-derived cultured mast cells (BMCMCs) and peritoneal cell-derived mast cells (PCMCs), and the human mast cell line LUVA were exposed to ZIKV FSS13025 (Cambodian strain) and Brazil Fortaleza (Brazilian strain). We observed that ZIKV infection induced significant β-hexosaminidase and chymase release.

To test whether ZIKV can induce mast cell activation in vivo, we used a pigtail macaque (Macaca nemestrina) model of congenital ZIKV infection, in which fetal brain malformations and lesions have been demonstrated.

In ZIKV-exposed fetuses, we observed moderate levels of mast cell degranulation in the meninges. Only minor mast cell degranulation was observed in the meninges of mock treated animals. Among 37 cytokines tested, only VEGF-A showed significantly increased titers upon ZIKV infection in the fetus meninges. We tested whether mast cells can secrete VEGF-A in vitro and found that both PCMCs and LUVA cells were able to secrete significant amounts of VEGF-A upon incubation with ZIKV.

Conclusions: Our findings provide evidence of increased mast cell function in ZIKV infection and pregnancy, which may contribute to fetal infection and brain injury.

18.

New insights into the intracellular regulation of human basophil responses

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Background: Basophils crucially contribute to allergies and other Th2-driven diseases by rapidly releasing both inflammatory (e.g. histamine) and immunomodulatory (e.g. IL-4) mediators following high-affinity IgE-receptor crosslinking. Although basophil-mediated responses depend on sensitization with antigen-specific IgE on these cells this does not predict the severity of

clinical symptoms of allergy per se. The aims of this study were to determine the intracellular mechanisms involved in the control of human basophil reactivity.

Methods: Primary human basophils were obtained either from buffy coats or from peanut allergic donors, following research ethics approval, and purified to over 90% purity by immunomagnetic cell sorting (negative selection). Basophils were incubated with IgE-dependent stimuli, together with unstimulated controls, for varying periods after which histamine releases and intracellular signalling protein expressions were assessed by spectrofluorometric autoanalysis and Western blotting, respectively.

Results: We observed that the constitutive phosphorylation of SH-2 containing inositol 5' phosphatase 1 (SHIP-1), but not total SHIP-1 or Syk expressions, in unstimulated basophil preparations correlated closely with maximum basophil histamine release to anti-IgE. Moreover, there was a striking inverse correlation between basophil responsiveness to IgE-dependent stimulation and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) expression, a protein which regulates intracellular calcium concentrations.

Conclusions: These results demonstrate that SERCA2 plays a critical role in basophil reactivity alongside early excitatory or inhibitory signal transduction pathways. Enhancement of SERCA2 expression or activation of this protein could therefore be considered as a novel therapeutic approach for the treatment of basophil-driven diseases.

19.

Skin barrier released Sphingosine -1 Phosphate Promotes Antimicrobial Activity in Mast Cells

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Background: MCs are best known for their capacity to release mediators in allergic reactions; however, their cytokines and antimicrobial peptides are also key in innate immunity and bacteria resistance. Despite their importance in innate immunity, the regulation of antimicrobial functions in skin mast cells are still only partially described. TLR2 ligands are potent stimulators that trigger MCs to produce antimicrobial peptides. However, since MCs in the dermis lose their TLR2 receptor; we propose Sphingosine -1 Phosphate (S1P) to function as an alternative pathway of antimicrobial activation in MCs in the skin.

Methods: Human MCs (hMC) and mouse MCs (mMC) were differentiated in vitro and stimulated with (S1P). Mouse MCs were also differentiated from *Lipocalin 2-/-* ($Lcn^{-2/-}$) and *Cathelicidin-/-*(Camp $^{-/-}$) mouse bone marrow. After S1P stimulation, supernatants were used in an agar and liquid antimicrobial assay against *S. aureus* and *E. coli*. S1P production from human keratinocytes (NHEK) was analyzed by ELISA after stimulation with commensal supernatants.

Results: We describe LCN2 activity in hMCs and mMCs for the first time. Commensal bacteria induce S1P production from NHEK and this leads to a direct increase in CAMP and LCN2 antimicrobials in MCs, expanding MC capacity to kill pathogenic bacteria. More specifically, Lipoteichoic acid (LTA), a byproduct of gram positive bacteria, and commensal bacteria *S. epidermidis, independently* induce NHEK to release S1P as a result of NHEK Sphingokinase 1 activation. NHEK release 450 pg/ml and 850 pg/ml of S1P in vitro when stimulated with LTA and *S. epidermidis* respectively. Sphingokinase 1 mRNA showed a 4 fold increase at 4 and 10 hours. Our data show that S1P antimicrobial stimulation leads to increased mouse, and human MC capacity to directly kill *S. aureus* and *E. coli*. Using *Lcn-2-/-* and *Camp -/-* we demonstrated that Lcn-2 is the main contributor to enhanced MC antimicrobial capacity against *E. coli*. In conclusion, we report, for the first time, that mast cells express LCN-2, in addition to the already known cathelicidin antimicrobial peptide, and that S1P is an important messenger that delivers commensal signals to the dermis, increasing MC antimicrobial activity and preparing MCs for fighting infections.

20.

A polymorphism in thymic stromal lymphopoietin (TSLP) is associated with chronic rhinosinusitis with nasal polyps and aspirin-exacerbated respiratory disease in the Japanese population

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Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common inflammatory disease associated with asthma, and nasal polyps cause impaired quality of life. Aspirin-exacerbated respiratory disease (AERD) is a syndrome that results in CRSwNP and asthma. AERD and CRSwNP have closely related phenotypes, suggesting an overlapping disease etiology. Recent genome-wide association studies (GWASs) have identified overlapping susceptibility loci across the allergic diseases. A significant association of the thymic stromal lymphopoietin (TSLP) locus with asthma, allergic rhinitis, allergic sensitization, asthma with hay fever, eosinophil counts, eosinophilic esophagitis and self-reported allergy has been reported. However, influences of genetic polymorphisms in the TSLP locus on susceptibility to CRSwNP or AERD are unclear. To investigate whether polymorphisms of TSLP gene affect susceptibility to CRSwNP or AERD, we conducted an association study.

Methods: We conducted an association study of CRSwNP and AERD using the eight GWAS-reported genetic polymorphisms in the TSLP locus using Japanese population (CRSwNP, 303 cases and 904 controls; AERD, 68 cases and 904 controls). We also counted eosinophils in mucosal tissues from a total of 240 patients with CRSwNP. Mucosal tissues were removed from the nasal polyps or mucosa of the ethmoid cavity.

Results: Among eight SNPs, we found significant associations between CRSwNP and rs1837253, rs380632, and rs3806933, with the most significant association being observed at rs1837253 ($P = 1.27\times10^{-6}$). We conducted conditional logistic regression analysis for rs1837253 and observed no independent association signals. We further performed an association study of AERD and observed significant associations of rs1837253 with AERD ($P = 1.16\times10^{-3}$). The direction of association of CRSwNP and AERD was consistent with findings in previous GWASs for asthma. We assessed whether the number of eosinophils in mucosal tissues of CRSwNP patients correlated with polymorphisms in TSLP gene. A significant correlation between rs1837253 genotype and the number of eosinophils was observed in linear regression analysis.

Conclusion: rs1837253 of TSLP gene is significantly associated with CRSwNP and AERD in the Japanese population. Although further genetic and functional analyses are needed, these findings improve our understanding of common genetic factors for asthma, CRSwNP, and AERD.

21.

Allergen-nanoparticle conjugates at the lung epithelial barrier – insights into particle uptake and immune responses

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Background: Inhalation represents one of the main routes for the uptake of allergens and also for engineered nanoparticles (NPs) by humans. Due to their high surface energy NPs can bind all sorts of biomolecules, including allergens. The present study focused on detailed investigations of uptake and biological effects of allergen-NP conjugates at the lung epithelial barrier using different advanced in vitro models.

Method: Fluorescently labeled TiO2 and SiO2 NPs were taken up in a time-dependent manner by human lung epithelial cells (A549, hAELVi), as shown by confocal laser scanning microscopy, flow cytometry and transmission electron microscopy.

Result: Both NPs did not induce cytotoxicity. However, it could be shown that fluorescently labeled Bet v 1 can be attached non-covalently to the NPs. These NP-allergen conjugates were taken up by type II human lung epithelial A549 cells indicating that NPs can function as delivery vehicles for allergens. Furthermore, the recently established hAELVi cell line (which represents alveolar type I cells) was able to accumulate substantial amounts of Bet v 1 – soluble or bound to mesoporous SiO2 NPs. Interestingly, the uptake kinetics of fluorescently labeled Bet v 1 into hAELVi cells was strongly modulated when bound to the SiO2 NPs: NP-conjugated allergen was taken up quickly during the first hour of incubation, reaching a saturation after 2 h, whereas free allergen was taken up slowly and steadily for at least 24 h.

Conclusion: Engineered NPs provide a model for other particles to which allergens are attached at delivery. The novel insights into NP uptake and allergen delivery at the lung epithelial barrier should be considered for assessing safety in occupational settings where co-exposure may occur.

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22.

Post-transcriptional gene dysregulation as a novel mechanism underlying severe asthma corticosteroid non-responsiveness

Jennifer Rynne, Peter H. Howarth and Rocio T Martinez-Nunez

Background: Asthma is a chronic inflammatory disease of the airways affecting ~350 million people. 5-10% of patients are considered severe asthmatics, whose asthma remains uncontrolled despite maximum therapy including high-dose of inhaled and oral corticosteroids. The underlying mechanisms of corticosteroid non-responsiveness in severe asthma remain poorly understood.

Method: We applied Frac-seq in bronchial epithelial cells isolated from human healthy controls (HC) and severe asthma patients (SA) to investigate genome-wide mRNA expression (transcription and translation), combined with small RNA-sequencing to determine microRNA expression. Frac-seq combines subcellular fractionation and RNA-sequencing, comparing total (transcribed) with polyribosome-bound (translated) mRNA. Knock-down experiments were undertaken to demonstrate the role of microRNAs and RNA binding proteins SA biology.

Results: Our work demonstrates that while mRNAs differentially expressed at the level of transcription (319 mRNAs) map to corticosteroid pathways, corticosteroid pathways are absent in mRNAs differentially translated (335 mRNAs) between HC and SA. We show that a network of only six microRNAs (small non-coding RNAs that inhibit mRNA expression post-transcriptionally) potentially regulates ~50% of differentially translated mRNAs. Transfected into primary bronchial epithelial cells from HC, this microRNA network renders bronchial epithelial cells insensitive to corticosteroids, measured by their inability to inhibit IL-1β-dependent IL-6 expression. As microRNAs regulate ~50% translational changes in SA, the remaining 50% of changes may be due to dysregulated RNA binding proteins (RBPs). We found two RBPs, ZFP36L1 and ZFP36L2, down-regulated in bronchial epithelial cells from SA patients. The levels of these RBPs correlate positively with FEV1 and inversely with airways reversibility in our cohort, suggestive of their clinical importance in SA. Data from bronchial brushes from U-BIOPRED confirms that these RBPs present lower levels in SA patients taking oral corticosteroids as compared with moderate and SA on inhaled therapy. We also find targets of ZFP36L2 up-regulated in polyribosome-bound mRNAs as compared to total mRNA. Down-regulation of these RBPs in bronchial epithelial cells abrogates the inhibition by corticosteroids of TNF-α-dependent IL-6 and IL-8 expression.

Conclusions: Together, our data suggest RNA dysregulation is at the heart of severe asthma pathophysiology and we uncover a novel post-transcriptional mechanism that may underlie corticosteroid non-responsiveness in this patient group.

23.

Reduced bitter taste perception of intranasal azelastine in chronic rhinosinusitis without nasal polyps (CRSsNP)

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Bitter taste receptors are widespread throughout the airways. Perception of bitter products secreted by microbes may be important in stimulating innate immune responses including ciliary beating and nitric oxide production.

We undertook a retrospective telephone audit of patients treated with azelastine, an anti- histamine nasal spray. They were asked to rate their taste experience on a scale of 0 to 2: 0 being no taste , 1 mild bitterness, and 2 very bitter. Outcomes were compared with the final rhinological diagnosis reached after clinic assessment. Differences in proportion of tasters (score of either 1 or 2) between groups were analysed by two-sided Chi-squared tests, using GraphPad Prism 6 (La Jolla, CA, USA), p-values <0.05 were considered statistically significant.

One-hundred and one patients were contacted , all completed the survey, 51 were male, age range 13-81. Forty-nine patients had a diagnosis of allergic rhinitis, 24 chronic rhinosinusitis with nasal polyps (CRSwNP), 15 CRSsNP, 11 non-allergic rhinitis, 2 recurrent acute rhinosinusitis (Table 1).

Only 4 (27%) of CRSsNP patients reported a bitter taste of azelastine, compared with 18 (75%) of patients with CRSwNP (p=0.031) and 36 (73%) of patients with allergic rhinitis (p=0.001). None of the 4 CRSsNP tasters reported a strong taste (score of 2); 7 (14%) of allergic rhinitis patients and 5 (21%) of CRSwNP patients did so. Seven (64%) of non-allergic rhinitis patients were tasters, as were both of the two recurrent acute rhinosinusitis patients. Age and sex did not appear to influence the results.

This supports the hypothesis that a defect in innate immunity related to bitter taste perception may be relevant to the pathophysiology of CRSsNP.

24.

Low-cost Sensor Array Devices as a Method for Reliable Assessment of Exposure to Traffic-related Air Pollution on Development of Allergy and Asthma

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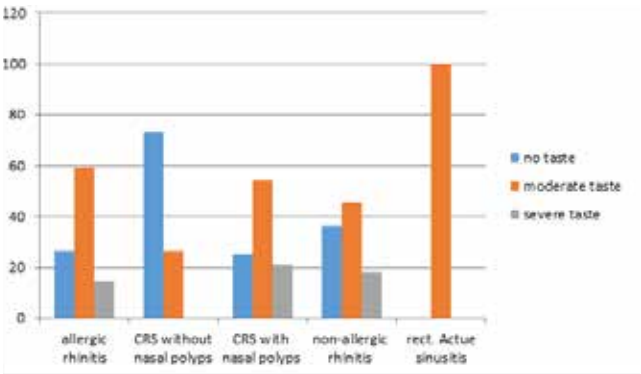
The exposure to air pollutant mixtures is a well-known risk factor for inducing and increasing the severity of diseases such as allergies and asthma. For real-time detection and monitoring of pollutant exposure, sensor arrays are an optimum choice because of versatility and aptitude for tracking composite multi-pollutant exposure. While many low-cost air pollution monitoring devices have been proposed, several underexplored opportunities remain, including sensor-derived pollution indices, source analysis and exposure assessment.

A thorough investigation of different low-cost commercial gas and particulate matter sensors from 5 manufacturers has been conducted and best-performing sensors were identified. A device for monitoring air quality has been developed and tested. Each device consists of an array of commercially available metal oxide semiconductor for monitoring NOx and O3, CO, CO2 and optical sensors for monitoring PM2.5. Level of pollutant exposure has been characterized at different locations in Toronto over 3 different campaigns between 2013 and 2016. These deployments allowed long-term sensor performance to be evaluated under different meteorological conditions as well as different ranges of pollutant concentrations.

Analysis of a large range of gas sensors revealed several key challenges, including high intra-sensor variability, interference from temperature and nonlinearity. Air quality health index estimation from sensor readings was successfully demonstrated. Three aspects of device reproducibility were evaluated: drift over time, impact of interferences and impact of site-specific mixtures. Three categories of approaches for improving sensor accuracy and reproducibility were tested: nonlinear calibration models, variable transformations and training data selection. Model reproducibility, ability to adjust for multiple combinations of interferences and ability to resolve sites was improved when devices were calibrated at multiple sites. Analysis showed that both short-term and long-term temporal patterns could be resolved and compared at different sites. Background subtraction helped further emphasize the differences and rank sites in terms traffic-related pollution.



25.

Gastrointestinal *Staphylococcus aureus,* immune responses to its enterotoxins and regulatory T cell dysfunction in childhood food allergy.

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Background: Staphylococcus aureus (SA) has been associated with several atopic diseases, including atopic dermatitis. SA is associated with disease severity and onset, and may be an important trigger of allergic sensitization. Although food allergy is often co-expressed with atopic dermatitis in early life, the role of S. aureus in food allergy remains uncertain. We sought to determine whether exposure to SA is associated with food allergy and to elucidate the potential role of its toxins on immune regulation and allergic immune responses in children with and without food allergy.

Methods: Sixty-seven children with and without food allergy were studied. The presence of S. aureus in the stool by microbial culture, specific circulating IgE and IgA antibody levels and cytokine responses of peripheral blood mononuclear cells (PBMCs) to staphylococcal enterotoxin were assessed. The effect of staphylococcal enterotoxin on regulatory T cells was evaluated by flow cytometry.

Results: S. aureus was detected in the stool in 53.3% of children with food allergy, compared to 15.4% of children without food allergy (p=0.04). Food allergic children also had higher levels of sIgA to staphylococcal enterotoxins (SEB: 1.35+/- 0.50 vs 2.21+/11.00 kU/L, p=0.003; SEA: 1.97+/-0.70 vs 2.59 +/-1.30 kU/L, p=0.003). PBMC exposure to staphylococcal enterotoxin B (SEB) promoted regulatory T cell dysfunction in food allergic children, with increased Il-17 expression of CD25+CD127loFoxp3+ cells that express SEB responsive T cell receptors (5.65% vs 16.6% p=0.01). In vitro exposure of PBMC to SA toxins uniquely revealed enhanced Th2 and Th17 responses and reduced IFNγ responses in children with food allergy, compared to responses from non-food allergic children.

Conclusion: Food allergy was associated with increased presence of S. aureus in the stool, regulatory T cell dysfunction, enhanced Th2 and Th17 cytokine responses, and IgA rather than IgE responses. These results suggest that S. aureus may participate in promoting allergic inflammation and regulatory T cell dysfunction in food allergy.

26.

Interleukin 23; a glimpse into the understanding of nonallergic eosinophilic asthma

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Introduction: We previously reported that eosinophilic airway inflammation significantly reduced by blocking IL-23 in a murine model of asthma. In addition, we observed that IL-23 expressions in induced sputum of patients with non-allergic eosinophilic (NAE) asthma were significantly increased compared to those with non-allergic non-eosinophilic asthma. Based on these findings, we performed this study to test our hypothesis that intranasal administrations of nonspecific airway irritants with IL-23 in mouse resulted in airway hyperresponsiveness (AHR) and eosinophilic airway inflammation which mimicked NAE asthma in human.

Methods: We selected a synthetic analogue of dsRNA [polyinosinic-polycytidylic acid (poly IC; 0.01 μg], lipopolysaccharide (LPS; 0.1 μg), and diesel exhaust particles (DEPs; 10 μg) as nonspecific airway irritants. Each nonspecific irritant was administered to mice intranasally with IL-23 (0.1 μg) at day (D)1, D2, D3, D10 and D14. Mice then were sacrificed and methacholine AHR, inflammatory cells in brochoalveolar lavage (BAL) fluid, and innate lymphocyte (ILC) and innate cytokine profiles in lung homogenate were measured at D21.

Results: An intranasal co-administration of poly IC and IL-23 showed significantly increased AHR and eosinophils in BAL fluid compared to IC only, IL-23 only, or sham administration. These changes were accompanied by significant increases in ILC2 proportion and IL-33 level. A co-administration of DEP and IL-23 showed similar phenotypes, but ILC3 proportion and IL-1b level were significantly increased in this model. Meanwhile, LPS with IL-23 did not show any significant phenotypical and immunological changes.

Conclusions: Intranasal co-administrations of poly IC with IL-23 or DEP with IL-23 in mouse resulted in AHR to methacholine and eosinophilic airway inflammation, although mediating innate cells were different between two models (ILC 2 in poly IC with IL-23 model and ILC3 in DEP with IL-23 model). This is the first study to show a key role of IL-23 in a murine model of NAE asthma. Given that IL-23 expressions significantly increased in induced sputum of patients with NAE asthma, this murine model provides new insight in understanding NAE asthma in human.

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27.

Environmental factors controlling the peripheral differentiation of ILC2

Shigeo Koyasu

Group 2 innate lymphoid cells (ILC2) are derived from common lymphoid progenitors (CLP) via several specific precursors, and the transcription factors essential for ILC2 differentiation have been extensively studied. However, the external factors regulating commitment to the ILC lineage and the sites and stromal cells that constitute the optimal microenvironment for

ILC2-specific differentiation are not fully defined. Here, we demonstrate that three key external factors, the concentration of IL-7 and strength and duration of Notch signaling, coordinately determine the fate of CLP toward the T, B, or ILC lineage. Additionally, we identified three stages of ILC2 in the fetal mesentery that require STAT5 signals for maturation—ILC progenitors, $CCR9^+$ ILC2 progenitors, and $KLRG1^-$ immature ILC2. We further demonstrate that mesenteric mesenchymal cells support ILC2 development. Collectively, our results suggest that early differentiation of ILC2 occurs in the fetal liver via IL-7 and Notch signaling, while final differentiation occurs in the periphery with the aid of mesenchymal cells.

28.

HLA I shield tumor skin T lymphocytes from NK-cell-mediated elimination

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Targeted therapies against cell surface molecules open a new perspective in the management of cutaneous T-cell lymphoma (CTCL). Antibody-dependent cellular cytotoxicity (ADCC) mediated by natural killer (NK) cells is a key mode of action of these drugs. However, since progressive impairment of cellular immunity is a hallmark of CTCL, we questioned the fact that patients with late stage CTCL will still be in a possession of fully functional ADCC.

We isolated NK cells from patients with MF stage I-IV, Sézary Syndrom (SS) patients and healthy individuals. An aCella-TOX GAPDH assay was used to detect the amount of endogenous glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the level of ADCC in each individual patient.

Indeed, ADCC in CTCL patients was severely abrogated. The percentage of NK cells in the blood of CTCL patients was within normal limits. Trogocytosis, a mechanism of cellular communication that can hamper ADCC by cleaving the surface of the tumour cells from the targeted molecule, did not play an essential role in CTCL. However, overexpression of MHC I on the malignant skin tumour cells in CTCL was important factor in helping them escape NK-cell activity. Furthermore, MHC I blockade could restore impaired ADCC.

Understanding the immunological mechanisms behind ADCC may help improve NK cell activity in CTCL patients and overcome resistance to treatment.

29.

Galectin-3 Activates Human IgE-bearing cells to Secrete Pro-inflammatory Cytokines

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Background/Rationale: Human basophils release histamine and secrete IL-4/IL-13 when co-cultured with the lung epithelial cell line, A549 –an adenocarcinoma (J Immunol 199:855, 2017). In that these responses proved dependent on cell-to-cell contact, basophil expression of IgE, and were inhibited by n-acetyllactosamine, we hypothesize involvement of a galectin (Gal). Indeed, studies show that both gal-3 and gal-9 bind IgE, potentially modulating the function of a variety of IgE-bearing cells. To investigate this possibility, we tested these lectins for their capacity to activate human basophils, monocytes and dendritic cells (DC), comparing responses to those following IgE-dependent activation. **Methods:** Cells were prepared using established protocols. rhGal proteins were tested in soluble form and immobilized on microspheres (MS) to better mimic cell-to-cell contact. IL-3 and anti-IgE were tested for co-stimulatory effects. Supernatants were tested for cytokines after 1-20h. **Results:** In the presence of IL-3 (10 ng/ml), basophils (n=5-8) secreted ~3-fold more IL-4 and IL-13 when co-cultured with 5μM-sized MS coated with gal-3 (MS-gal-3), compared to MS with BSA (MS-BSA) [$P \leq 0.007$] or with gal-9 (MS-Gal-9) [$P \leq 0.047$]. Soluble gal-3 (sGal-3) had minimal effect on basophil IL-4/IL-13 and only at 1mg/ml. Likewise, monocytes (n=4) and DC subtypes [both plasmacytoid, pDC (n=8) and myeloid, mDC (n=6)] showed marked secretion of TNF-α/IL-6 when co-cultured with MS-Gal-3, with levels (0.4-30ng/10^6 cells) averaging 20-1000-fold greater than those induced by MS-Gal-BSA or MS-Gal-9. These monocyte/DC responses did not require IL-3, although this cytokine significantly enhanced MS-GAL-3-dependent TNF-α/IL-6 from pDC, which knowingly express CD123 ($P=0.017$). Ranked responses to MS-Gal-3 indicated: monocytes≥pDC>mDC. sGal-3 stimulated monocytes/DC, but only at concentrations ≥100ng/ml. Overall. MS-Gal-3 activated basophils/monocytes/DC for cytokines consistent with IgE-dependent activation. **Conclusions:** Gal-3 is implicated in many diseases ranging from asthma to cancer. Identifying this lectin as a modulator of basophil/monocyte/DC function, gives plausibility as to how it might activate these IgE-bearing cells for pathogenesis extending beyond allergic disease.

30.

High fat diet exacerbates skin inflammation independent of obesity: Saturated fatty acids as key players

Jan C. Simon

Background: Epidemiological evidence has linked obesity to the risk and severity of various inflammatory disorders, including type II diabetes, cardiovascular diseases, hepatic steatosis, asthma, neurodegeneration, inflammatory bowel disease, arteriosclerosis, and psoriasis. Consequently, interactions between the adipose tissue, metabolism and the immune system are postulated to be of importance in the pathogenesis of obesity-associated inflammatory diseases. In obesity, hypertrophic adipocytes secret high amounts of adipocytokines resulting in low-grade inflammation amplified by infiltrating pro-inflammatory macrophages, oxidative stress, hypoxia and lipolysis. It is known, that these chronic pro-inflammatory conditions support the development of type II diabetes and cardiovascular diseases, while mechanisms of obesity-related exacerbation of chronic inflammatory disorders are still unclear.

Results: As shown in previous human studies, in the present study waist-to hip-ratio positively correlated with disease severity in plaque type psoriasis patients. Consistently, high fat diet

induced obese mice develop a more pronounced psoriasis-like skin inflammation. Obesity per se did not alter the pro-inflammatory status of skin and immune cells, but rather renders them more susceptible to pro-inflammatory stimuli.

Correlation analyses in a cohort of psoriasis vulgaris patients and in our mouse model revealed free fatty acid (FFA)-serum-levels as the only obesity-associated parameter affecting disease severity. Importantly, an increase of FFAs in healthy, lean mice alone was sufficient to induce an exacerbation of psoriasiform inflammation. Consequently, reduction of nutritional saturated fatty acids (SFA) alone diminished the psoriatic phenotype in obese mice.

Mechanistic studies revealed that SFA alone did not affect the pro-inflammatory immune response of myeloid cells but renders them more susceptible to pro-inflammatory stimuli. In detail, SFA sensitize DCs resulting in augmented secretion of TH1/TH17-instructive cytokines upon pro-inflammatory stimulation resulting in amplification of TH1/TH17 immune responses. Similarly, SFAs sensitize macrophages to an increased inflammatory response in answer to pro-inflammatory stimuli which in turn augments the activation of keratinocytes in an IL-1β dependent manner.

Conclusion: In our studies, we uncover nutritional SFAs as major risk factors for the amplification of skin inflammation, independent of obesity-related parameters, like fat mass extension, adipocytokines and glucose homeostasis. Thus, our findings open new perspectives for adjuvant dietary measures accompanying anti-inflammatory psoriasis therapies in lean and obese patients.

31.

Innate immune signals induce the accumulation of lung mast cells during influenza infection in mice

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Background Mast cells accumulate at specific sites of the lung and release symptom-causing mediators thereby participating in the pathogenesis of allergic asthma. The most common cause behind the exacerbations of asthma symptoms is respiratory virus infections. Still, very little is known about how respiratory virus infections influence the mast cell populations in the lung. We recently demonstrated that influenza infection stimulates the recruitment of mast cell progenitors to the lung. In the present study, we tested whether the accumulation of mast cells upon influenza infection was due to innate stimulation or whether the development of an adaptive immune response was required for the influenza-induced recruitment of mast cell progenitors to the lung to occur.

Method $RAG2^{-/-}$, $TLR3^{-/-}$ or $ST2^{-/-}$ mice and their wild type controls were given a H1N1 influenza infection (Puerto Rico 8) adapted to mice or receptor agonists intranasally. In some experiments, anti-CD4 depleting antibodies were used. Lung mast cell subpopulations were quantified by flow cytometry.

Results We found that the increase in lung mast cells upon influenza infection occurs independently of adaptive immune cells, as mice depleted of $CD4^{+}$ cells and $RAG2^{-/-}$ mice had an intact influenza-induced recruitment of mast cell progenitors to the lung. Nevertheless, the development of adaptive immune responses after the first exposure to influenza virus protected the mice from a second wave of mast cell progenitors infiltrating the lung upon re-infection. The mice exposed to primary influenza infection still harbored more lung mast cells than mice that were unexposed to influenza virus seven weeks after the primary infection. Poly I:C, a synthetic analogue of viral dsRNA, or IL-33, induced a TLR3- or ST2-dependent increase in lung mast cell progenitors, respectively. However, the recruitment of mast cell progenitors to the lung in response to influenza infection was intact in mice lacking TLR3 or ST2 alone.

Conclusions We conclude that multiple innate receptors likely trigger the influenza-induced recruitment of mast cell progenitors to the lung and that some of the emerging lung mast cells remain in the lung after the resolution of inflammation.

32.

In Vivo Mast Cell Activation by the MRGPRX2 Receptor Ligands in CSU and Healthy Subjects

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Background: The MRGPRX2 (MAS-related G protein-coupled receptor X2) receptor activates mast cells in a non-IgE mediated manner, and is upregulated 2 fold by protein expression the skin of patients with chronic spontaneous urticaria (CSU). While in vitro studies show MRGPRX2 activation by substance P, VIP, compound 48/80 and therapeutic drugs associated with pseudoallergic reactions, no human studies of MRGPRX2 activation have been performed. We examined skin mast cell responses to MRGPRX2 activation using 2 known MRGPRX2 ligands (icatibant and atracurium) in healthy and CSU subjects.

Methods: Healthy controls (n=10) and urticaria subjects (n=8) underwent serial intradermal skin. Five, 10-fold serial dilutions to histamine, insulin (negative control) atracurium and icatibant (1×10^{-6} to 1×10^{-1} mg/ml) were tested on the volar aspect of the forearms. Wheal size was calculated after 15 minutes. The area under the curve (AUC) was calculated for histamine and the 2 ligands.

Results: The AUC for healthy and urticaria subjects for icatibant was 9.8 ± 1.76 mg/ml and 23.2 ± 4.53 ($P < .01$) and 9.7 ± 2.31 and 21.5 ± 3.96 ($p=.02$), respectively. No major differences were noted in the histamine dose response curve between subject groups.

Conclusion: The AUC for atracurium and icatibant supports that there is a significant heightened sensitivity to MrgX2 ligands in CSU subjects as compared to healthy controls. Further studies are needed to establish whether MrgX2 inhibition could be a potential disease target in CSU.

33.

Inhibiting Protein Isoprenylation Suppresses IgE- and IL-33-induced Mast Cell Function

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Mast cell activation by IgE or IL-33 elicits powerful inflammatory responses contributing to allergic disease, a significant health burden in need of novel therapies. Cholesterol-lowering statin drugs targeting HMG Co-A reductase (HMGCR) suppress allergic inflammation in some clinical trials, but effects are inconsistent. Our data suggest this variability is due to statin resistance that can be circumvented by targeting geranylgeranyl transferase (GGT) downstream of HMGCR. We find that GGT inhibitors suppress IgE- and IL-33-mediated degranulation and cytokine secretion. These results are consistent in vivo, as GGT inhibitors suppress passive systemic anaphylactic shock, even in mouse strains that are resistant to statins. We postulate that Ras family geranyl modification is required for IgE- and IL-33-induced functions, including enhanced glycolysis used to generate ATP needed for inflammation. These data suggest that GGT may be an effective target in allergic disease.

34.

Cockroach Extract Down-regulates Interleukin-13(IL-13)-induced CCL26 expression in human Airway Epithelial Cells

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Background: The airway epithelium is the main barrier between the host and the external environment, and its activation by allergens is a key step towards activation of innate and adaptive immunity in asthma. Inhaled allergens activate epithelial cells through multiple mechanisms to induce pro-inflammatory effects that in many cases function to polarize the immune system towards a Th2 response. IL-13 is a pleiotropic Th2 mediator with multiple effects on the airway epithelium in asthma. Little is known, however, regarding the interactions between allergen- and IL-13-mediated epithelial cell activation in asthma. Here we studied the interactions between IL-13 and cockroach extract (CE) (a mix of multiple allergens) on the release of CCL26 (a potent eosinophil chemoattractant) from human airway epithelial cells.

Methods: A bronchial epithelial cell line (BEAS-2B) and normal human bronchial epithelial cells (NHBE) were stimulated with IL-13, CE or both. CCL26 mRNA induction was quantitated using qRT-PCR and the release of CCL26 protein by ELISA. Direct effects of CE on IL-13 were studied by Western blotting.

Results: CE prevented IL-13-mediated induction of CCL26 mRNA and protein in BEAS-2B and NHBE cells in a time and dose dependent manner. Heat inactivated CE and CE pre-incubated with aprotinin, an inhibitor of trypsin-like serine proteases, did not prevent IL-13-mediated CCL26 mRNA induction, indicating that the CE effect is mediated by a trypsin-like serine protease. However, trypsin, the prototype mammalian serine protease, did not prevent IL-13-induced CCL26 upregulation at concentrations with similar protease activity, indicating that CE serine proteases are unique in mediating this effect. CE did not inhibit early steps of IL-13 signaling and did not affect IL-13 receptor expression on epithelial cells. CE degraded IL-13 in a time-depended fashion, but IL-13 degradation by CE was only partially inhibited by heat inactivation or aprotinin.

Conclusion: CE inhibits IL-13-mediated CCL26 up-regulation in human airway epithelial cells. This effect is mediated by CE serine proteases. The ability of CE to prevent the release of eosinophil chemotactic factors by IL-13 may dampen the local effects of Th2 mediators in the airways in favor of a stronger CE-initiated inflammatory response. The mechanisms and biological significance of this effect require further study.

35.

Structural alterations of allergens at the surface of engineered nanoparticles can modify their biological impact on phagocytic cells

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In occupational settings association of allergens with engineered nanoparticles (NPs) may occur. Due to their high surface energy NPs have the capacity of binding allergens non-covalently, leading to formation of an allergen corona. In this context, the binding affinity and specificity of allergens compared to bystander substances in crude extracts were studied on solid and mesoporous SiO2 NPs. Notably, we found selective binding of the native major birch pollen allergen Bet v 1. This allergen showed very slow replacement kinetics when incubated with solutions having a high protein excess, such as serum-containing cell culture medium, demonstrating a high stability of the allergen corona (depot effect). Surface association of recombinant allergens onto different NPs can alter allergen conformation, as was studied on different solid vs. mesoporous SiO2 NPs with a set of structure analysis techniques. These structural alterations can impact the allergic response by interfering with the binding of the IgE molecules to the allergenic epitopes recognized by sensitized individuals. Using the human macrophage-like THP-1 cell line we observed a time-dependent uptake behavior of allergen-NP conjugates employing life cell imaging, confocal laser scanning microscopy and flow cytometry. Furthermore, we investigated alterations in the cytokine secretion profile of treated THP-1 cells. In order to mimic an ongoing inflammatory process type 1 and type 2-polarized states were induced and characterized by mRNA expression and ELISA. The combination of allergens with NPs induced an elevation of the pro-inflammatory cytokines TNFα and IL1β, whereas secretion of the anti-inflammatory cytokine IL-10 remained unaffected in unpolarized, type 1-, and type 2-polarized macrophage-like cells. The here presented effects of allergen retention and pro-inflammatory potential of non-covalent allergen-NPs conjugates may suggest an adjuvant function of SiO2 NPs.

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36.

No difference in human mast cells derived from peanut allergic versus non-allergic subjects

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37.

Novel mimotopes bind to IgE and block peanut allergen-induced activation of sensitized RBL SX-38 cells

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Rationale. IgE-mediated immediate hypersensitivity reactions occur when an allergen effectively cross-links IgE/FcεR1 complexes on the surface of mast cells and basophils. We hypothesized that linear mimotopes of binding of allergen-specific IgE and selected human monoclonal antibodies to Ara h 2 can block allergen-induced activation of sensitized RBL SX-38 cells.

Methods. We screened a phage peptide library (12-mer peptides) with Ara h 2/6-affinity-purified serum IgE and Ara h 2-specific human IgG monoclonal antibodies (mAb) obtained from the B cells of patients undergoing oral immunotherapy. For these studies, all mimotopes were expressed on phage. Binding to PN-sIgE was confirmed by ELISA. The ability of these constructs to inhibit allergen-induced cross-linking of IgE/FceR1 complexes was assessed using RBL SX-38 cells sensitized by a serum pool from 10 highly peanut allergic donors. Mimotopes were sequenced and aligned with the primary structure of Ara h 2.

Results: We identified over 100 unique mimotopes. These mimotopes are variably recognized by peanut specific IgE from 52 individual patients with approximately 10% of the mimotopes being identified by all subjects and 70% of the sera containing IgE that recognizes ~70% of the mimotopes. Also the mimotopes are recognized by a panel of 19 Ara h 2-specific IgG mAb. Preincubation of Ara h 2 with individual phage expressing a unique mimotope inhibited Ara h 2-induced activation of sensitized RBL cells by up to 80%. If 2 different phage, each expressing a unique mimotope were used, inhibition was 85-95%. Finally, selected combinations of mimotope-expressing phage could inhibit crude peanut extract induced activation of sensitized RBL cells by up to 85%. Upon sequencing, these mimotopes fell into two categories: those with homology to one of four linear sequences and those that had no association with linear sequences and presumably represent conformational sequences.

Conclusions: We have identified novel 12mer peptides that, when expressed on bacteriophage, are able to interfere with peanut allergen-induced IgE/FceR1-induced activation of sensitized RBL cells.

38.

Surfactant protein D alleviate ozone and cigarette-induced lung inflammation and emphysema in murine model of COPD.

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Background: Chronic obstructive pulmonary disease (COPD) is characterized by lung inflammation that persists after smoking cessation. This inflammation is heterogeneous but the key inflammatory cell types involved are macrophages, neutrophils and T cells. Other lung cells may also produce inflammatory mediators, particularly the epithelial cells. Surfactant protein D (SP-D) is innate immune modulator, which acts as the first line defense against lung pathogens and prevents inflammatory responses. Previous studies showed there is increased expression of SP-D in the patients of COPD as compared to healthy people, suggesting that SP-D may play an important role in the development of COPD. Accordingly, we hypothesized that SP-D improved the symptoms of COPD by decreasing inflammation and reducing ROS in the lung.

Methods: To examine the role of SP-D in COPD, we established the mouse model of cigarette smoke-induced, and ozone-induced COPD.

Results: We found that ozone exposed, and cigarette smoke-induced SP-D$^{-/-}$ mice have reduced the lung functions, increased the number of macrophages and neutrophils infiltration and the expression of inflammatory cytokines/chemokines in the lung as compared to ozone exposed wild type mice. Moreover, our results showed that intra-tracheal administration of exogenous SP-D in the ozone-induced mouse model can improve lung functions, decreased the number of inflammatory cells infiltration and reduced the production of inflammatory cytokines. To explore the mechanism of anti-oxidant role of SP-D in COPD, A549 and BES-2B cell lines were pretreated with SP-D before subjected to H2O2 exposure, the results showed that SP-D pretreatment can counteract and decreased H2O2-induced ROS production by increasing the expression of Nrf2, which is a transcriptional factor regulated the antioxidant gene expression.

Conclusions: Taken together, our results indicate that SP-D may play a protective role through ROS inhibition and reduce cell apoptosis via increasing the Nrf2 protein expression to alleviate the symptoms of COPD.

39.

Role of epithelial cells in aspirin-exacerbated respiratory disease

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Background and hypothesis: Clinical features of aspirin-exacerbated respiratory disease (AERD) are characterized by overproduction of cysteinyl leukotrienes (LT) E4 and persistent eosinophilia where we hypothesized airway epithelial cells (AECs) have key roles. Therefore, we investigated the roles of 4 epithelial cell-derived molecules, periostin, TGFβ1, surfactant protein D(SPD) and folliculin(FCN) in the pathogenic mechanism of airway inflammation/remodeling in AERD compared to aspirin-tolerant asthma(ATA)and normal control(NC) in in vivo/ in vitro/ex vivo settings.

Results: Serum levels of periostin, TGF- β1and FCN measured by ELISA were significantly higher in AERD than in ATA and NC groups, while serum surfactant protein D (SPD) level was significantly lower in AERD group. The subjects with high periostin level had higher prevalence of severe asthma, higher peripheral eosinophil counts and serum TGF-β1 level($P<0.05$, respectively). The patients with high FCL level had significantly lower PC20 methacholine. In in vitro setting, when AECs (both A549 cell line and primary epithelial cells) were co-cultured with peripheral eosinophils from AERD patients and/or LTE4, periostin, TGF-β1 and FCLN production increased from AECs along with increased production of IL-8, increased expression of α-smooth muscle actin and disruption of epithelial tight/adherens junctions. Significant correlations were noted among IL-8/periostin/TGF-β1/FCN release from AECs. LTE4-exposed mice showed increased eosinophil counts with decreased SPD level in bronchoalveolar lavage fluid/sera, which was attenuated by nintedanib (enhancing the production of SPD) through protecting AECs against eosinophils. In a mouse model of ovalbumin-induced asthma, levels of periostin and TGF-β1 as well as Th2-cytokines remarkably increased in bronchoalveolar lavage fluid, in which SPD treatment reduced the production of these cytokines through enhancing Th1 response.

Conclusions: These findings suggest that persistent exposure to LTE4 and eosinophilia reduces SPD production, but drives periostin/TGF-β1 production from AECs , which leads to Th2-driven inflammation/remodeling in AERD; SPD treatment may provide a potential benefit through shifting to Th1 response. As increased FLCN induces activation and disintegrity of AECs, the modulation of FLCN may be a potential target for AERD.

40.

Phenotypes of Allergen-specific T-cells in Peanut and Tree Nut Allergic Patients, Asymptomatically Sensitized and Non-Sensitized Tolerant Subjects

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Background: An imbalance in the Th1/Th2 cell expression is observed in allergic diseases, where Th cell polarization has been shown to correlate with clinical reactivity. We aimed to investigate the polarity of tree nut and peanut allergen-specific Th cells in subjects with confirmed tolerance or allergy to multiple nuts, and hereby detect differences in allergen-specific Th cells within the same study population.

Methods: PBMCs from 35 donors, all assessed for clinical reactivities to hazelnut, walnut, cashew nut, pistachio nut and peanut, were stimulated with whole nut extracts for 7 days. CD4+ Th cell polarity and cytokine production in the proliferation assays was analyzed by flow cytometry and multiplex software. Basophil activation tests on blood from 12 allergic and 12 healthy donors were used to validate the nut-extracts' stimulatory potentials.

Results: The relative contribution of highly differentiated pathogenic ($IL\text{-}4^+IL\text{-}5^+$) Th cells was increased in donors with allergy to a given nut compared to tolerant donors. When subdividing the tolerant donors into asymptomatically sensitized or IgE-negative, increased pathogenic $IL\text{-}4^+IL\text{-}5^+$ Th cells were found in asymptomatically sensitized compared to tolerant-IgE-negative donors. For some of the nuts, elevated levels of the IL-5 and IL-13 were also observed in allergic subjects versus asymptomatically sensitized, and in asymptomatically sensitized subjects versus tolerant-IgE-negative subjects.

Conclusion: An overrepresentation of allergen-specific pathogenic $IL\text{-}4^+IL\text{-}5^+$ Th2 cells and an elevated IL-5 production was observed in allergic subjects compared to subjects that tolerated the nuts. Subjects asymptomatically sensitized to nuts differed from tolerant-IgE-negative subjects by having relatively more Th cells producing IL-5, suggesting that during the development of food allergy, allergen-specific inflammatory Th cells together with specific IgE are prerequisites for the breakthrough of clinical reactivity to food.

41.

Neutralizing natural anti-IL-17F autoantibodies may protect from asthma.

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The autoimmune regulator (AIRE) gene is critical for the development of central tolerance. APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) patients have a mutation in the AIRE gene, resulting in an autoimmune attack against multiple endocrine organs and the intestine. APECED patients display high-affinity neutralizing antibodies against several cytokines in addition to self-antigens, however, the influence of these antibodies on allergy and asthma has not been characterized.

To advance our understanding on the relationship between allergy and asthma symptoms, and the circulating cytokine autoantibody levels, we investigated 29 APECED patients. Allergy symptoms, medication and previous prick test, peak expiratory flow (PEF), and spirometry results were recorded. Sensitization to the 112 most common allergen components was tested with ImmunoCAP ISAC sIgE-microchip and autoantibodies against 52 cytokines were measured with ELISA using recombinant antigens. Statistical comparisons were performed with T-test

and Wilcoxon rank-sum test. P-values were adjusted for multiple hypothesis testing using Benjamini-Hochberg false discovery procedure.

Four of the 29 (14%) APECED patients were sensitized to IgE chip allergens as follows: birch pollen (rBet v 1), common wasp (rVes v 5), timothy (nPhl p 4,) and domestic cat (rFel d 1). However, thrice more (12/29) patients reported clinical symptoms: eight reported rhinoconjunctivitis from birch pollen, three of which were sensitized to rBetv1, six reported symptoms from cat but only one was sensitized. Oral/oropharyngeal symptoms from various fruits, nuts, and fish were also reported but could not be confirmed in the sIgE chip.

Comparing clinical allergy symptoms, verified asthma, and IgE sensitization to the presence of various anti-cytokine and anti-chemokine autoantibodies, the presence of anti-IL-17F autoantibodies seemed to be associated with the lack of asthma symptoms, since asthmatic APECED patients had a statistically lower mean OD level of anti-IL-17F antibodies compared to the non-asthmatic APECED patients (0.53 +/- 0.43 vs. 2.11 +/- 1.02, $p<0.05$, respectively). Other statistically significant associations were not found.

The high neutralizing potential of these fully human anti-IL-17F antibodies derived from AIRE-deficient patients has been previously shown *(Meyer et al., Cell 2016)*, and we now show for the first time in humans, the protective role of IL-17F blocking in asthma induction or exacerbation.

42.

Analysis of microRNA expression in the lung and bone marrow of IL-33 challenged mice

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Background: MicroRNAs (miRs) are small noncoding RNAs that regulate gene expression and are emerging as modulators of immune responses. We have recently identified miR-155 as a critical regulator of IL-33 signaling in experimental models of allergic airway inflammation. In addition, we have also identified IL-33 responsive bone marrow innate lymphoid cells as an early source of IL-5, a crucial factor for eosinophil hematopoiesis. Little is currently known about IL-33-induced miRNA expression in vivo. In this study, we aimed to define miRNA expression patterns in airways and bone marrow in response to IL-33 challenge.

Methods: Wild type mice were challenged intranasally with rmIL-33 (1µg) or saline as a control on day 1, 3 and 5. Lung tissue and bone marrow were sampled 24h after the final challenge. Total RNA was isolated and miR microarray was performed. A miRNA was determined to be differently expressed if the fold change were > 2 and p-value < 0.05 (n=4). KEGG pathway analysis was performed using miRSystem.

Results: Analysis of miRNA expression in IL-33 challenged mouse lungs demonstrated a significant upregulation of several miRNAs including miR-204, miR-21, miR-290a and miR-155, whereas in the bone marrow, a down regulation of miRNAs was more evident. Furthermore, KEGG pathway analysis demonstrated mTOR and MAPK signaling as top candidate pathways for the differentially expressed miRNAs.

Conclusions: Our data suggest a regulatory role of several miRNAs in IL-33-induced airway inflammation. Furthermore, specific miRNAs might regulate mTOR signaling pathway downstream IL-33. However, further studies are needed to understand the contribution of miRNAs in allergic and non-allergic IL-33 driven diseases.

43.

Cutaneous immune responses to external stimuli in terms of inducible skin-associated lymphoid tissue (iSALT)

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In the 1978, Streilein et al. introduced the term 'skin associated lymphoid tissue (SALT)' based on observations that revealed the existence of T cells and dendritic cells (DCs) in the skin and that T cells are activated in the skin draining lymph nodes. However, it remains unclear whether and how cellular components interact each other in the skin. In addition, how the memory T-cell activation occurs in the skin in situ has been unrevealed. With the close observation of a skin specimen obtained from a patient with contact dermatitis, we discovered that dermal DCs (dDCs) clustered and closely attached to T cells. Through the detailed examination of the elicitation phase of contact hypersensitivity as a murine model of contact dermatitis, we demonstrated the formation of sequential leukocyte clusters at the postcapillary venules. The structure does not exist in the steady state, but is 'induced' in response to local inflammatory conditions. Herein, we propose that this structure to be termed as 'inducible SALT (iSALT)'. In this symposium, I will introduce the underlying mechanism of cutaneous immune responses to external stimuli in terms of iSALT, PNAd positive cell induction, and cutaneous antigen presenting cell subsets.

44.

Role Of Histaminergic H4 Receptors in Anti-Inflammatory And Lung Anti-Fibrotic Activity Of GLUCOCORTICOIDS

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Histamine, a mediator of inflammation and immune-allergic responses, regulates cellular immunity by controlling the production of pro-inflammatory cytokines, chemokines and the migration of inflammatory cells. Histamine and H4 Receptor (H4R) ligands regulate T lymphocyte responses and promote differentiation of $CD4^+$ lymphocytes into Th2 profile. Glucocorticoid-induced leucine zipper (GILZ), a dexamethasone inducible gene that mediates glucocorticoid (GC) action in immune system, controls T cell activation and differentiation, through dimerization with NF-κB, and the regulation of pro-inflammatory target genes. An anti-inflammatory role for GILZ in various mouse models of inflammatory diseases has been demonstrated.

This research aims to study the role of histaminergic H4R in the anti-inflammatory activity of GC in a murine model of bleomycin-induced lung fibrosis.

C57BL/6 GILZ$^{-/-}$ mice and their wild-type (WT) littermates were treated with bleomycin (0.05 IU) or saline intratracheally to induce lung fibrosis. Immediately after, mice were treated with vehicle, JNJ7777120 (2 mg/kg b.wt.) or dexamethasone (2 mg/kg b.wt.), released by micro-osmotic pumps for 15 days. We assayed airway resistance to inflation and lung samples were processed to measure H4R and GILZ expression and pro-inflammatory cytokines production. Fibrosis and airway remodeling were evaluated by measuring TGF-β production and α-SMA deposition; as well as, the percentage of positive Goblet cells, and smooth muscle layer thickness. CD4^{+}, CD8^{+} and CD25^{+} T-cells from spleen and inguinal lymphnodes were collected to analyse FoxP3, a Treg marker, TGF-β, TNFα, IL-1β, IL-6, IL-17 expression by realtime PCR

Our results indicate that in WT mice, but not in GILZ$^{-/-}$ mice, GC and JNJ decrease the airway resistance to inflation. These results are in agreement with the expression levels of pro-inflammatory (TNFα, IL-1β, IL-6) and pro-fibrotic (TGF-β) cytokines.

Based on these results, the interactions among histamine, GC and GILZ, should be hypothesized in order to validate new therapeutic targets for pulmonary fibrosis.

45.

Retinoic acid controls lung homeostasis by converting group 2 innate lymphoid cells to a regulatory phenotype

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Background: Group 2 innate lymphoid cells (ILC2s) play critical roles in induction and exacerbation of allergic airway inflammation. Thus, clarification of the mechanism that underlies the regulation of ILC2 activation has been receiving a broad attention. Although ILCs are divided into three major subsets that mirror helper T-cell subsets, counterpart subsets of regulatory T (Treg) cells have not been well characterized. Therefore, in the present study, we sought to determine the factors that induce regulatory ILCs (ILCregs).

Methods: Human ILC2s were stimulated with Treg-associated factors and analyzed. IL-10^{+} ILCregs converted from ILC2s by retinoic acid (RA) were analyzed using RNA-sequencing and flow cytometry. ILCregs were evaluated in human nasal tissues from healthy individuals and patients with chronic rhinosinusitis with nasal polyp (CRSwNP), and in lungs from house dust mite (HDM)- or saline-treated mice.

Results: RA induced IL-10 production by human ILC2s, but not such type-2 cytokines as IL-5 and IL-13. IL-10^{+} ILCregs, converted from human ILC2s by RA stimulation, expressed such Treg-associated markers as IL-10, CTLA-4, CD25 etc., and down regulated effector type 2-related markers such as CRTH-2 and ST2, and suppressed activation of CD4^{+} T cells and ILC2s. ILCregs were rarely detected in human nasal tissues from healthy individuals or lungs from saline-treated mice, but were increased in nasal tissues from patients with CRSwNP and in lungs from HDM-treated mice. Enzymes for RA synthesis were upregulated in airway epithelial cells during type-2 inflammation in vivo and by IL-13 in vitro.

Conclusion: We have identified a unique anti-inflammatory pathway by which RA converts ILC2s to ILCregs. Interaction between airway epithelial cells and ILC2s may be important in the generation of ILCregs that results in avoidance of excessive tissue injury and inflammation.

46.

The role of RORα and innate lymphoid cells in mucosal inflammatory disease

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Background: Innate Lymphoid Cells (ILCs) are a newly discovered population of immuno-modulatory cells with a potent ability to produce cytokines in the absence of antigen specific receptors. Recently, we showed that the transcription factor, retinoic acid receptor-related orphan receptor alpha (RORα) is essential for the development of IL5 and IL13 producing group 2 ILCs (ILC2). Accordingly, we have used RORα-deficient mice (Rorasg/sg) to evaluate the role of ILCs in acute Th2- or Th17-driven lung inflammatory disease and in intestinal fibrotic disease.

Methods: Hematopoietic chimeric mice were generated by transplanting wild type or Rorasg/sg bone marrow cells into lethally-irradiated recipients. Following stable engraftment (12 weeks), the inflammatory responses of these chimeric mice were evaluated in 4 models of mucosal inflammation: 1) House dust mite-induced asthma (local priming and challenge, Th2 inflammation) 2) Ovalbumin-induced asthma (systemic priming and local challenge, Th2 inflammation), 3) Hypersensitivity pneumonitis (local priming and challenge, Th17 inflammation) and 4) Salmonellainduced intestinal fibrosis (a model of Crohn's disease). In each case, inflammatory infiltrates, cytokine production and local tissue remodeling and pathology were assessed.

Results: We found that RORα-dependent ILC2s play an essential role in the local priming of Th2-driven allergic airways disease but are completely dispensable for systemically-primed Th2 disease. We also found that these cells are dispensable for Th17-driven lung inflammation in a farmer's lung model of hypersensitivity pneumonitis. In the intestine we found that loss of RORα potently protected mice from fibrosis in a Salmonella-driven model of Crohn's disease. Surprisingly, we found that this was due to an unanticipated role for RORα in the function of group 3 ILCs. Although ILC3s are present in normal frequency in RORα-deficient chimeric mice, they are severely impaired in their ability to produce IL17 and other ILC3-associated cytokines. Correspondingly, we found that IL17-neutralizing antibodies dampen intestinal fibrosis. Conclusions: Our data suggest that RORα-dependent ILC subsets play key roles in the priming of Th2 allergic disease in the lung and the development of fibrotic disease in the gastrointestinal track. They also suggest that targeting RORα would be of therapeutic benefit in several forms of mucosal inflammatory disease.

47.

IL-33 promotes the egress of group 2 innate lymphoid cells from the bone marrow

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Group 2 innate lymphoid cells (ILC2) are effector cells within the mucosa that are key participants in type 2 immune responses in the context of allergic inflammation and infection. ILC2 develop in the bone marrow from common lymphoid progenitor cells, but little is known about how ILC2 egress from the bone marrow for hematogenous trafficking. In this study, we identified a critical role for IL-33, a hallmark peripheral ILC2-activating cytokine, in promoting the egress of ILC2 lineage cells from the bone marrow. Mice lacking IL-33 signaling had normal development of ILC2 but retained significantly more ILC2 progenitors in the bone marrow via augmented expression of CXCR4. Intravenous injection of IL-33 or pulmonary fungal allergen challenge mobilized ILC2 progenitors to exit the bone marrow. Finally, IL-33 enhanced ILC2 trafficking to the lungs in a parabiosis mouse model of tissue disruption and repopulation. Collectively, these data demonstrate that IL-33 plays a critical role in promoting ILC2 egress from the bone marrow.

48.

Pathogenic Th2 (Tpath2) cells in airway inflammation: Fibrosis inducing Tpath2 cells

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To develop more effective vaccines and strategies to regulate chronic inflammatory diseases, it is important to understand the mechanisms underlying the generation and maintenance of immunological memory. In 2011, we identified a highly pathogenic IL-5-producing memory Th2 (Tpath2) cell subset in allergic airway inflammation (Endo et al. Immunity, 2011). The IL-33/ST2 axis has been shown to be important for the induction of Tpaht2 cells (Endo et al. Immunity, 2015). Based on these data, we propose a new model called "Pathogenic Th population disease induction model" in the pathogenesis of Th1/Th2/Th17 diseases (Endo et al. Trends in Immunology, 2014, Nakayama et al. Ann. Rev. Immunol. 2017). We extended the study and identified Fibrosis inducing pathogenic Th2 cell subsets (Morimoto et al. Immunity in press 2018). Asthma is a chronic allergic inflammatory disease with airway remodeling including fibrotic changes. We found that IL-33 enhanced Amphiregulin production by $ST2^{high}$ memory Th2 cells. Amphiregulin-EGFR-mediated signaling directly reprogramed eosinophils to an inflammatory state with enhanced production of Osteopontin, a key profibrotic immunomodulatory protein. IL-5-producing memory Th2 cells and Amphiregulin-producing memory Th2 cells appear to cooperate to establish lung fibrosis. The analysis of polyps from patients with eosinophilic chronic rhinosinusitis revealed fibrosis with accumulation of Amphiregulin-producing $CRTH2^{high}CD161^{high}CD45RO^{+}CD4^{+}$ Th2 cells and Osteopontin-producing eosinophils. Thus, the IL-33-Amphiregulin-Osteopontin axis directs fibrotic responses in eosinophilic airway inflammation and is a novel potential target for the treatment of fibrosis induced by chronic allergic disorder.

49.

Crucial roles for basophils in Th2 and non-Th2 immune responses

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Background Basophils had long been overlooked or underestimated in immunological studies, because of their minority status and some similarity with mast cells. Recent development of novel analytical tools, including genetically-engineered mice deficient for basophils, has advanced our understanding of basophil biology and pathology. Here we show crucial roles for basophils in Th2 and non-Th2 immune responses.

Methods The surface expression of MHC class II (MHC-II) on basophils and the function of basophils as antigen-presenting cells (APCs) were revisited. The roles of serine proteases stored in secretory granules of basophils was analyzed in allergic inflammation. The contribution of basophils to the development of chronic obstructive pulmonary disease (COPD) was examined by using a mouse model.

Results and conclusions (1) It remained controversial whether basophils express MHC-II and function as APCs, promoting Th2 cell differentiation. We found that basophils produce few or no MHC-II molecules by themselves while they can acquire peptide-MHC-II complexes from dendritic cells (DCs) through trogocytosis. Peptide-MHC-II-dressed basophils were able to stimulate naive $CD4^{+}$ T cells to proliferate and differentiate into Th2 cells. These observations appear to reconcile some discrepancy observed in previous studies reporting basophils as important APCs under some but not other experimental conditions, likely depending on the basophil-DC interaction and the extent of MHC-II trogocytosis. (2) Among the mouse mast cell protease family, mMCP-8 and mMCP-11 are preferentially expressed by basophils rather than mast cells, suggesting their contribution to basophil-mediated immune responses. Indeed, mice deficient for mMCP-11 showed much milder inflammation in basophil- and IgE-mediated chronic allergic inflammation. Intradermal administration of mMCP-8 induced skin inflammation. These results indicated that mMCP-8 and mMCP-11 are basophil-derived effector molecules contributing to basophil-mediated allergic inflammation. (3) COPD is characterized by the progressive airflow limitation associated with chronic inflammation and emphysema formation. It is generally considered as a non-allergic lung disorder. Unexpectedly, we identified the essential role of basophils in the pathogenesis of COPD.

50.

Generation of a novel Der p 1-specific CD8 T cell receptor transgenic mouse

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Modern mouse models of allergy and asthma use inhalant allergens such as Dermatophagoides pteronyssinus, cockroach and Blomia tropicalis. However, a limitation of these models is the paucity of T cell receptor (TcR) transgenic mice specific for inhalant allergens. The aim of this project was to generate a Der p 1-specific CD8 TcR transgenic mouse. C57BL/6 mice were

immunized with Der p $1^{111-119}$ plasmid DNA vaccine by skin tattoo. Professor Ton Schumacher of the Netherlands Cancer Institute kindly provided 1TTFC-NP DNA vaccine plasmid and Der p $1^{111-119}$ was engineered into the 3' end of the TTFC sequence. Responder cells were identified using MHC I Der p $1^{111-119}$ tetramer with 1.39% of $CD3^+CD8^+$ T cells positive, compared with 0.25% from control animals. These cells were expanded with Der p $1^{111-119}$ peptide pulsed CD90 depleted splenocytes in RPMI 1640 supplemented with IL-2, IL-7 and IL-15 through 4 weekly cycles to 93%. TcR alpha and beta chains from these cells were cloned using 5' RACE. We selected and sequenced close to 20 colonies for each TCR chain. 11 out of 18 colonies yielded the same TCR-α sequence TRAV7-5*01/TRAJ26*01 with only a single colony returning a productive TCR combination of TRAV16N*01/TRAJ34*02. Sequencing results returned a single productive combination of TCR-β genes, TRBV5*01/TRBD1*01/TRBJ2-3*01/TRBC2. This indicated that the Der p 1 specific CD8 T cell line had been grown close to clonality. TRAV7-5*01/TRAJ26*01 and TRBV5*01/TRBD1*01/TRBJ2-3*01/TRBC2 were inserted into two gene cassettes, pTacass and pTbcass (Kind gift from Diane Mathis, Harvard Medical School. The cassettes were microinjected into mouse oocytes from pseudo pregnant mice and progeny tested. After screening 32 and 61 mice from two separate attempts, a single male was identified 41% of whose CD8 T cells expressed the correct TcR-α and -β chains and bound Der p $1^{111-119}$ tetramer. This mouse was crossed with wild type mice and 8 mice identified whose CD8 T cells bound between 33% and 58% of the tetramer. This was increased to 94% by crossing with $RAG^{-/-}$ mice. This is the first description of inhalant allergen-specific transgenic CD8 TcR mice. These animals should facilitate further investigation of asthma mechanisms in mice.

51.

Thymic stromal lymphopoietin (TSLP) confers steroid resistance to airway lymphoid cells in asthma by engaging a novel MEK-ERK kinase 2 (MEK2)-chromobox 7 (CBX7) signaling pathway

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Background: Steroid resistance is a major cause of refractory asthma. The mechanism of steroid resistance is poorly understood.

Methods and Results: We examined steroid sensitivity of ILC2s. Dexamethasone (Dex) inhibited type 2 cytokine production in IL33-stimulated ILC2s but not in TSLP- and IL7-stimulated ILC2s from the blood. Unlike blood ILC2s, which were generally sensitive to Dex, bronchoalveolar lavage (BAL) ILC2s from refractory asthma patients were basally resistant to Dex and had elevated TSLP. The latter correlated with steroid resistance of ILC2s. Mechanistically, Dex upregulated ILC2 expression of IL7Rα (the common receptor for TSLP and IL7), which augmented MEK1 and STAT5 signaling by TSLP. MEK1 and STAT5 were important as their inhibitors Trametinib and Pimozide reversed steroid resistance of BAL ILC2s.

A previous study identified 50 steroid resistant genes in leukemic cells using a genome-wide gene knockdown approach. We selected top 15 steroid resistant genes from this screen and examined their Dex sensitivity in lymphoid cells. Three of these 15 genes— MEK2, CBX7 and TLR2 were resistant to Dex. Dex, through its upregulation of IL7Rα, protagonized TSLP but not IL33 to induce MEK2, CBX7 and TLR2. CBX7 is a member of the polycomb repressor complex 1 (PRC1). MEK2 translocated to the nucleus and formed a multimolecular complex with the glucocorticoid receptor, CBX7 and other PRC1 members. We demonstrated the importance of CBX7 for type 2 genes by showing 1) Its direct binding to the promoters of GATA3, IL4 and IL5 by ChIP; 2) Inhibition of type 2 cytokine production in lymphocytes treated with a shRNA and MS37452, a pharmacological inhibitor of CBX7; and 3) Inhibition of airway hyperreactivity, eosinophilic inflammation and type 2 gene expression by MS37452 in a mouse model of asthma. We demonstrated the importance of MEK2 and CBX7 for steroid resistance by showing 1) Their increased expression in BAL lymphoid cells from refractory asthmatic patients; and 2) Reversal of steroid resistance by Trametinib and MS37452, inhibitors of MEK2 and CBX7, respectively.

Conclusions: TSLP, unlike IL33, induces steroid resistance. TSLP engages a novel steroid resistant pathway involving MEK2 and CBX7. MEK2 and CBX7 inhibitors are likely to benefit steroid-resistant asthma.

52.

Use of classical and non-classical initiation codons for translation of human thymic stromal lymphopoietin (TSLP) determines its secretory pathway

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Introduction: TSLP is a member of the IL-7 cytokine family that has been associated with both T2- and non-T2 asthma, as well as other allergic inflammatory diseases. Most cytokines possess a signal peptide that directs their sorting to the endoplasmic reticulum (ER) and transport to the extracellular space via the ER–Golgi secretory pathway. Others, including IL-1 family members, lack a signal peptide and are secreted via ER/Golgi-independent mechanisms, termed 'unconventional protein secretion'. While murine TSLP has a conventional signal peptide, this has not been formally studied for human TSLP.

Methods: Bioinformatic tools were used to compare mouse and human TSLP mRNA and protein sequences. Expression constructs and primary cells were used to investigate whether these differences were of functional consequence.

Results: Bioinformatic analyses of human protein TSLP compared with its murine homologue, identified a 10 amino acid N-terminal extension that would render the proposed signal peptide inactive; furthermore, mRNA analysis predicted that the accepted AUG translation start site of human TSLP would be inefficient due to absence of a basic Kozak sequence (purine -3 with respect to AUG) resulting in poor ribosome recognition. We identified two in-frame non-classical CUG initiation sites downstream of the AUG, both possessing Kozak sequences, the first of which occurred at Leu10. Using a series of expression constructs and site-directed mutagenesis, we demonstrate that all three initiation codons are functional and there is significant read through from the primary AUG site to the CUG sites. Product from the first CUG site was predicted to restore a functional signal peptide. In contrast, TSLP produced from classical AUG site was released via a glyburide-sensitive unconventional secretory pathway involving exosomes in response to stimulation with TNFα/IL-4. Release of endogenous TSLP from primary human fibroblasts confirmed use of both conventional and unconventional secretory pathways.

Conclusions: Unlike mouse TSLP, the potential to utilise alternative translational start sites and distinct mechanisms for release of human TSLP may influence its site and/or mechanism of action, with product from the non-classical CUG1 codon resulting in release of free cytokine whilst that originating from the AUG codon is packaged as cargo in exosomes.

53.

Lack of gut lactobacilli in early life associates with a skewed cytokine/chemokine profile in plasma, elevated FeNO levels as well as allergy at 1-10 years of age

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Background: Genes and environment interact in a multifaceted interplay important both for initiation and maintenance of allergic disorders. Early-life gut microbes influence the developing immune system and may impact future immune-mediated diseases like allergy.

Study aims: 1) To investigate gut colonization with lactobacilli during infancy in relation to immune profile and allergy-development during the first 10 years of life.
2) To seek mechanistic insights into why presence of lactobacilli in the early gut is associated with being non-allergic. Here this was approached by studying transcriptional events in dendritic cells (DC) following lactobacilli exposure in vitro.

Method: The study subjects (n=281) of mixed heredity were followed from birth and evaluated for allergic symptoms and IgE-sensitization at 1, 2, 5 and 10 years of age. FeNO was measured at 10 years of age according to standardized criteria. DNA was extracted from fecal samples from a subgroup of 72 children from four occasions between 1 week and 2 months of age. Bacterial DNA was analyzed with real-time PCR targeting a group of lactobacilli (L. casei, L. paracasei and L. rhamnosus). Cytokines and chemokines were quantified in plasma by ELISA or Luminex. Transcriptional regulation in DC derived from healthy blood donors was studied by chromatin immunoprecipitation and q-PCR.

Results: Lactobacilli colonization in infancy associated with a reduced allergy prevalence up to 10 years of age; also in high-risk children. Lactobacilli colonization associated with lower circulating levels of the chemokines BCA-1/CXCL13 and MDC/CCL22; factors that were clearly elevated in the circulation of children that developed allergy. Lactobacilli-colonized children also had higher levels of circulating IFNγ. Further early-life colonization was associated with lower FeNO values at 10 years of age, irrespective of allergy. In addition, in vitro experiments with DC revealed that lactobacilli enhance their immune maturation at a transcriptional level.

Conclusions: A lack of lactobacilli in feces during infancy associates with allergy-development, elevated FeNO levels and a skewed cytokine/chemokine profile in children. Together with our findings on how lactobacilli influence DC maturation at a transcriptional level *in vitro*, this might be indicative of a lactobacilli-mediated immune maturation that is beneficial to prevent allergy.

54.

A potential role for soluble Toll-like receptor 2 in the regulation of oral tolerance development

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Background: Multiple studies have implicated TLR2 in the regulation of allergic disease. We have previously demonstrated that Toll-like receptor 2 (TLR2) activation can limit the development of oral tolerance. Soluble toll-like receptor 2 (sTLR2) that acts as a decoy receptor is found in both human and cow's milk. We have investigated the impact of sTLR2 in milk, in regulating the development of food sensitization using murine models.

Methods: Oral tolerance was established in mice through one week of oral antigen administration, with or without additional cow's milk. Oral tolerance was assessed through analysis of antigen-specific antibody responses after systemic (i.p) antigen immunisation and challenge. The role of sTLR2 in milk on tolerance to OVA in early life was more directly assessed by cross-fostering 1-3 day old TLR2+/- mice onto wild type or TLR2$^{-/-}$ dams. sTLR2 levels were assessed cow's milk and milk-based baby formulas by ELISA

Results: Oral administration of antigen was sufficient to induce the development of tolerance in both wild type and TLR2 deficient mice. Tolerized mice produced 5 fold less allergen-specific IgE after antigen challenge. Treg cells and tolerogenic-dendritic cells, in Peyer's patches and mesenteric lymph nodes were increased significantly ($p<0.05$) in cow's milk fed adult mice. Cow's milk feeding blocked the anti-tolerogenic effects of oral TLR2 activator (Pam3CSK4) administration. The development of oral tolerance, pre-weaning, was significantly impaired in mice receiving milk from TLR2-/- dams regardless of whether they were born from TLR2+/+ or TLR2 -/- mothers. 15-45 ng/ml sTLR2 was detected in both cow's milk and in milk based baby formulas with greater amounts in human breast milk.

Conclusions: Our results indicate an important role for sTLR2 in milk in promoting the development of oral tolerance during early life and adulthood. These findings could be critical to understanding the mechanisms of sensitisation to foods and the role of TLR2 in allergy development.

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55.

Antibody repertoires in the gastrointestinal tract of peanut allergic individuals

Scott D. Boyd

Background: Many studies of human allergy have relied heavily on sampling of cell populations and secreted molecules from the blood, leaving significant gaps in our understanding of the immunobiology at other tissue sites. Defining the phenotypes and antigen receptors expressed by B-lineage cells in the mucosal and systemic immune system in the allergic disease state is a first step for identifying the mechanisms responsible for successful allergy immunotherapy.

Methods: We have applied second-generation DNA sequencing of antibody repertoires of B cells and plasma cells in the peripheral blood and biopsies of the esophagus, stomach and duodenum of peanut allergic subjects in a cohort of 19 adult patients to evaluate the antibodies expressed in all isotype compartments.

Results: We identified large numbers of IgE+ clones in the allergic patient specimens, particularly the stomach (median 438, range 31-2217 IgE+ clones per biopsy) and duodenum (median 179, range 4-574 IgE+ clones per biopsy), compared to biopsies from 6 non-allergic stomach controls (median 0 IgE+ clones). Somatic mutation positions in the IgE sequences suggested that the IgE+ B-lineage cells were most likely to similar in their mutation state to IgA+ members of the same clone. Members of IgE+ clones that expressed non-IgE isotypes were much more frequently co-localized in the same tissue biopsy compared to other anatomical sites in the same patient, providing evidence for local class switching clonal expansion in the tissue sites. Phage display of IgE antibody sequences identified numerous clones that were specific for the Ara h 2 peanut allergen, and these clones showed similar sequence and clone isotype sharing features to the total IgE repertoires in these subjects.

Conclusions: Together, our data suggest that IgE+ B-lineage cells can be produced in gastrointestinal tissues by local class-switching, and are often members of expanded clonal lineages containing IgA and IgG expressing members that may represent precursors to the IgE+ clone members. Identifying the behavior of these clones during allergy immunotherapies may shed light on the cellular mechanisms associated with successful desensitization and potential longer duration unresponsiveness following therapy.

56.

Evidence of an abnormal epithelial barrier in active, untreated and corticosteroid-treated eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic, immune/antigen-mediated disease characterized by symptoms related to esophageal dysfunction and an eosinophil-predominant inflammation. This study has aimed to investigate whether the recently observed sensitization to Candida albicans in EoE patients is owing to pre-existing disease and its underlying abnormal epithelial barrier or, alternatively, is linked to corticosteroid (CS) therapy.

Methods: Medical histories, as well as serum and tissue samples of 60 EoE patients (15 CS-naive, 45 with current or previous CS therapy) and 20 controls, stored in the Swiss Eosinophilic Esophagitis Database (SEED) and Biobank, were analyzed. We applied ImmunoCAP to measure IgE levels and immunofluorescence techniques to examine epithelial barrier components.

Results: EoE patients had higher total IgE levels and were more frequently sensitized to Candida albicans than controls. In EoE tissue specimens, increased numbers of eosinophils and mast cells, a higher expression levels of thymic stromal lymphopoietin (TSLP), cathelicidin, proteases, i.e. the kallikreins (KLK)-5 and KLK-7, were observed as compared with controls, while reduced expression of lympho-epithelial Kazal-type-related inhibitor (LEKTI), filaggrin, E-cadherin, claudin, occludin, demoglein-1 was found, independent of CS therapy. In CS-treated EoE, significantly lower numbers of CD1a+ cells and cathelicidin expression were noted as compared to CS-naive EoE.

Conclusion: This study provides further evidence that EoE is associated with an abnormal epithelial barrier and assumes that CS therapy, by reducing innate immune mechanisms, may promote Candida albicans colonization and likely subsequent sensitization.

57.

Cow's milk proteins in pediatric eosinophilic esophagitis

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Background: We have previously reported that low-level IgE antibodies to cow's milk (CM) are common in children with eosinophilic esophagitis (EoE). Specific IgG4 antibodies (sIgG4) to causally relevant foods have recently been reported in adult EoE. We sought to investigate serum food-sIgG4 with component diagnostics in children with EoE and children from an unselected birth cohort and to relate the sIgG4 results with IgE to the same proteins.

Methods: Sera from 71 cases of pediatric EoE and 210 early-adolescent children from an unselected birth cohort (Project Viva) were assayed for sIgG4 and specific IgE (sIgE) with ImmunoCAP to major CM proteins (α-lactalbumin, β-lactoglobulin, caseins) and to wheat, soy, egg, and peanut proteins.

Results: In the EoE cohort high-titer sIgG4 ($\geq$ 10 µg/mL) to CM proteins were more common than in control sera and had odds ratios (OR) for EoE ranging from 5.5 - 8.4. Levels of sIgE to CM proteins were mostly $\leq$ 4 IU/mL in EoE, such that mean sIgG4:sIgE ratios were in many cases $\geq$10,000. When adjusted for age and milk consumption, high-titer sIgG4 to CM proteins were strongly associated with EoE, with an OR > 14 to caseins (95% CI 6.7-32). In a sub-analysis we found that the presence of antigen-specific IgE (often low-level, ie < 1 IU/mL) correlated strongly with the magnitude of the IgG4 response to the same antigen, an effect that was more dramatic in EoE subjects than controls.

Conclusions: Specific IgG4 to CM proteins are common and high-titer in children with EoE. While it is not clear that this response is pathogenic, the levels of sIgG4 imply that these antibodies are an important feature of the local immune response that gives rise to EoE. The fact that IgG4 levels were most pronounced in those with detectable levels of IgE to the same antigen provides more evidence for a link between these two antibody isotypes, speaks to the specificity of the immune response and is consistent with our earlier reports that low-titer IgE, especially to milk, is an important feature of pediatric EoE.

58.

Immunoprofile of α-Gal- and B-antigen-specific responses differentiate red meat allergic patients from healthy individuals

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Background: The carbohydrate epitope galactose-α-1,3-galactose (α-Gal) is involved in red meat allergy, an IgE-mediated disease where patients develop severe reactions (angioedema, urticaria, gastrointestinal symptoms and in its most severe form, anaphylaxis involving several organ systems including the respiratory tract) 2 to 6 hours after red meat consumption. Reports of red meat allergy have increased worldwide over the past few years. As α-Gal is structurally similar to the blood group B-antigen, we explored the relationship between the immune responses to α-Gal- and the B-antigen in red meat-allergic patients compared to healthy A/O or B blood donors.

Methods: Sera from 51 red meat-allergic patients IgE-positive to α-Gal suffering from urticaria, angioedema, gastrointestinal symptoms and/or anaphylaxis involving respiratory symptoms, and 102 healthy blood donors (51 blood group A/O; 51 blood group B) were included. α-Gal- and B-antigen-specific IgE (ImmunoCAP) as well as IgG/IgG1-4 (ELISA) responses were determined. Basophil activation tests were performed with α-Gal and B-antigen.

Results: Fifteen healthy donors were IgE positive to α-Gal, of which 3 had blood group B. The allergic patients had significantly higher α-Gal IgE levels compared to the healthy donors. The majority of the allergic patients, but none of the healthy donors, had IgE against the B-antigen. Inhibition studies revealed cross-reactivity between α-Gal and the B-antigen. The biological activity of the B-antigen was confirmed by basophil activation tests. Anti-α-Gal IgG1 and IgG4 levels were significantly higher in the patients compared to the healthy donors. Moreover, the IgG response to the B-antigen was comparable between the allergic patients and healthy A/O donors.

Conclusions: Red meat-allergic patients showed significantly higher α-Gal IgE, IgG1, and IgG4 levels, reflecting a Th2 response, compared to healthy blood donors. Blood group B donors had significantly reduced antibody responses to α-Gal, due to similarities with the B-antigen, resulting in a lower risk of sensitization to α-Gal and development of red meat allergy.

59.

IgE to the mammalian oligosaccharide α-Gal is associated with coronary atheroma volume and plaques with unstable characteristics

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Background: Emerging evidence suggests a link between atherosclerosis and type-2 immune mediators, including mast cells and total IgE levels. The oligosaccharide galactose-α-1,3-galactose (α-Gal) is present in mammalian foods such as red meat and dairy and is the target of IgE in cases of delayed anaphylaxis to red meat. Sensitization to α-Gal is particularly common in the southeastern United States, a region with high incidence of cardiovascular disease. We sought to test the hypothesis that sensitization to α-Gal represents an independent risk factor for coronary artery disease (CAD).

Methods: Total IgE and specific IgE (sIgE) to α-Gal were assayed on sera from 118 subjects who had symptoms suggestive of possible coronary heart disease and underwent coronary imaging with intravascular ultrasound (IVUS). IgE to peanut and common inhalants were also assayed.

Results: Of the cohort 26% had detectable titers of detectable IgE to α-Gal (cut-off 0.1 kU/L) and atheroma burden was higher in sensitized subjects (p=0.02). Total IgE was also significantly higher in the α-Gal sensitized group (p<0.001). Because α-Gal sensitization relates to an environmental exposure that could be a risk factor for early-onset CAD (ie, tick bites), we age-stratified the cohort. Focusing on subjects 65 and younger the strength of the association with atheroma burden was stronger (p<0.001) and atheromatous plaques in the sensitized group also had less stable features based on IVUS virtual histology. In subjects 65 and younger sensitization to peanut (8%) and inhalants (32%) was not associated with atheroma burden and in regression modeling the strength of the relationship with atheroma burden was stronger for α-Gal sIgE than total IgE. The association of atheroma burden with αGal sIgE was significant when adjusted for sex, diabetes, hypertension, triglycerides, statin use and total IgE (regression coefficient 11.9, SE 5.2, p=0.03).

Conclusions: Over 25% of the subjects in this cohort in central Virginia who presented for cardiac evaluation were positive for IgE antibodies to α-Gal. Increased atheroma burden and plaques with more unstable features were associated with IgE to εGal, an effect most pronounced in subject 65 and younger. IgE sensitization to α-Gal may represent a novel, and potentially modifiable, risk factor for coronary atherosclerosis.

60.

Holo- beta-lactoglobulin prevents allergic sensitization and promotes arylhydrocarbon receptor activation.

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Background: We have shown in vitro that the allergenic potential of the major lipocalin allergen beta-lactoglobulin (BLG) depends on the absence of its molecular ligand in vitro. Our hypothesis therefore was i) that vice versa, BLG loaded with iron complexed with catechol-type siderophores should harbour allergy preventive effects, and ii) that the arylhydrocarbon receptor (AhR) could play a pivotal role in the immune suppressive properties of holo-BLG.

Methods: Quercetin-iron complex formation and allergen binding was assessed by spectral analysis. BALB/c mice were sensitized nasally 6 times with apo- BLG, holo- BLG or controls before assessing reactivity by i.p.-challenge with the allergen. In a prophylactic approach, mice were pre-treated 3 times nasally with either form of BLG before they were sensitized with the respected antigen in combination with alum. Immune reactivity was assessed by specific serum antibodies and cytokines from splenocytes by ELISA. AhR activation was evaluated by stimulation of the reporter cell line AZ-AhR with combinations of quercetin, iron and BLG.

Results: Spectral analysis revealed complex-formation of quercetin with iron as well as binding into BLG. While holo-BLG suppressed specific antibody and cytokine formation, and protected against clinical hyperreactivity, the apo-BLG induced allergic sensitization, specific antibodies and cytokines, and caused anaphylaxis. Holo- BLG, but not apo- BLG significantly enhanced AHR-activation indicating active shuttling of quercetin into the intracellular compartment.

Conclusion: Apo-BLG exhibits the characteristics of the major milk allergen Bos d 5. In contrast, holo- BLG is immune-suppressive and non-allergenic in vivo. This may due to the fact that holo-BLG delivers its ligand quercetin to the anti-inflammatory and immunoregulatory AhR-pathway.
The study was supported by the Austrian Science Fund FWF, grant SFB F4606-B28. SA was supported by a grant of the Ministry of Higher Education, Egypt.

Conflict of Interests: EJJ, LFP and FRW are inventors of EP2894478, owned by Biomedical International R+D GmbH, Vienna, Austria. The other authors declare no conflicts of interests.

61.

Two oral daily doses of ibrutinib, an FDA-approved irreversible inhibitor of Bruton's tyrosine kinase (BTK), markedly inhibits or eliminates skin test responses and IgE-mediated basophil activation ex vivo in adults with food allergy.

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Background: There are no therapies capable of preventing anaphylaxis other than allergen avoidance. BTK is an enzyme that is required for FcεRI signaling. Published work suggests that pharmacologic BTK inhibition blocks IgE-mediated degranulation responses in vitro and in vivo. There are two oral, irreversible BTK inhibitors approved for the treatment of various lymphoproliferative disorders with favorable side effect profiles. Because BTK inhibitors have the potential to prevent anaphylaxis, we hypothesized that short-term ibrutinib use would reduce allergic responses in food allergic adult subjects.

Method: After obtaining local IRB approval, six adults with a history of IgE-mediated systemic reactivity to peanut and/or tree nuts were enrolled. After screening, subjects were given standard doses of ibrutinib (420 mg daily) for 2 to 7 days. Skin prick tests (SPTs) to relevant foods and basophil activation testing (BAT) were performed at baseline, during ibrutinib treatment, and after cessation of therapy.

Results: Two days of ibrutinib treatment significantly and consistently reduced SPT wheal and flare area (77 and 86% reductions, respectively; $p<0.0001$, n=25 food allergens). Overall, 44% of all skin tests became negative (wheal < 3mm diameter). Additional doses of ibrutinib for 4 or 7 days maintained a similar degree of reduced SPTs, but did not provide any additional suppression compared to what was observed after 2 days. Histamine control SPTs were unaffected. Anti-IgE induced (but not fMLP-induced) BAT became completely negative after 2 doses ($p=0.0002$) and remained negative at 4 and 7 days. The majority of SPTs and BAT returned to baseline levels within 1 week of cessation of ibrutinib therapy. No clinical or serologic toxicity from ibrutinib treatment was observed, nor were there any changes in total or food-specific IgE levels during the study.

Conclusions: As few as two standard daily doses of ibrutinib 420 mg eliminates or drastically reduces SPT responses to foods in food-allergic adult subjects, and completely abolishes IgE-mediated BAT responses. Ibrutinib could potentially be used either prophylactically or chronically to prevent allergic responses including anaphylaxis to foods, drugs and other substances. Ongoing studies are testing whether ibrutinib pretreatment can favorably alter food challenge responses in food-allergic adults.

62.

Tissue-specific stem cell origin of allergic and autoimmune diseases

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Both allergic and autoimmune diseases have increased in their prevalence for the last several decades. Both diseases are chronic inflammatory diseases. Homeostasis of the inflicted tissues is chronically disturbed in these diseases. Long-term homeostasis of normal tissues is kept at the level of tissue-specific stem cells. We hypothesize that some persistent inflammatory diseases such as allergic and autoimmune diseases may have a root cause at the level of tissue-specific stem cells. Some examples supporting this hypothesis exist: atopic dermatitis (AD) is related to perturbations in Th2, ILC2, and mast cells, whose development and activities are controlled by hematopoietic stem cells (HSCs). Inflammatory bowel diseases (IBDs) seems to have abnormal intestinal epithelium that is under the control of intestinal stem cells (ISCs).

Phospholipase C (PLC) is a family of enzymes that hydrolyze phosphatidyl 4,5-bisphosphate into diacylglycerol and inositol 1,4,5-trisphosphate. PLC-β isoforms are a small subgroup of the PLC family composed of 4 members. PLC-b3 is expressed ubiquitously. Young Plcb3-/- mice were fertile and apparently normal, but developed myeloproliferative neoplasm, an HSC tumor, when old. In addition, Plcb3-/- mice exhibited both aspects of allergic and autoimmune diseases when subjected to disease models. The mutant mice developed severe AD-like inflammation in a mast cell-dependent manner in a house dust mite allergen-induced AD model. Plcb3-/- mice also exhibited a more severe phenotype of ovalbumin-induced food allergy than did WT mice. They also exhibited an extreme sensitivity to oral exposure to dextran sodium sulfate (DSS), a model of IBD. This DSS susceptibility was partly due to increased permeability of

intestinal epithelium and these mice had a drastically reduced proliferative activity of ISCs in the small intestine. Transcriptome analyses revealed a high similarity in gene expression patterns between these mice and human patients with IBD. We found that PLC-b3 controls STAT5 activity in HSCs and mast cells as well as WNT signaling pathway in intestinal epithelial cells. Thus, our study demonstrates that an abnormality in tissue-specific stem cells causes chronic inflammatory diseases that have been traditionally categorized into allergic vs. autoimmune diseases.

63.

Identification of 2 distinct early life eczema and non-eczema phenotypes with high risk for asthma development in a prospective birth cohort: redefining the atopic march

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Abstract (Word count: 279)

Background. The atopic march is postulated to be initiated with infantile eczema, which then leads to the development of other allergic co-morbidities. The causal nature of this progression, however, remains a topic of considerable debate. Systematic prospective studies that integrate mechanistic and epidemiologic are needed to better understand the pathogenesis of allergic co-morbidities.

Objective: We dissected the interactions between eczema and the timing and pattern of food and aeroallergen sensitization on asthma development in a prospective birth cohort. Given the reported associations of KIF3A genetic variation with the atopic march phenotype, we also examined the impact of KIF3A genetic risk on our analyses.

Methods. We studied 505 participants in the Cincinnati Childhood Allergy and Air Pollution Study, a well-phenotyped birth cohort with longitudinal eczema and asthma outcomes as well as prospective data regarding timing of sensitization to foods and aeroallergens. KIF3A genotypes were available on all children.

Results. Two high-risk groups were identified. Only one of the high-risk groups had eczema, while both groups had early sensitization, albeit different patterns of sensitization. The high-risk group with eczema was more likely to be sensitized to food allergens, while the high-risk group without eczema was more likely to be poly-sensitized to aeroallergens. The KIF3A rs12186803 risk allele was found to be an eQTL and it differentially interacted with sensitization pattern to modify asthma risk in children with eczema vs.non-eczema.

Conclusions. Early polysensitization to food or aeroallergens results in high risk for asthma even without clinical eczema. Thus, the atopic march only represents one phenotype of asthma, and the other early life high-risk group has been largely ignored previously. Asthma risk in both groups was modified by the functional KIF3A risk allele.

64.

Diminished IL-6 responses during picornavirus-induced asthma exacerbations are associated with chronic airway obstruction

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Background. Patients with asthma and airflow limitation have more severe disease and are at risk for developing chronic obstructive pulmonary disease. Recurrent asthma exacerbations are associated with decline in lung function, but the molecular mechanisms involved have not been explored. Interleukin- (IL-) 6 is a pleiotropic cytokine involved in both acute inflammatory responses and in epithelial repair processes during infection-induced airway injury. Our goal was to assess the role of IL-6 and other cytokines produced during acute asthma exacerbations as determinants of chronic airflow limitation in children with asthma.

Methods. We performed spirometry at enrollment in 218 children with asthma aged 6-18 years and followed them until they developed a moderately severe exacerbation or for 18 months. In 87 participants we measured induced sputum cells, IL-6, soluble IL-6 receptor (sIL-6R), IL-8, and IL-13 during an exacerbation and/or at convalescence.

Results. Sputum IL-6 was highly correlated with sputum neutrophil counts, IL-8 and sIL-6R, and negatively correlated with sputum IL-13. There was a significant ($p=0.004$), positive correlation between FEV1/FVC ratio at baseline and IL-6 levels in sputum obtained during the exacerbation but not in convalescence. The association between FEV1/FVC ratio and sputum IL-6 was strongest ($r^2= 0.35$, $p=0.001$) and only present among subjects whose nasal secretions were positive for picornaviruses. Neither acute nor convalescent sputum neutrophils, sIL-6R, IL-8 or IL-13 were associated with baseline lung function.

Conclusions. Insufficient IL-6 production during acute exacerbations due to rhinovirus and other picornaviruses may predispose for the development of airflow limitation in children with asthma.

65.

Atopic dermatitis subjects colonized with staphylococcus aureus have a distinct phenotype and endotype

Short Title: The phenotype/endotype of AD S. aureus+ subjects

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Background: Atopic dermatitis (AD) patients are commonly colonized with Staphylococcus aureus (ADS.aureus+) but what differentiates this group from noncolonized AD subjects (ADS. aureus-) has not been well-studied.

Objective: To evaluate whether these two groups have unique phenotypic or endotypic features we performed a multi-center, cross-sectional study enrolling ADS.aureus+ (N=51) and ADS.aureus- (N=45) subjects defined by the presence or absence of S. aureus by routine culture techniques and nonatopic, noncolonized controls NAS.aureus- (N=46).

Materials: Filaggrin (FLG) genotypes were determined and disease severity (EASI, RJL, IGA and NRS) was captured. Skin physiology was assessed (transepidermal water loss [TEWL], stratum corneum integrity, hydration and pH) and serum biomarkers measured.

Results: We found that ADS.aureus+ had more severe disease based on all scoring systems except itch (NRS). They had higher levels of type 2 biomarkers (eosinophil count, tIgE, CCL17, and periostin). Additionally, ADS.aureus+ had significantly greater allergen sensitization (Phadiatop and tIgE), barrier dysfunction (TEWL and SC integrity) and serum LDH than both ADS.aureus- and NAS.aureus- groups. FLG mutations did not associate with S.aureus+ colonization.

Conclusion: In conclusion, adult AD participants who are colonized on their skin with S. aureus have more severe disease, greater type 2 immune deviation, allergen sensitization, barrier disruption, and LDH elevation than noncolonized AD subjects.

66.

Lipid-mediator governed molecular phenotyping of asthma sub-phenotypes

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Background: We hypothesised that sub-phenotypes of asthma could be identified by the profiles of urinary eicosanoids. We therefore quantified the urinary excretion of the main metabolites of cysteinyl-leukotrienes (CysLTs), prostaglandins (PGs) and isoprostanes (IPs) in the U-BIOPRED cohort.

Methods: The cross-sectional baseline visit included 598 subjects: non-smoking severe asthma (SAn; n=302), smoking SA (SAs/ex; n=109), mild-to-moderate asthma (MMA; n=86), and healthy controls (HC; n=101). The main eicosanoid metabolites were quantified in spot urine samples by tandem mass spectrometry (Balgoma et al Anal Chem 2013). Unbiased consensus clustering (Lefaudeux et al JACI 2017) was made on baseline values and for the severe asthmatics also on samples collected one year later (n=302).

Results: In the HC, tetranor-PGE was the most abundant metabolite, followed by several IPs, the two major PGD metabolites (PGD-M), whereas LTE4 had the lowest concentration. Levels of LTE4 and the PGD2 metabolites were however progressively higher in SA than in MMA and HC, respectively. The levels of these two mast cell derived metabolites furthermore correlated with measures of Type 2 inflammation such as blood or sputum eosinophils, exhaled nitric oxide, serum IL-13 and periostin. Clustering using only the profile of lipid mediators in the urine as input generated a stable five-cluster model (U1-U5) of baseline data for all asthmatics which in SA was replicated in the one year follow-up. The clusters were significantly differenent with respect to other outcomes. For example, U3 (n=97) was dominated by women (86 %) with high BMI, many exacerbations and high levels of IPs and PGD-M, whereas U1 (n=55) had more men (64%) with mild asthma. The largest cluster U3 (n=153) included typical Type 2 asthma with high eosinophils, LTE4 and PGD-M, whereas U4 (n=83) contained patients with late onset obstructive asthma. Finally, U5 (n=109) had the lowest levels of PGD-M and a non-Type 2 profile.

Conclusions: Molecular clustering based on urinary eicosanoid profile can identify distinct and clinically meaningful sub-phenotypes of asthma. Mast cell over-activation with high levels of PGD-M and LTE4 was associated with Type 2 inflammation, indicating that urinary PGD-M has potential as non-invasive marker for precision medicine.

67.

An SP-A Peptide Modulates Responses to IL-13 in Asthma and Offers a Novel Therapeutic Alternative

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Background: Surfactant protein A (SP-A) regulates a variety of immune cell functions within the lung. Humans SP-A is a complex oligomeric structure that is encoded by SP-A1 and SP-A2 genes. We have previously reported that oligomeric SP-A is dysfunctional in type 2 asthma. Genetic variation in SP-A2 (rs1965708), which corresponds to a Q (Gln) to K (Lys) amino acid substitution at position 223 of the lectin domain, is associated with lower lung function and worse asthma control. We hypothesized that SP-A disrupts IL-13 signaling pathways and a shortened peptide expressing the major allele of SP-A2 (223Q) would be as effective as full-length SP-A.

Methods: Mice sufficient and deficient in SP-A, and mice expressing either the Q of K allelic variant of SP-A2 were exposed to IL-13 (3.9 µg via oropharyngeal delivery x 4d). In separate experiments WT mice were given the SP-A2 peptide prior to either IL-13 or HDM via oropharyngeal delivery; airways resistance to methacholine was measured by flexivent. In human studies, airway epithelial cells from participants with and without mild to moderate type 2 asthma not on controller therapy (n=5/group) were cultured at an air liquid interface for two weeks. Cells were exposed to 20 µg/ml of full length SP-A or the peptide for 30 minutes followed by IL-13 exposure for 48 hours. MUC5AC and IL-8 were determined by RT-PCR and ELISA, respectively.

Results: IL-13 increased airway eosinophils and neutrophils, MUC5AC mRNA and mucin in SP-A deficient mice compared to WT. Mice expressing the Q allele of SP-A2 or given the Q peptide were protected compared to mice expressing the K allele. In WT mice, the SP-A2 peptide significantly reduced airways resistance following both HDM and IL-13 exposure. In humans, full-length SP-A and the SP-A peptide decreased IL-13-induced MUC5AC mRNA and IL-8 in asthmatic cells; no significant effect was seen in normal cells.

Conclusion: SP-A modulates a key cytokine in asthma, IL-13. Its effects are altered by genetic variation of SP-A2. Use of a peptide expressing the Q variant at position 223 of SP-A2 restores function and may offer a unique therapeutic alternative for asthma.

68.

Circulating miRNAs and airways responsiveness in asthma

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Introduction: MicroRNAs (miRNAs) are small, epigenetic modifiers of transcriptomic activity. They function in intercellular communication and are released into the circulation during inflammation or cellular necrosis. MiRNAs are stably bound to plasma proteins or within vesicles, making them excellent biomarker candidates for disease. We hypothesized that circulating miRNAs would be associated with airways responsiveness, a hallmark of asthma.

Methods: We sequenced small RNA from the serum obtained at baseline for 96 participants in the Childhood Asthma Management Program (CAMP). RNA was extracted using the Qiagen miRNA-Easy Kit (Germantown, MD). Sequencing was performed on a HiSeq 2500 (Illumina, San Diego, CA) at Norgen Biotek (Thorold, Canada) using the Norgen Small RNA Library Prep Kit. Following QC, data was log transformed, quantile normalized, and analyzed using linear regression.

Results: The median number of miRNAs detected in the serum samples was 407. Of the 241 miRNAs detected in at least 80% of the samples, 27 were associated ($p<0.05$) with the provocative concentration of methacholine required for a 20% decrease in FEV1 (PC20). Pathway analysis indicated an enrichment for focal adhesion, MAP kinase signaling, and WNT signaling. Airway smooth muscle culture with miR-mimics revealed that 5 of the CAMP associated miRNAs (miR-10a, miR-181a-5p, miR-181b-5p, miR-21-5p, and miR-99b-5p) led to significant changes in average airway smooth muscle cell number.

Conclusion: MiRNAs can be readily detected in the serum of childhood asthmatics, and can directly reflect both clinical and functional severity within asthma.

69.

Antisense Technologies – An emerging field for the development of new therapeutic molecules: Anti-GATA3 DNAzyme as a prototypic example

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Transcription factors play a central role in the regulation of cellular function and activities. In this context GATA3 has been recognized as the master-transcription factor in the regulation of Th2 development and Th2 functions. GATA3 is expressed not only in CD4-Th2 cells, but also in ILC2 cells, eosinophils mast cells, and basophils, among others. We have developed an antisense molecule belonging to the DNAzyme family which specifically and selectively targets GATA3 mRNA. DNAzymes are characterized by target-specific binding domains and an intrinsic catalytic nucleic acid sequence. Following extensive preclinical analysis, toxicology studies and three clinical safety studies in healthy and asthmatic individuals, a phase IIa proof-of-concept clinical trial was performed in mild asthmatic patients. Treatment was carried out for 28 days by daily inhalation of a single dose of 10 mg SB010. Allergen provocations were performed before and after the treatment cause. The primary endpoint of this study, significant reduction of the late phase asthmatic response, was reached, and highly surprisingly, also significant effects on the early phase allergic response was measured. The degree of improvement could be furthermore related to the level of blood eosinophils before treatment. Based on these encouraging results, SB010 treatment has been recently clinically tested in patients with atopic dermatitis (NCT02079688), eosinophilic COPD (DRKS00006087), and ulcerative colitis (NCT02129439), all of these conditions with predominant type 2 inflammation. Topical administration of SB010 on lesional skin for 14 days significantly improved skin barrier function. 28 days of inhalation of SB010 in eCOPD patients significantly reduced sputum eosinophils and shifted systemic IL-5 and IFN-εproduction; following encouraging results in animal models we have recently completed a clinical trial in UC patients with highly significant effects on the total Mayo score as the primary endpoint. In addition, target regulation was observed in local tissues.These data indicate for the first time that the transcription factor GATA3 represents potential targets for topical pharmatherapy in asthma, eosinophilic COPD, atopic dermatitis, and ulcerative colitis. However, additional clinical trials are warranted to firmly establish this new treatment modality.

70.

The functional implications of airway epithelial cell remodeling

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Purpose: The respiratory epithelium maintains a critical homeostasis, balancing the need for a robust host defense while preventing exuberant inflammatory responses. These functions are realized by the coordinated actions of specialized epithelial cell subsets including ciliated, secretory, neuroendocrine and tuft cells, along with basal epithelial cell progenitors. In the setting of type 2 inflammation, the composition of the respiratory epithelium is altered, but apart from changes in ciliary function and goblet cell metaplasia, the functional sequelae of airway remodeling are poorly defined.

Methods: Here we assessed sino-nasal tissue from patients with chronic rhinosinusitis (CRS) with and without nasal polyposis (NP), using the Seq-Well platform for massively-parallel single-cell RNA-sequencing (scRNA-seq), bulk RNA-seq, and ex-vivo primary airway epithelial cell cultures.

Results: Here we report the first single-cell transcriptomes for human respiratory epithelial cell subsets, immune cells, and parenchymal cells (18,036 total cells) from a type 2 inflammatory disease, and map key mediators. We find a striking contribution of stromal and innate immune cells to the nasal polyp environment and find that epithelial cells carry the footprint of these inflammatory diseases. Furthermore, we detect an aberrant basal progenitor program which persists in the absence of type 2 inflammation.

Conclusions: Our data demonstrate that immune memory may be retained in structural airway cells and that airway remodeling may have broader functional consequences than previously understood.

71.

Mechanisms of allergen-induced activation of "Itch Nerves" terminating in mouse ski

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Scratch-inducing "itch" nerves terminating in mouse skin can be differentiated from pain fibers based on their expression of MrgprA3 and MrgprC11 receptors (Cell, 2009,139:1353; Nat. Neurosci, 2013,16:174). Action potential (AP) discharge in single afferent itch and pain C-fibers in the skin of mice actively sensitized to ovalbumin (OVA) was evaluated by recording from individual neurons within the dorsal root ganglia (DRG), using our innervated isolated skin preparation (J. Physiol 2017, 595: 3651). OVA, applied to the skin, caused a strong response in the majority of itch C-fibers evoking the discharge of 117± 22 APs (n=17). In contrast, OVA failed to evoke appreciable responses in "pain" type C-fibers (n=19).

The itch C-fibers responded to histamine with AP discharge, but not to serotonin (n=7). The H1 receptor antagonist pyrilamine (1 μM) blocked the histamine response and reduced the OVA response by about 50% ($P < 0.05$). We have previously noted that activated mouse mast cells release a mediator(s) that can stimulate MrgprA3 and MrgprC11 receptors (J. Immunol, 2008,180:2251). The response of itch fibers in Mrgpr-cluster $\Delta^{-/-}$ mice to OVA was reduced (65±21 APs, n=5, $P>0.05$). When pyrilamine was studied in the Mrgpr-cluster $\varepsilon^{-/-}$ mice, the OVA response was nearly abolished (12 ± 5 action potentials; $P < 0.05$).

The membrane depolarizations induced by histamine and Mrgpr receptor agonists will be inconsequential unless they generate APs by activating voltage-gated sodium channels (NaVs). There are nine NaV subtypes (NaV 1.1 – 1.9). We carried out single cell RT-PCR on MrgprA3 expressing DRG neurons retrogradely labeled from the skin (n=18 neurons). Itch neurons expressed NaV 1.7 (83%), NaV 1.8 (100%) and NaV 1.9 (100%); other NaV1 genes were relatively rarely expressed. Blocking NaV 1.8 had no effect on AP discharge in the itch fibers; blocking NaV 1.7 silenced AP discharge in 46% of fibers; a combination of an NaV 1.8 and NaV 1.7 blockers silenced 100% of the itch fibers (n=19). We hypothesize that in mice, acute allergic itch can be prevented by blocking the relevant mast cell mediators (histamine and MrgprA3/C11 agonists) or by blocking NaV 1.7 and 1.8 channels.

72.

The role and relevance of IgE, FceRI, and mast cells in the pathogenesis and treatment of chronic urticaria

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Background: Chronic urticaria (CU) is a common disease with signs and symptoms reminiscent of allergy. CU, however, is not an allergy, i.e. not due to sensitization and reaction to environmental allergens. Several independent lines of evidence support the concept that CU is an autoimmune disease, with IgG autoantibodies against IgE or its receptor, FceRI, or with IgE autoantibodies to self (autoallergy). The targets of IgE to self and their role and relevance in the pathogenesis and treatment of chronic urticaria remain to be characterized in detail. Objective: To characterize the role and relevance of autoallergy (Type I autoimmunity) in the pathogenesis and treatment of chronic urticaria.

Methods: Autoantigen array and ELISA analyses in patients with chronic spontaneous urticaria, randomized controlled trials and retrospective studies of IgE-targeted treatment in patients with chronic urticaria, clinical profiling of patients with chronic urticaria.

Results: We present published and unpublished findings that show: 1) IgE levels are elevated in patients with CSU. 2) Total IgE levels are linked to the presence and expression levels of IgE to autoantigens. 3) Most patients with CSU express IgE to autoantigens. 4) CSU patients, but not control subjects, have IgE to more than 200 IgE autoantigens. 5) Most of the IgE in CSU patients is directed to autoantigens. 6) Of the IgE autoantigens detected in most patients with CSU, many are soluble or membrane bound and expressed primarily in the skin. 7) The IgE to autoantigens in patients with CSU is functional, i.e. causes FceRI-dependent degranulation of mast cells. 8) IgE-anti-self levels in CSU patients are linked to disease activity and reduced basophil counts. 9) IgE is a predictor of the response to IgE-targeted therapy with omalizumab in patients with CSU. 10) Omalizumab is effective in the treatment of chronic inducible urticarias.

Conclusion: Our findings support the concept that CU, in most patients, is an autoallergy, i.e. due to IgE autoantibodies against autoantigens (autoallergens). These results call for the development of novel treatment options for patients with CU that target the development and function of IgE autoantibodies.

73.

Ceramide-CD300f interaction in mast cells inhibits IgE-dependent and independent anaphylactic responses CD300f inhibits IgE-independent pseudo-allergic reactions ae well as IgE-dependent anaphylactic responses

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CD300 (also called leukocyte mono-immunoglobulin-like receptor (LMIR)) is a paired activating and inhibitory receptor family. CD300f (also called LMIR3) is an inhibitory receptor containing immunoreceptor tyrosine-based inhibitory and switch motif (ITIM and ITSM) in the cytoplasmic region. CD300f is mainly expressed in myeloid cells, including mast cells. We have recently identified ceramide as a ligand for CD300f and demonstrated that ceramide-CD300f binding inhibits IgE-mediated mast cell activation and the related allergic responses in mice (Izawa et al., Immunity, 2012). In addition, we have shown that ceramide-CD300f binding inhibits ATP-, LPS-, or E. coli-stimulated mast cell activation in vivo (Matsukawa et al., Gut, 2016; Shiba et al., J Biol Chem, 2017; Izawa et al., Sci Rep, 2017).

According to a recent report, basic secretagogues including inflammatory peptides and drugs associated with allergic-type reactions (c. g., compound 48/80, Ciprofloxacin, or Icatibant) induced peudo-allergic reactions by directly activating connective tissue-type mast cells via Mrgprb2 in mice or via MRGPRX2 in human. To investigate a role of CD300f in pseudo-allergic reactions, compound 48/80, Ciprofloxacin, or Icatibant together with Evans blue dye was intradermally injected into wild-type or CD300f-deficient mice. The results showed that CD300f

deficiency enhanced vascular permeability in skin treated with these basic secretagogues. Interestingly, ceramide-CD300f interaction inhibited compound 48/80-stimulated degranulation of mouse peritoneal mast cells or human mast cell line LAD2. Moreover, pretreatment with anti-ceramide antibody or ceramide vesicles enhanced or inhibited vascular permeability in compound 48/80-stimulated skin of wild-type mice, but not of CD300f-deficient mice. Thus, ceramide-CD300f interaction inhibits IgE-independent pseudo-allergic reactions as well as IgE-dependent allergic reactions.

74.

Antigen rapid IgE desensitization is driven by differential phosphorylation of SHIP1, Lyn, Syk and p38 MAPK at suboptimal antigen doses.

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Background: Antigen/IgE desensitizations to drug and food allergens have protected thousands of patients from anaphylaxis and allowed for improved life quality and survival in cancer patients by permitting first-line therapies. Although studies have previously shown that rapid IgE desensitization of murine bone marrow-derived mast cells (BMMCs) impairs pathways of mast cell activation, the actual roles of SHIP1, which co-localizes with FceRI and has a negative regulatory role, and the Src-family kinases Syk and Lyn remain unknown in desensitization.

Methods: BMMCs were cultured with IL-3, sensitized with anti-dinitrophenyl (DNP) IgE, and challenged with single or serial doses of DNP-HSA or HSA for activation or rapid desensitization. Desensitization was done by 11 suboptimal doses (Sancho Serra et al 2011). Cells underwent either β-hexosaminidase assay to assess degranulation, sonication for western blot (phospho-Syk Tyr525/526, phospho-SHIP1 Tyr1020, phospho-Src Tyr416, or phospho-p38 D-8), or flow cytometry analysis (phospho-Syk Tyr348).

Results: In BMMCs stimulated with single doses, SHIP1, Syk, Src/Lyn and p38 MAPK proteins showed a dose-response phosphorylation, with minimal phosphorylation at low doses, increased after 60 pg, and maximum at the target dose of 1 ng, when β-hexosaminidase release was highest (30-40%). FACS in permeabilized BMMCs confirmed Syk dose-response phosphorylation at 0.5ng, 1ng, 2.5ng, and 5ng DNP (rMFI: 1.28, 1.75, 2.24, 2.30). In contrast, during desensitization steps, SHIP1 and Lyn were phosphorylated at all steps, predominantly in the early 3- 4 steps, before 60 pg, when the antigen doses were suboptimal and did not induce significant β–hexosaminidase release. Additionally, Syk and p38-MAPK phosphorylation were inhibited at all steps of desensitization, in particular at optimal antigen doses that could induce ehexosaminidase release. FACS confirmed increased Syk phosphorylation in activated versus desensitized BMMCs (rMFI: 1.18, 0.979).

Conclusions: Negative regulatory signals associated with SHIP1 and Lyn phosphorylation in the early steps of IgE desensitization are associated with inhibition of Syk and MAPK p38 signaling - likely preventing further propagation of signal transduction and blocking calcium entry and the release of granule mediators. Whether SHIP1 and/or Lyn agonists would provide tolerization to suboptimal food and drug allergens in human desensitizations should be explored.

75.

The efficacy and safety of venom immunotherapy in patients with clonal mast cell disorders and hymenoptera venom anaphylaxis

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Background Clonal mast cell disorders (cMCD); systemic mastocytosis (SM), monoclonal mast cell activation syndrome (MMAS), are risk factors for severe systemic anaphylactic reaction to hymenoptera venoms. Lifelong venom immunotherapy (VIT) is recommended for these patients; however, its use has raised concerns about its efficacy and safety. The aim of this study was to determine the efficacy and safety of VIT in patients with cMCD and hymenoptera venom anaphylaxis (HVA).

Method Until 2018, 374 consecutive adult patients (≥ 18 yo) were referred to the Mastocytosis Centre Karolinska and investigated due to clinically suspected MCD. The final diagnoses were obtained after a bone marrow investigation following WHO-criteria.

Thirty patients with HVA and cMCD (21 with indolent SM, 9 with MMAS) who received VIT were enrolled in the study. All patients were allergic to vespula venom. Total IgE, venom-specific IgE, component-specific venom IgE, venom-specific IgG4 and serum baseline tryptase levels (sBT) were routinely assessed. The patients were thoroughly followed up during VIT both clinically and with above mentioned biomarkers.

Results The patients, 19 males (63%), received VIT for a median of 48 months (range 7-147 months). Eleven (37%) experienced adverse reactions; 8 during induction, 3 (including 1 anaphylaxis) during maintenance phase. Seventeen (57%) patients (total 22 episodes) were re-stung while undergoing VIT. Four (18 %) of these episodes presented with anaphylaxis (1 reaction during induction) phase, 13 (59 %) with local reaction, and 5 (23%) without any reaction. In 10 of the 22 episodes (45 %) the patient did not take epinephrine, of these 7 (70 %) presented with local reaction, and 3 (30 %) did not develop any symptoms at all. No significant changes were observed regarding levels of sBT, total IgE, venom-specific IgE or venom components during VIT compared to baseline levels. By contrast, we demonstrated that venom-specific IgG4 levels increased during VIT ($p = 0.012$).

Conclusion Our study has shown that VIT provides effective and safe treatment in most patients with clonal MCD, although the incidence of adverse reactions during VIT is increased. The increased levels of venom-specific IgG4 during VIT may correlate with treatment outcome.

76.

Alterations in cord blood hemopoietic progenitor cell surface receptor expression precede atopy: a 1 year follow up in a longitudinal birth cohort study

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Background: Numerous epidemiological studies demonstrate a rising incidence of asthma and allergic diseases. A variety of factors contribute to susceptibility to and severity of allergy and asthma. We have previously reported indications of the involvement of hemopoietic processes in the pathogenesis of atopy and asthma from pre-conception and birth. We have found a striking association between the expression levels of hemopoietic cytokines, innate immune surface receptors on cord blood (CB) $CD34^+$ hemopoietic progenitor cells (HPC), and either maternal atopic sensitization or infant atopic manifestations in several high-risk birth cohort studies. These findings suggest a key role for in utero factors (genetic and environmental) in regulating lineage skewing and mobilization of CB HPCs, thus providing key insights into their role in modulating the production of cell types that are characteristic of allergic inflammation.

Objective: To assess whether cell surface receptor profiles of CB HPC can predict subjects who will develop atopic traits by one year of age in a longitudinal population-based birth cohort.

Methods: We used six-color flow cytometry to compare cytokine and toll-like receptor expression levels in CB HPC from infants who developed atopy (defined as positive skin prick test and atopic dermatitis and wheeze) with those from infants without atopy at one year of age in the Canadian Healthy Infant Longitudinal Development (CHILD) Study.

Results: We found a significant increase in the population of IL5R and IL17RB-expressing HPC in the CB of children who became atopic at 1-year in comparison with those who did not. Conversely, GM-CSFR and ST2-expressing CB HPC were observed to be decreased in atopic children.

Conclusions: Children with atopy at one year of age exhibited altered HPC surface receptor expression patterns at birth compared to non-atopic children. This pattern of receptor expression suggests that Th2 skewing of CB HPC, before the actual onset of atopy, may lead to the development of atopy and/or atopic march in early life.

77.

Time to challenge food allergy diagnosis?

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Food allergies can substantially burden patients and families by negatively affecting finances, social relationships and personal perceptions of health. The study was performed under the Finnish Allergy Program which aims to reduce avoidance diets to foods in school children by 50%. The aim of this study was to investigate how many children that could be freed of a diet restriction in one school district through an extensive diagnostic work-up including Component Resolved Diagnostics and food challenge. Further, to provide a crude estimate of the burden of the elimination food diets in the region, and the savings associated to the proposed intervention and clinical procedures.

Method Two-hundred and five (7.2%) children, who had food avoidance diet according to the school register because of food allergy verified by a physician, were together with their parent invited into the study. One hundred fifty-seven children were interviewed, tested for IgE to extracts and allergen components and food challenged in respective order.

Results Twelve children had an avoidance diet and three had been treated succesfully with OIT after two years. The rest had had their avoidance diet suspended (n=134) or dropped out of the study (9). Suspended diet was due to tolerance development in egg- and milk allergic children. Bet v 1 homology and oral allergy syndrom was another reason for suspended food allergy. Cost of elimination diet was 177 700 Euro per year for the participating children at start, 13 200 Euro per year at the end of the study resulting in a cost saving of 159 500 Euro yearly.

Conclusion The study results demonstrate that it is possible with a 65 % reduction of avoidance diets to foods in schoolchildren.

78.

Comparative analysis of specific allergen levels in baked milk challenge materials

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Backgound: Oral food challenges are considered the 'gold standard' to determine allergic reactions to food. The recent death of a 3-year-old boy during a routine oral food challenge raises questions about whether this could be related to residual allergen in the baked milk challenge material. The aim was to compare the levels of major milk allergens in uncooked and baked milk containing foods, including recipes used for making oral food challenge materials. We also sought to determine the variability in allergen levels between different recipes and repeated batches of the same recipes.

Methods: Uncooked and baked muffin mix were compared using two-site monoclonal antibody ELISA for beta-lactoglobulin (Bos d 5) and for beta-casein (Bos d 11). The lower limit of detection (LLOD) of these assays were 0.19ng/ml and 3.9ng/ml respectively. Intra and inter muffin variability was compared. As well as muffin recipes from Mount Sinai and NHS.

Results: Bos d 5 (beta-lactoglobulin) was reduced from 658µg/g in uncooked muffin mix to 0.07µg/g in baked muffin, representing more than a 99% decrease after baking. Conversely the level of Bos d 11 (beta-casein) decreased from 3030µg/g in uncooked muffin mix to 2841µg/g. Representing only a 6% decrease in allergen after baking. The levels of Bos d 11 allergen varied significantly depending on whether the baked muffin was sampled from the top, middle or bottom of the baked muffin. There was a statistically significant difference in the level of allergen from muffins made according to different recipes.

Conclusions: The level of major milk allergen Bos d 11 remained high within the baked foods, including those used as oral food challenge material. These findings highlight the differences between specific milk allergen molecules and demonstrates the need to assess each potential allergen individually. These measurements could improve safety of food products in clinical practices for oral food challenges.

79.

A new set of cockroach allergens reveals new major components and different patterns of B and T cell immunodominance in a US population of cockroach allergic patients

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Rationale: Evidence in the literature shows that the "traditional" set of five cockroach (CR) allergens does not cover all IgE reactivity to CR. The aim was to create a comprehensive panel of CR allergens, including known or potential allergens, for assessing the importance of most identifiable allergens regarding B cell reactivity and T-cell epitopes on the allergic response to CR in US patients.

Methods: Eight German CR allergens were expressed in Pichia pastoris (groups 1, 2, 4, 6, 7, 9, 11) or Escherichia coli (group 5). The allergens were purified by specific-antibody affinity chromatography (Bla g 1, Bla g 2 and Per a 7), phenol-sepharose chromatography (Bla g 4), glutathione S-transferase affinity chromatography (Bla g 5), ion exchange and size-exclusion chromatography (Bla g 6) or metal-affinity chromatography (Bla g 9 and Bla g 11). IgE antibody levels to the biotinylated allergens were measured using streptavidin ImmunoCAPs. Ex vivo T cell reactivity to allergen-derived peptide pools was determined by flow cytometry.

Results: The pattern of IgE recognition of the new set of cockroach allergens was patient-specific, with the following IgE prevalences in US cockroach allergic patients (n=23): Bla g 1, 30%; Bla g 2, 57%; Bla g 4, 35%; Bla g 5, 39%; Bla g 6, 44%; Per a 7, 22%; Bla g 9, 44% and Bla g 11, 57%, using a conservative cut-off of <0.35 kU/L. For subgroups of patients with high cockroach-specific IgE (CAP classes 3-5), Bla g 2, Bla g 5, Bla g 6, Bla g 9 or Bla g 11 were major allergens. Bla g-specific T cell reactivity was also highly variable among patients: Bla g 1, 40%; Bla g 2, 25%; Bla g 4, 15%; Bla g 5, 15%; Bla g 6, 15%; Per a 7, 15%; Bla g 9, 40% and Bla g 11, 35%.

Conclusions: The results demonstrate the complexity of CR allergen-specific sensitization and T cell responses as highlighted by the variability in immunodominance observed in different patients. The individual sensitization pattern should be taken into account for disease diagnosis and allergy intervention, as well as for B cell component analysis and data interpretation in immunotherapy trials.

80.

Development of a potential allergic reaction predictor tool for peanut challenges

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Background: Reliable prognostic markers for predicting severity of allergic reactions during oral food challenges (OFC) have not been established.

Objective: We sought to develop a predictive algorithm of a food challenge severity score (CSS) to identify those at higher risk for severe reactions to a standardized peanut OFC.

Methods: Medical history and allergy tests were obtained for 120 peanut-allergic participants who underwent double-blind, placebo-controlled food challenges (DBPCFCs). Reactions were assigned a CSS between 1 to 6 based on cumulative tolerated dose and a "severity clinical indicator." Demographic characteristics, clinical features, peanut component IgE values, and a basophil activation marker were considered in a multi-step analysis to derive a flexible decision rule to understand risk of OFC.

Results: 18.3% participants had a severe reaction (CSS >4). The decision rule identified the following three variables (in order of importance) as predictors of reaction severity: ratio of $\%CD63^{hi}$ stimulation with peanut to $\%CD63^{hi}$ anti-IgE (CD63 ratio), history of exercise-induced asthma, and forced expiratory volume in 1 sec/forced vital capacity (FEV1/FVC) ratio. The CD63 ratio alone was a strong predictor of CSS ($p<0.001$).

Conclusion: The CSS is a novel tool that combines dose thresholds and allergic reactions to understand risks associated with peanut OFCs. Lab-values (CD63 ratio), along with clinical variables (exercise-induced asthma and FEV1/FVC ratio) contribute to the predictive ability of the severity of reaction to peanut OFC. Further testing of this decision rule is needed in a larger external data source before it can be considered outside of research settings.

81.

Robust marker selection and cost reduction for basophil activation test usage with high specificity and sensitivity

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Introduction The prevalence of allergies has tremendously increased over the past decades in most parts of the world. As an efficient treatment strategy is dependent on the accurate detection of the culprit allergen source, new approaches that do not simply document allergen-specific sensitization are urgently needed. Provocation tests are the only routine diagnostic measures that verify an allergy. During the last years, a diagnostic method that closely mimics the in vivo situation of an allergic reaction has gained much attention, the basophil activation test (BAT). However, high costs and the availability of well-trained staff restrict the use of BAT to only few institutions. Therefore, we aimed to reduce the complexity as well as the costs of the BAT while retaining a reasonably low cutoff to facilitate the usage of our protocol on instruments only minimally equipped. Moreover, we aimed at improving test specificity.

Methods Blood was drawn from peanut-allergic patients (n=15) and healthy controls (n=30) to monitor the basophil activation mediated by different stimuli including peanut allergens and stimulation controls (anti-IgE, fMLP). Flow cytometric analysis of peripheral blood cells applying different gating strategies was performed to identify mandatory and dispensable markers for the detection of basophils at high purity and the analysis of their activation status.

Results The application of a very comprehensive gating strategy that involves the use of 12 different antibodies as well as light scattering properties (area, width and height) enabled us to identify basophils at high purity and to document their status of activation. Based on this strategy, a clear discrimination between peanut-allergic patients and individuals with an asymptomatic sensitization to peanuts and healthy controls was achieved (sensitivity and specificity 100%). Furthermore, we were able to reduce the number of mandatory markers to 3 (CD63, CD203, FcεRIα) without having negative effects on accuracy or the low cutoff (2%) which saves up to 85% of the costs for antibodies.

Conclusion We have established a low cost method with a cutoff far better than that of commercial kits that is applicable on a wide variety of flow cytometers and seems to provide excellent diagnostic properties.

82.

Raster-scan optoacoustic mesoscopy for precision assessment in allergy patch testing of the skin

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Background: The differentiation between irritative and allergic skin reactions in epicutaneous patch testing is largely based on subjective clinical criteria and prone to a high intra- and interobserver variability.

Method: Dermatological imaging using Raster Scan Optoacoustic Mesoscopy (RSOM) allows three-dimensional assessment of microvascular reactions of the skin. For the first time, we investigated the potential of optoacoustic imaging to improve the precision of patch test evaluation by examining and analyzing a total of 69 test reactions and 48 healthy skin sections in 52 patients.

Results: We identified several relevant models from the optoacoustic images and tested for their diagnostic potential. Linear discriminant analysis was applied and receiver operating characteristic (ROC) curves were calculated to identify optimal cut-off values and quantify test quality. With respect to the "number of vessel fragments" (mean 19.5± 9.7 vs. 14.3 ± 3.7; p=0.01) and "ratio of high-to-low frequency signal" (mean 1.6 ± 0.5 vs. 2.0 ± 0.6, p=0.02) we observed statistically significant differences between allergic and irritative test reactions. Regarding the differentiation of allergic and irritative test reactions, we achieved an area under the ROC curve (AUC) of 0.80 (95% CI 0.64 – 0.91). Using appropriate cut-off values the test method reached a sensitivity of 81% and a specificity of 63%.

Conclusions: The observations correlate most likely with differences in vascular physiology such as vasodilation and vessel tortuosity as well as edema. RSOM can be used for high-resolution imaging of skin allergic reactions. In addition, used as a complementary diagnostic tool, RSOM holds potential to improve precision of allergy patch testing.

83.

Mugwort pollen is the main vector for outdoor endotoxin.

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Endotoxins (lipopolysaccharides, LPS) released from Gram-negative bacteria exert strong immunologic and inflammatory effects and when airborne may contribute to respiratory conditions such as allergic asthma.

We determined LPS in outdoor air on a daily basis for a period of 4 consecutive years in Munich and Davos. Air was sampled as Particulate Matter PM>10µm and 10>PM>2.5. Over 60%

of the annual endotoxin exposure was detected in the PM>10 fraction showing that bacteria do not aerosolize as independent units or aggregates, but adhered to very large particles. In Munich, the endotoxin exposure was highest in summer, with 70% of the annual exposure detected between June 12th and August 28th. Multivariate modelling showed that endotoxin levels could be explained by phenological parameters i.e. plant growth. Indeed, days with high airborne endotoxin levels correlated well with the amount of Artemisia pollen in the air. Pollen collected from plants across Europe (100 locations) showed that the highest levels of endotoxin were detected on Artemisia vulgaris (mugwort) pollen, with little on other samples. Microbiome analysis showed that LPS concentrations on mugwort pollen were related to the presence of Pseudomonas spp. and Pantoea spp. communities. In a mouse model of allergic disease, the presence of LPS on mugwort pollen was critical for allergic sensitization.

The majority of airborne endotoxin stems from bacteria dispersed with pollen of only one plant: mugwort. In addition of LPS being health relevant, we show that pollen is an important ecological vector for the airborne dispersal of bacteria.

84.

Calcium binding protein, Spermatid-Associated 1: A biomarker of stress with an anti-inflammatory motif

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Background: We previously established that rat SMR1 is under neuro-regulation and has anti-inflammatory activity in allergic and other inflammatory responses. We developed a mimetic of the anti-inflammatory motif, but this was unsuccessful in a human trial of allergic asthma. Interestingly, Smr1 is not in the human genome. Thus, we searched for a human orthologue using the sequence of the anti-inflammatory peptide. We identified CABS1 (Calcium Binding Protein, Spermatid-Associated 1) and established that its homologous peptide has anti-inflammatory activity. CABS1 is in the same region of the homologous human chromosome (4), as SMR1 in the rat (14), and its protein is found in salivary glands and saliva. If human CABS1 is neurally regulated, we postulated it would be elevated after acute (Trier Social Stress Test) and examination stress.

Methods: Using a polyclonal antibody to a CABS1 immunogen in semi-quantitative Western Blot (WB) analyses, we studied levels of CABS1 in human stress. We characterized three other polyclonal antibodies to CABS1 using WB, automated, quantitative immunoassay (Wes, ProteinSimple), and immunohistology.

Results: We identified a 27 kDa form of CABS1 in saliva of all participants, and additional smaller forms in some participants. Acute stress increased the level of the 27 kDa form in saliva. Participants with smaller forms of CABS1 had reduced stress and negative affect (increased resilience) in response to acute stress. We are testing comparability of WB and Wes in stress samples. If validated, we will use Wes to study CABS1 levels in stress and stress reduction in military personnel and ulcerative colitis.

Conclusions: The association of CABS1 with stress and potentially with resilience to stress may enable monitoring the effects of stress on allergic and other inflammatory diseases, and development of novel therapeutics targeting the stress-health relationship.

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85.

Periostin in allergic bronchopulmonary aspergillosis: serum levels and its expression in the lungs

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Allergic bronchopulmonary aspergillosis (ABPA) is an airway disease characterized by peripheral blood eosinophilia, elevated total IgE and appearance of Aspergillus-specific IgE/IgG in serum, and bronchiectasis/mucus plugs in the central bronchi. Eosinophils and IgE levels have been used as diagnostic biomarkers for ABPA, however, their usefulness are limited in the stable diseases under treatment with systemic corticosteroids. Serum periostin is a unique biomarker that reflects type 2 inflammation in the asthmatic airways, however, little is known about its involvement in ABPA. We examined serum periostin levels and its expression in the lungs with ABPA.

Serum periostin concentrations were measured with enzyme-linked immunosorbent assay in 44 patients with ABPA, 49 patients with severe asthma with fungal sensitization (SAFS), 32 and 44 patients with atopic/non-atopic severe asthma without fungal sensitization, respectively. Immunohistochemical analysis of periostin was performed in the lungs resected from the patients with ABPA or asthma.

Median concentration of periostin in serum was 107 (76-139) ng/mL (interquartile range) in ABPA, significantly higher than in all patients with severe asthma [n = 72, 72 (56-100) ng/mL, $p<0.0005$] or in patients with Aspergillus-sensitized SAFS [n = 24, 72 (56-84) ng/mL, $p<0.005$]. We then examined the usefulness of periostin, IgE, and eosinophils as biomarkers to discriminate ABPA from Aspergillus-sensitized SAFS. Area under the ROC curve (AUC) was 0.75 for periostin ($p < 0.001$), 0.71 for total IgE ($p = 0.005$), and 0.51 for peripheral blood eosinophil counts ($p = 0.92$). The optimal cut-off value was 100 ng/mL for periostin and 970 IU/mL for total IgE with the sensitivity and specificity of 83% and 57% for periostin and 75% and 70% for total IgE, respectively. The deposition of periostin immunoreactivity was observed in the sub-epithelial layer both in the lungs with ABPA and asthma. In addition, periostin was strongly expressed at the area of organizing pneumonia surrounding bronchocentric granulomatosis in the lungs with ABPA.

Serum concentrations of periostin were elevated in ABPA with the expression in the sub-epithelial basement membrane of the airways and at the organized lesions of the peripheral lungs.

86.

Grass pollen immunotherapy: relationships among clinical response to nasal allergen challenge, seasonal symptoms, nasal tissue eosinophils and the impact of treatment compliance.

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Background: Good compliance with allergen immunotherapy is important for optimal clinical efficacy. In the GRASS trial (Gauging Response in Allergic Rhinitis to Sublingual and Subcutaneous Immunotherapy, Scadding G JAMA 2017;317-615-25) two years sublingual (SLIT) or subcutaneous immunotherapy (SCIT) was effective in suppressing the clinical response to nasal grass pollen allergen challenge (NAC), but this was not sustained 12 months after discontinuation. Here we evaluate relationships among NAC, symptoms during the pollen season, peak nasal inspiratory flow (PNIF) post-NAC and local tissue eosinophils; we also assess the impact of treatment compliance.

Methods: In a randomised double-blind placebo-controlled trial of SCIT and SLIT grass pollen immunotherapy (n=106) we performed a post-hoc comparison between NAC and seasonal symptoms, PNIF and numbers of nasal mucosal biopsy eosinophils (immunohistochemistry using monoclonal antibody EG2). We also correlated NAC response with the degree of compliance with once daily sublingual grass allergen tablets, as measured by return of used and unused tablet blister packs.

Results: In 92 completed participants, 91.3% took more than 50% of study tablets (protocol adherent), 75.0% took more than 75%, and 46.7% took more than 90%. Greater compliance with sublingual tablets was associated with lower NAC responses at year 2 (whilst on treatment) ($r=-0.46$, $p<0.01$) and at year 3, 12-months after stopping treatment ($r=-0.32$, $p<0.10$). Positive correlations were observed between response to NAC and each of seasonal weekly visual analogue scores, rhinitis quality of life scores (mini-RQLQ) and a global evaluation after the season at year 2 ($r=0.32$, $p=0.002$; $r=0.33$, $p=0.002$ and $r=0.31$, $p=0.002$). For all 3 outcomes correlations remained significant at year 3, 12-months after stopping treatment ($r=0.22$, $p=0.04$; $r=0.25$, $p=0.01$ and $r=0.42$, $p<0.001$). At year 3, there was an inverse relationship between post-NAC PNIF and nasal tissue eosinophils after both SLIT ($r=-0.46$, $p=0.01$) and SCIT ($r=-0.36$, $p=0.06$).

Conclusion: Good compliance with sublingual tablet immunotherapy correlated with enhanced suppression of the clinical response to NAC. In turn, NAC correlated with seasonal symptoms during natural pollen exposure. Improvements in nasal airflow correlated inversely with nasal tissue eosinophils. These data support the use of nasal allergen challenge as a useful clinical surrogate in immunotherapy trials.

87.

Diverse and highly cross-reactive T cell responses in ragweed allergic patients from different geographical regions

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Background: Ragweed is one of the primary causes of seasonal allergies in the United States. Several closely related ragweed species are known to cause allergic symptoms and their distribution varies across geographical regions. The cross-reactivity of IgE causing these diverse sensitizations and the cross-reactivity of IgG4 induced by ragweed tablet treatment has been demonstrated previously. However, little is known about the underlying cross-reactivity between ragweed species regarding T cell responses.

Purpose: To determine the level of cross-reactivity of T cell responses towards important ragweed epitopes from Amb a allergens and their homologous epitopes from related weed species.

Methods: Sequences of Amb p and Amb t allergens were identified by transcriptome and proteome analyses of pollen extracts. T-cell epitopes of Amb a allergens (Amb a1, 3, 4, 5, 8, and 11) were mapped for 20 American ragweed allergic individuals by fluorospot and proliferation assays towards 20-mer overlapping (10aa) peptides. T-cell responses to 50 frequently recognized T-cell epitopes and homologoes peptides from Amb p, Amb t, and Art v were further investigated in 32 American and 14 Slovakian ragweed allergic individuals. Bioinformatic tools were used to investigate possible associations between T-cell specificities, IgE sensitivities (ImmunoCAP), HLA expression (genotyping), and geographical origin.

Results: Various isoform-specific and common T-cell epitopes with response frequencies above 30% were identified for Amb a1. T-cell epitopes recognized by more than 20% of the donors were observed for Amb a3, 4, 8 and 11 whereas Amb a5 epitopes were recognized by 10% or less. Extensive cross-reactivity was observed for the majority of the T-cell epitopes investigated. Several HLA DR molecules may be involved in the responses to most of the epitopes. In general, T cell cross reactivity correlated with sequence homology.

Conclusion: T-cell response in ragweed allergic individuals are polyclonal and directed towards multiple epitopes positioned primarily in group 1, 8 and 11 allergens. T-cell lines from individual patients were highly cross-reactive towards other homologous ragweed species as well as towards allergen isoforms without any apparent geographical bias. These conclusions are consistent with the use of Amb a as the representative allergy immunotherapy species for this homologous group of weeds.

88.

Hypoallergenic properties of a hybrid protein from Dermatophagoides pteronyssinus allergens

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Background: More than 30 allergens from the house dust mite Dermatophagoides pteronyssinus have been characterized. They induce different profiles of sensitization in atopic individuals and allergic patients, which determine the IgE reactivity to the whole extract, the reagent currently used for immunotherapy. However, to overcome the limitations of using extracts for immunotherapy, modified recombinant allergens exhibiting reduced allergenic activity have been proposed. The aim of this study was to evaluate the allergenic properties of a hybrid protein composed by segments of four allergens from D. pteronyssinus.

Methods: A hybrid protein (DPx4) made up by four segments of allergens from D. pteronyssinus (Der p 1, Der p 2, Der p 7 and Der p 10), was expressed in E. coli. The protein fold and cysteine protease activity were analysed by circular dichroism and kinetic assay respectively. The IgE reactivity was determined by ELISA and non-denaturing dot-blot using sera from house dust mite allergic individuals. Basophil activation was detected by flow cytometry. Epitopes on DPx4 structure were analysed by ELISA inhibition assay with human sera and dot blot with anti-Der p 1 and anti-Der p 2 monoclonal antibodies. Induction of passive cutaneous anaphylaxis (PCA) and the production of specific IgG blocking antibodies were investigated using BALB/c mice.

Results: The hybrid DPx4 showed a beta structure partially folded and had no cysteine protease activity. The frequency of IgE reactivity and antibody levels were significantly lower than those obtained with Der p 1, Der p 2 and D. pteronyssinus extract. In the ELISA inhibition, the IgE reactivity to allergen extract was inhibited (35%) by the hybrid protein. DPx4 reacted with the monoclonal antibodies and induced lower basophil activation than Der p 2 and the allergen extract. Immunization of mice induced specific IgG antibodies that reacted with Der p 1 and Der p 2, and these antibodies inhibited the binding of allergic patients' IgE to allergen extract. The capacity to induce PCA by specific antibodies to DPx4 was significantly lower than observed with antibodies to D. pteronyssinus extract. Altogether, our results suggest that DPx4 could be useful for the development of a mite allergy vaccine.

89.

Immunological biomarkers of successful immunotherapy

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BACKGROUND: Allergen-specific immunotherapy (AIT) represents the only curative and specific way for the treatment of allergic diseases, which have reached a pandemic dimension in industrial countries. Besides our growing knowledge concerning mechanisms we still lack objective, validated biomarkers that would allow efficacy monitoring as well identification of responder patients preferably at the beginning of therapy.

OBJECTIVES: Search for immunological biomarkers defining clinical responder patients.

METHODS: PBMCs from birch pollen allergic patients that participated in a double blind, placebo controlled, phase II study (NCT02271009) were collected before, during and after COPs AIT and were stimulated with recombinant Bet v 1 for 7 days. Frequencies of Th1, Th2, Th17, Th22 and Treg cells were analysed by flow cytometry. Cytokine levels were measured in supernatants after 5 days of culture. Clinical results of the therapy were evaluated by subjective global efficacy score assessment.

RESULTS: We found significant differences between responder and non-responder patient groups (Global Evaluation of Treatment Efficacy) for selected immune parameters. Responder patients were characterized by significant increase in allergen-specific T-regulatory cell frequency during AIT. Additionally we were able to detect differences between responder and non-responder patients at the therapy onset. Clinical responder patients had significantly lower levels of allergen-specific T regulatory cells than non-responder patients. **CONCLUSION:** Allergen specific T-regulatory cell frequency at the onset of therapy together with immunological parameter change at the early stages of immunotherapy can thus be a potential biomarker that can be used to design a clinical algorithm that may define responder patients before and during AIT.

90.

The immunomodulation effect of secreted peptide, LGp40, from Lactobacillus gasseri LGP40 in allergic diseases

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Background: Probiotics are normal inhabitants in the gastrointestinal tracts of man and are widely considered to exert a number of beneficial roles including immunomodulation, interference with enteric pathogens, and maintenance of a healthy intestinal microflora. In recent years, studies of probiotics have also confirmed their extra-intestinal effects, particularly for the prevention of allergic diseases. However, the anti-allergy mechanism of probiotics is still unclear. Our previously studies found oral intake of Lactobacillus gasseri (L. gasseri) for 2 months improved mucosal allergic symptoms in asthmatic children with allergic rhinitis in a double-blind, randomized controlled (DBRC) trial. L. gasseri can attenuate allergen-induced airway inflammation in mouse model of asthma and suppress Th17 cell infiltration in the lungs (Bri J Nutrition 2011). We also identified a novel mechanistic pathway through PPARγ activation in dendritic cells, by which probiotics help maintain host homeostasis and for the treatment and prevention of allergic asthma (J Mol Med 2017). In this study, we plan to identify and characterize the anti-allergic effect of the secreted peptide, LGP40, from L. gasseri that can activate PPARγ and enhance IL-12 production in dendritic cells.

Methods: LGp40 is purified from L. gasseri culture supernatant. Immmuno-modulation effect of LGP40 was evaluated in in vitro on marrow-derived DCs (BMDC) and macrophages, and administered in murine model of allergic diseases.

Results: We found LGp40 significantly increased the expression of IL-12p40 in mouse BMDC. LGp40 is also binder of plasminogen, and this binding activity was inhibited by G3PDH enzyme activity inhibitor (IAA).LGp40 was intraperitoneally administrated in a preventive or therapeutic manner in HDM-sensitized mice with allergen-induced airway inflammation. Mice administered with medium doses of LGp40 had significantly reduced airway resistance, inflammatory cells infiltrations, total IgE levels and TH2 inflammatory cytokines in BALF as compared to non-treated asthmatic mice.

Conclusions: We believe our results may provide a new therapeutic alternative for allergic asthma.

91.

Knowledge of pulmonologists, allergists, ENTs and paediatricians related to maintenance treatment of asthma

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Background: April 2017 the Mexican Asthma Guidelines (GUIMA) were published, using the ADAPTE approach, fusing evidence from the BTS/SIGN, GEMA and GINA guidelines, adapted for Mexico. Before launch physicians' knowledge was explored related to key-issues of the guideline.

Methods: A Surveymonkey® survey was sent out to board-certified physicians of 4 medical specialties treating asthma. Replies were analyzed per specialty against the GUIMA evidence-based recommendations.

Results: A total of 364 allergists (ALERG), 161 pulmonologists (PULM), 34 ENTs and 239 pediatricians (PED) replied. Spirometry is not routinely indicated when asthma is very probable by ALERG54%, PULM47%, ENT39%, PED65%. A fictitious case proposed to the physicians with intermittent asthma was erroneously treated with ICS by ALERG33%, PULM38%, ENT55%, PED30%, while a mild persistent case received mistakenly ICS-LABA by ALERG35%, PULM37%, ENT39%, PED32%. The 1st-line option for moderate persistent asthma was ICS(median dose) instead of ICS(low)+LABA for ALERG29%, PULM25%, ENT17%, PED27% and in severe asthma maintenance treatment ALERG29%, PULM-ENT-PED 20-30% failed to indicate LABA. Concerning the guidelines' recommendation to use one inhaler for maintenance&rescue in moderate-severe asthma, ALERG57%, PULM45%, ENT72%, PED80% erroneously indicated ICS-salmeterol could be used, instead of ICS-formoterol. Oral b2 or theophylline are no longer recommended, but PULM37% and ALERG-ENT-PED 42-62% still indicate their use. In severe asthma 61-73% of physicians consider adding LTRA to the treatment, but only 6-17% consider adding omalizumab.

Conclusion: an online survey could detect specialty-specific knowledge-gaps related to asthma treatment. Even amongst allergists and pulmonologist there is ample room for improvement.

92.

Cyclophilin – a novel cross-reactive determinant in peanut

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Background: Component-resolved diagnostics is a frequently used tool to distinguish between primary and cross-reactive peanut sensitization. Known targets of cross-reactive pollen sensitization in peanut are PR-10 (Ara h 8), profilin (Ara h 5) and cross-reactive carbohydrate determinants (CCD). In a subset of subjects, we found pollen-dependent IgE binding to peanut that could not be explained by either of these targets. The aim of this study was to identify an hitherto unknown determinant of cross-reactivity between pollen and peanut.

Method: Sera of 15 peanut sensitized subjects that tested negative to Ara h 1, 2, 3, 6, 8, 9, profilin and CCD, but whose IgE binding to peanut extract could be inhibited by grass pollen extract, were used in this study. Peanut extract was separated by different chromatographic methods and fractions displaying IgE binding activity were analysed by MS/MS using an Orbitrap Fusion instrument. A novel peanut protein was expressed in E. coli and purified by IMAC and gel filtration. IgE antibody measurements were performed using ImmunoCAP.

Results: MS/MS analysis of a basic, IgE reactive fraction, purified by anion and cation exchange, size exclusion and reversed phase chromatography, produced a perfect match (100% coverage) to a cyclophilin (peptidyl-prolyl cis-trans-isomerase)-like amino acid sequence predicted from peanut EST record GO340500. The 172-residue sequence was predicted to have no signal peptide, a molecular weight of 18.3 kDa and an isoelectric point of 8.4. It showed 86% sequence identity with carrot cyclophilin, a previously described IgE reactive protein. Recombinant peanut cyclophilin could completely outcompete IgE binding to the isolated peanut fraction of interest and bound IgE antibodies from 13 of the 15 sera studied (87%), at a level which was on average 9-fold higher than to peanut. The results suggest that cyclophilin is a low-abundance protein in peanut and probably not a primary sensitizer.

Conclusion: Cyclophilin was identified as a novel IgE binding protein in peanut, forming a previously unknown cross-reactive overlap with pollen. Alongside PR-10, profilin and CCD, cyclophilin may become a valuable marker of pollen-related food sensitization which represents a lower risk of severe food allergy than primary food sensitization.

93.

Multidimensional endotypes in patients with allergic reactions revealed by topological data analysis of clinical and immunological parameters: Associations between mast cell mediator release, atopy and reaction severity

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Background: Allergic reactions can present with diverse symptoms ranging from a mild skin rash to a life-threatening anaphylaxis. However, little is known of how such heterogeneity can relate to underlying cellular and biochemical events. We have investigated serum levels of markers of mast cell activation and a panel of cytokines in patients with allergic reactions to drugs or food aiming to identify clinico-immunological endotypes.

Method: Patients (n=321) and control participants (n=22) were recruited from two separate centres, the Allergy Clinic of Southampton University Hospital, UK (n=202) and Queen Medical Hospital, Qatar (n=119), where detailed clinical assessment and phlebotomy were performed. Serum levels of mast cell tryptase and carboxypeptidase A3 (CPA3) were determined using sensitive enzyme linked immunosorbent assays (ELISA) that were developed and validated, using specific antibodies (lower limits of detection of 0.4 ng/ml and 0.2 ng/ml, respectively). A panel of pro-inflammatory mediators were determined in serum using electro-chemiluminescence-based V-plex assays. Clinical and biochemical parameters were analyzed using topological data analysis (TDA) to identify multidimensional endotypes.

Results: Patients who had suffered allergic reactions (concurrent with asthma, hay fever, or atopic dermatitis) had significantly higher baseline levels of tryptase (p=0.04), CPA3 (p=0.04) and IL-4 (p=0.03) than in subjects without an allergic history. There was a strong association between the severity of historical reactions and atopy (Pearson Chi square p<0.0001). Levels of IL-6 and IL-8 at baseline were significantly higher in cases of drug allergy than those with food allergy (p<0.0001). TDA identified four clinico-immunological clusters: high CPA3 - high IL-13, high CPA3 - low IL-13, low CPA3 – high IL-13, low CPA3 – low IL-13. These four clusters were replicated in both geographic cohorts.

Conclusions: The identification of novel clinico-immunological endotypes in two quite distinct populations of allergic patients highlights associations between levels of specific mast cell mediators, atopy and the severity of allergic reactions, and may provide insights into new approaches for diagnosis and management.

94.

Interaction patterns between component-specific IgE antibodies in component-resolved diagnostics and prediction of asthma: Machine learning approach

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Background: The relationship between allergic sensitisation and asthma is complex, and the data about the strength of this association are conflicting. We propose that discrepancies arise in part because sensitisation is not a single entity (as considered conventionally), but a collection of several different sensitisation classes. We hypothesised that interaction patterns between IgE antibodies to individual allergenic molecules (components), rather than IgE responses to "informative" molecules, are hallmarks of different types of sensitisation, and are linked to the increased risk of asthma.

Methods and findings: We measured IgE to 112 allergen components using a multiplex array (ISAC®) among 461 children aged 11 years in a population-based birth cohort. We analysed data for 44 active components (sIgE≥0.30 ISU in at least 5% of children), and among 213 participants sensitised to least one active component. Firstly, we applied network analysis and hierarchical clustering to explore the connectivity structure of component-specific IgEs and identify clusters of component-specific sensitisation ("component clusters"). Of the 44 components included in the model, 33 grouped in seven clusters (C.sIgE-1-7), and remaining 11 formed singleton clusters. Cluster membership mapped closely to the structural homology of proteins and their biological source. Components in the PR-10 proteins cluster (C.sIgE-5) were central to the network, and mediated connections between components from grass (C.sIgE-4), trees (C.sIgE-6) and profilin clusters (C.sIgE-7) with those in mite (C.sIgE-1), lipocalins (C.sIgE-3) and peanut clusters (C.sIgE-2). We then used hierarchical clustering to identify four "sensitisation clusters" among study participants: (1) Multiple sensitization (sIgE to multiple components across all 7 component clusters and singleton components); (2) Predominantly dust mite sensitisation (IgE mainly to C.sIgE-1 components); (3) Predominantly grasses/trees sensitisation (sIgE to multiple components across C.sIgE-4-7); and (4) Lower-grade sensitisation. We used bipartite network to explore the relationship between component clusters, sensitisation clusters and asthma, and the joint density-based non-parametric differential interaction network analysis and classification (JDINAC) to test whether interactions of component-specific IgEs are associated with asthma. JDINAC outperformed logistic regression in predicting asthma, with area-under-the-curve of 0.86 compared to 0.59. Logistic regression had low sensitivity (0.66) and low specificity (0.52); in contrast, JDINAC provided a good balance between sensitivity (0.84) and specificity (0.89). We then inferred the differential network of pairwise component-specific IgE interactions which demonstrated that interactions between 16 pairs of allergen components predicted asthma. These findings were confirmed in an independent sample of children aged 8 years.

Conclusions: Interactions between pairs of sIgE components alter the risk of asthma and provide the basis for designing diagnostic tools for asthma.

95.

Basophil activation as determined by basogranulin release, and altered expression of membrane-bound and intracellular basogranulin stores: Sensitive means for diagnosing allergic sensitivity

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Basophil activation tests can provide valuable information on allergic sensitivity to a range of different allergens. The most widely employed involve flow cytometric detection of increased expression of membrane-bound CD63 or CD203c following addition of allergen to basophils in vitro. The identification of basogranulin, a unique basic protein stored in basophil granules, has opened the way for new basophil activation methods to be explored.

Blood was collected from subjects with a history of allergy to food, drugs, house dust and grass pollen. Basophils were stimulated with specific allergen, anti-IgE antibody, or the peptide fmetleuphe (FMLP). Flow cytometry was performed with non-permeabilised and permeabilised cells with antibodies specific for basogranulin or CD63, and data analysed with CellQuest software. In separate experiments, cell supernatants were collected and levels of basogranulin and histamine determined by dot blotting and enzyme immunoassays respectively.

Basogranulin was released into cell supernatants of allergen-stimulated basophils with bell-shaped concentration response curves similar to those observed for histamine. Flow cytometry with permeablised cells indicated that intracellular stores of basogranulin were depleted following basophil activation. Associated with basogranulin release was the presence of increased quantities of this marker on the basophil membrane following cellular activation. Increased membrane expression of basogranulin mirrored that for CD63, and with allergens and other stimuli tested the measurement of cell surface basogranulin represented a more sensitive means for assessing basophil activation in vitro. The flow cytometric assays for basogranulin were optimised for use with samples of whole blood so as to avoid the need for basophil purification.

The rapidity, simplicity and reproducibility of basogranulinbased methods for measuring basophil activation will facilitate their application to clinical samples and allow better assessment for allergic sensitivity.

96.

Epithelial barrier dysfunction in asthma: new role for protein kinase D

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Background: Epithelial barrier dysfunction is increasingly associated with asthma, but the mechanisms involved are poorly understood. Although early studies indicated that allergen proteases degrade tight junction proteins, recent research has uncovered several protease-independent mechanisms of epithelial tight junction dysfunction.

Methods: Using monolayers of human 16HBE epithelial cells grown in vitro, we compared the barrier disruptive effects of allergen extracts (house dust mite and ragweed, from Indoor Biotechnologies) and a panel of pattern recognition receptor ligands (including lipopolysaccharide, the double-stranded RNA poly:IC, flagellin, and CpG oligonucleotides). Barrier dysfunction was analyzed using both trans-epithelial electrical resistance (TEER) and paracellular permeability to macromolecules. Experiments were repeated at least six times.

Results: Of all of the compounds examined, polyI:C was far and away the most potent barrier disruptive, causing marked reductions in TEER (~70-80%) and increased paracellular permeability ($p<0.05$). Dust mite extracts and recombinant protease-high Der p 1 caused modest reductions in TEER (~20-30%) but did not significantly increase permeability. We explored the mechanisms of polyI:C-induced barrier dysfunction using a panel of chemical inhibitors, and found that the protein kinase D (PKD) antagonist was strikingly barrier protective (CRT0066101). By Western blot and RT-PCR, PKD3 was the most highly expressed PKD isoform in the airway epithelium. PKD3 knock-out mice were obtained and found to develop normally. In order to monitor airway barrier function in living animals, we instilled FITC-dextran into the airway and measured its disappearance from airspaces (and accumulation in serum) over time. In separate studies, we found that this approach is reliable measure of "outside/in" barrier function in the lung. Using a mouse model of polyI:C inhalation, PKD3 knock-out mice were protected from polyI:C-induced barrier dysfunction measured in vivo ($n=12$ mice per genotype, $p<0.05$). Interestingly, PKD3 knock-out mice were also protected from polyI:C-induced airway neutrophilia ($n=10$ mice per genotype, $p<0.05$).

Conclusions: These results point to a new role for PKD3 in regulating double stranded RNA-induced airway epithelial barrier function as well as airway inflammation. Since epithelial barrier dysfunction and inflammation likely contribute to virally mediated asthma exacerbations, further study of PKD antagonists in models of asthma and asthma exacerbation seem warranted.

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97.

IL-24 causes epidermal barrier dysfunction downstream of the IL-13/periostin pathway in allergic skin inflammation

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Background: Barrier dysfunction is an important feature of atopic dermatitis (AD) in which IL-4 and IL-13, signature type 2 cytokines, are involved. Periostin, a matricellular protein induced by IL-4 or IL-13, plays a crucial role in the onset of allergic skin inflammation, including barrier dysfunction. However, it remains elusive how periostin causes barrier dysfunction downstream of the IL-13 signal.

Methods: We systematically identified periostin-dependent expression profile using DNA microarrays. We then investigated whether IL-24 downregulates filaggrin expression downstream of the IL-13 signals and whether IL-13–induced IL-24 expression and IL-24–induced downregulation of filaggrin expression are dependent on the JAK/STAT pathway. To build on the significance of in vitro findings, we investigated expression of IL-24 and activation of STAT3 in mite-treated mice and in AD patients.

Results: We identified IL-24 as an IL-13–induced molecule in a periostin-dependent manner. Keratinocytes are the main IL-24–producing tissue-resident cells stimulated by IL-13 in a periostin-dependent manner via STAT6. IL-24 significantly downregulated filaggrin expression via STAT3, contributing to barrier dysfunction downstream of the IL-13/periostin pathway. Wild-type mite-treated mice showed significantly enhanced expression of IL-24 and activation of STAT3 in the epidermis, which disappeared in both STAT6-deficient and periostin-deficient mice, suggesting that these events are downstream of both STAT6 and periostin. Moreover, IL-24 expression was enhanced in the epidermis of skin tissues taken from AD patients.

Conclusions: The IL-13/periostin pathway induces IL-24 production in keratinocytes and IL-24 plays an important role in barrier dysfunction in AD.

98.

Novel role for the acute phase protein serum amyloid A in the initiation of type 2 immunity

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Background: Our understanding of how innate type 2 immunity wards off harmful environmental triggers has increased tremendously with the discovery of the type 2 promoting cytokine IL-33 and IL-33-activated lung innate lymphoid cells (ILC2). However, when mounted against innocuous environmental proteins such as allergens or exaggerated, this response can harm the host. Yet, the knowledge of how such allergens are sensed at mucosal surfaces is limited. This work identifies a novel, evolutionary conserved innate immune sensing pathway that is associated with the development of allergic inflammation.

Results: Specifically, we identify the formyl-peptide receptor 2 (FPR2) and its endogenous ligand, the acute phase protein serum amyloid A1 (SAA1) as major drivers of house dust mite (HDM)-induced IL-33 release in vitro and in vivo. In mice, local inhibition of FPR2 in the lungs abrogated HDM-induced airway hyperresponsiveness, IgE synthesis, and bronchoalveolar lavage (BAL) eosinophilia, concomitant with reductions in Th2 cytokine levels, IL-13^{+} innate lymphoid cells (ILC2s), and BAL IL-33 levels in allergen-exposed mice. Similarly, Saa1/2 deficiency or antibody blockade of SAA1 abrogated HDM-induced IL-33 secretion and ILC2 recruitment. This was dependent on SAA1 recognition of the cytosolic fatty acid binding protein (FABP) Der p 13 contained in HDM extract. Importantly our findings in mice translate to human allergic diseases including asthma and chronic rhinosinusitis (CRS). Notably, the FABP sensing pathway is upregulated in respiratory epithelial cells from asthmatic and CRS patients resulting in increased IL-33 secretion associated with enhanced SAA1 monomer formation and FPR2 signaling.

Conclusion: Taken together, we here report a novel mechanism of allergenicity which involves SAA1-facilitated allergen recognition via FPR2 leading to aberrant IL-33 release and type 2 responses. This novel paradigm allows for a new view on SAA1 as a potent driver of type 2 allergic immune responses at mucosal surfaces.

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99.

The leukotriene E4 receptor CysLT3R regulates airway brush cell function and type 2 inflammation

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Background: Cysteinyl leukotrienes (cysLTs) are pro-inflammatory lipid mediators with recently identified innate functions in promoting type 2 inflammation. CysLT3R, the receptor for LTE4, is expressed on nasal goblet cells and mediates their release of mucus. How this epithelial cell (EpC) receptor might promote type 2 inflammation in the lung has not been identified.

Methods: Wild-type (WT), LTC4 synthase-deficient (Ltc4s$^{-/-}$) and CysLT3R-deficient (Oxgr1$^{-/-}$) mice received a single intranasal dose of aeroallergen (Alternaria alternata or Dermatophagoides farinae) or saline and airway EpC remodeling and lung inflammation was assessed by immunofluorescence and flow cytometry, respectively. WT, Oxgr1$^{-/-}$, IL-25 reporter (Il25F25/F25) and Stat6$^{-/-}$ mice were given 4 daily doses of LTE4 and analyzed 24 h after the last inhalation as above. Antibody blockade with intraperitoneal anti-IL25 was performed on days 0 and 3 in conjunction with Alternaria and LTE4 challenges and analyzed as above. RNA sequencing was performed on sorted airway EpC subsets.

Results: Here we find that murine tracheal brush cells (BrCs), a chemosensory EpC subset, express CysLT3R and high levels of IL-25 and the biosynthetic machinery for cysLT generation, including Alox5, Alox5ap, and Ltc4s. Aeroallergen inhalation leads to the rapid expansion of BrCs, which is attenuated in mice lacking either LTC4 synthase or CysLT3R. LTE4 inhalation is sufficient to elicit CysLT3R-dependent expansion of IL-25+ BrCs through an IL-25-dependent but STAT6-independent signaling pathway. Finally, blockade of IL-25 attenuated both aeroallergen and LTE4-elicited CysLT3R-dependent type 2 lung inflammation.

Conclusions: These results demonstrate that LTE4 is a novel driver of BrC expansion, activation, and subsequent IL-25-dependent type 2 inflammation mediated through CysLT3R.

100.

Cysteinyl leukotriene receptor 2 drives lung immunopathology through a platelet and high mobility box 1-dependent mechanism

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Cysteinyl leukotrienes (cysLTs) act at both structural and hematopoietic cells to facilitate mucosal type 2 (eosinophilic) immunopathology. LTC4, the parent cysLT, acts at platelet-associated type 2 cysLT receptors (CysLT2R) to upregulate lung endothelial adhesion receptors essential for antigen-induced eosinophil recruitment. We now demonstrate that CysLT2R signaling on platelets induces a high mobility box 1 (HMGB1)/receptor for advanced glycation end products (RAGE)-dependent autocrine circuit leading to both endothelial priming and IL-33-dependent mast cell activation/aspirin sensitivity. LTC4, but not its metabolites LTD4 or LTE4, induced surface expression of HMGB1 by mouse platelets in a CysLT2R-dependent manner. Blockade of RAGE with FPS-ZM1, but not of toll like receptor 4 with LPS-RS, prevented LTC4-induced platelet activation ex vivo. The administrations of either LTC4 or the CysLT2R-selective agonist N-methyl LTC4 to ovalbumin-sensitized WT mice increased BAL fluid levels of HMGB1 in parallel with the platelet products CXCL7 and thromboxane B2 in a CysLT2R-dependent manner. Neutralization of HMGB1 with a monoclonal Ab or blockade of RAGE with FPS-ZM1 prevented LTC4-induced airway eosinophilia and platelet activation, while also preventing increases in the levels of soluble vascular cell adhesion molecule-1 and intracellular adhesion molecule-1. Depletion of platelets or deletion of T prostanoid receptors eliminated the LTC4-induced increase in HMGB1. Finally, Ptges$^{-/-}$ mice, which exhibit endogenous LTC4 hypersynthesis and a phenotype similar to aspirin exacerbated respiratory disease, develop a rapid, platelet-dependent increment in lung IL-33 levels and resultant mast cell activation in response to inhaled lysine aspirin challenges that depended CysLT2R and HMGB1/RAGE. Thus LTC4 activates activates platelets in an HMGB1/RAGE-dependent autocrine loop that can drive type 2 lung immunopathology and IL-33-mediated mast cell activation. Antagonists of HMGB1 or RAGE may be useful to treat AERD and other disorders associated with type 2 immunopathology.

101.

Differential prostaglandin E homeostasis explains sex differences in aspirin-exacerbated respiratory disease

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Background: Aspirin exacerbated respiratory disease (AERD), a triad of late-onset asthma, chronic rhinosinusitis with nasal polyposis and respiratory reactions to inhibitors of cyclooxygenase-1, disproportionately affects females. Prostaglandin E2 and downstream signaling through E prostanoid (EP)2 receptors and protein kinase (PK)A negatively regulates leukotriene production and mast cell activation. Impairments in PGE2 production and EP2/PKA signaling are implicated in the pathobiology of AERD.

Methods: Self-reported clinical characteristics from subjects with AERD enrolled in the Brigham and Women's Hospital AERD patient registry were stratified by sex. 51 subjects with AERD underwent an oral aspirin challenge protocol followed by desensitization. From a subset, urinary and nasal fluid eicosanoids were determined by UPLC-MS/M or GC-MS and stratified by sex. Plasma and nasal fluid tryptase was monitored.

Results: In our cohort of 386 subjects with AERD, females (n=221, 57.3%) reported the development of asthma (29.8±13.1 versus 37.0±14.7 years, P<.0001), nasal polyps (34.6±11.8 versus 40.1±12.3 years, P<.0001), and NSAID-induced respiratory reactions (34.4±12.6 versus 40.6±12.9 years, P<.0001) younger than males. Females were more likely than men to have been hospitalized for asthma (P<.0001). Among 51 subjects formally challenged, females developed respiratory reactions to aspirin earlier than males (144±59 vs 181±63 minutes, P<.05). Nasal fluid (n)PGE2 declined at the onset of respiratory symptoms in females (P<.001), but not in males, and remained reduced at all time points up to 3 hours (P<.01). Lower nPGE2 production in females was associated with higher nasal fluid tryptase release during the aspirin induced reaction (P<.05). Peak nasal tryptase showed a trend toward inverse correlation with the decline in nPGE2 (rs=-.771, P=.07) and correlated with peak nLTC4 (rs=.846, P<.05) in females. Baseline levels of the urinary PGE2 metabolite (uPGE-M) tended to be higher in males (n=22) than females (n=26, 8.99±4.96 versus 5.52±7.06 ng/mg creatinine (Cr), P=.058). At the onset of an aspirin-induced reaction, uPGE-M decreased by 33±24% in females (P<.01) but did not decline significantly from baseline in males. Females showed a trend toward higher peak levels of urinary leukotriene (LT)E4 (P=.12) during reactions.

Conclusions: Sex differences in PGE2 homeostasis may account for the greater prevalence, earlier age of onset, and increase morbidity in females with AERD.

102.

Genetic restriction of antigen-presentation dictates allergic sensitization and disease in humanized mice

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Background: Immunoglobulin(Ig)E-associated allergies result from misguided immune responses against innocuous antigens. $CD4^+$ T lymphocytes are critical for initiating and perpetuating that process, yet the crucial factors determining whether an individual becomes sensitized towards a given allergen remain largely unknown.

Objective: To determine the key factors for sensitization and allergy towards a given allergen.

Methods: We here created a novel human T cell receptor (TCR) and human leucocyte antigen(HLA)-DR1 (TCR-DR1) transgenic mouse model of asthma, based on the human-relevant major mugwort (Artemisia vulgaris) pollen allergen Art v 1 to examine the critical factors for sensitization and allergy upon natural allergen exposure via the airways in the absence of systemic priming and adjuvants.

Results: Acute allergen exposure led to IgE-independent airway hyperreactivity (AHR) and T helper(Th)2-prone lung inflammation in TCR-DR1, but not DR1, TCR or wildtype (WT) control mice, that was alleviated by prophylactic interleukin(IL)-2-αIL-2 mAb complex-induced expansion of Tregs. Chronic allergen exposure sensitized one third of single DR1 transgenic mice, however, without impacting on lung function. Similar treatment led to AHR and Th2-driven lung pathology in >90% of TCR-DR1 mice. Prophylactic and therapeutic expansion of Tregs with IL-2-αIL-2 mAb complexes blocked the generation and boosting of allergen-specific IgE associated with chronic allergen exposure.

Conclusions: We identify genetic restriction of allergen presentation as primary factor dictating allergic sensitization and disease against the major pollen allergen from the weed mugwort, which frequently causes sensitization and disease in humans. Furthermore, we demonstrate the importance of the balance between allergen-specific T effector and Treg cells for modulating allergic immune responses.

103.

Genomic and transcriptomic analyses of Clara Cell Secretory Protein (CC16) as a biomarker for asthma and chronic obstructive pulmonary disease (COPD)

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Background: CC16 is a protein produced by non-ciliated bronchial epithelial cells that participates in host defense. In this report, we use systems biology in the SARP asthma cohort and SPIROMICS COPD cohort to determine if CC16 gene expression is associated with important clinical and cellular outcomes in asthma and COPD.

Methods: Genome-wide eQTL analysis of CC16 mRNA expression levels was performed in cells from human bronchial epithelial biopsy (BEC, n=121) using Agilent microarrays in SARP. Genome-wide pQTL analyses of CC16 serum protein levels were performed in non-Hispanic Whites (n=1,874) in SPIROMICS. Spearman correlation analyses between CC16 mRNA levels and other protein-coding genes were performed using microarray data from BEC in SARP and RNAseq data from BEC (n=131) in SPIROMICS. Association analyses between CC16 SNPs/mRNA/protein and asthma-related or COPD-related phenotypes were performed using a generalized linear model.

Results: In asthma, the A allele of rs3741240 was suggestively associated with lower CC16 mRNA levels in BEC (β=-0.25 and p=0.066). CC16 mRNA levels were positively correlated with MUC5B, IL12A, and C3, and were negatively correlated with MUC5AC, POSTN and IL18R1 ($p<10^{-5}$). Lower CC16 mRNA levels were significantly associated with asthma (p=0.039) and asthma severity (p=0.026), with lower baseline % predicted FEV1 (p=0.0097), and with a higher proportion of ER or urgent care visits (p=0.05) and treated with systemic corticosteroids (p=0.0042) in the last year. In COPD, The A allele of rs3741240 was associated with lower CC16 protein levels ($p=9x10^{-6}$). CC16 mRNA levels were positively correlated with CC16 protein levels (p=0.02). CC16 mRNA levels were positively correlated with MUC5B, IL12A, C3, TLR5, and IL6, and negatively correlated with MUC5AC, IL18R1, and TLR6 ($p<10^{-5}$). Lower CC16 protein levels were associated with lower post-bronchodilator % predicted FEV1 and COPD (p=0.003) in former smokers, and associated with prospective frequent exacerbations for three years after recruitment (Exacerbations>1 vs. 0, OR=0.8, p=0.02).

Conclusion: rs3741240 is an eQTL/pQTL SNP for CC16 mRNA/protein levels. CC16 mRNA levels are positively correlated with Th1 genes and negatively correlated with Th2 genes. CC16 mRNA levels are associated with asthma, asthma severity, and exacerbation. CC16 protein levels were associated with reduced future COPD exacerbations.

104.

Adam33 null mice do not exhibit post-natal airway hyperresponsiveness as a consequence of maternal allergy.

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Background Maternal allergy is a strong risk factor for developing asthma and airway hyperresponsiveness (AHR). ADAM33 has been identified as an asthma susceptibility gene and is associated with AHR and impaired lung function in early life. Our aim was to investigate a potential in utero gene-environment interaction involving Adam33 by determining the effects of maternal murine allergic airway inflammation on the lungs of offspring before and after birth. We hypothesised that the effects of maternal allergy will be modified in Adam33 knock out (KO) compared to wild-type (WT) offspring.

Methods We established a model of maternal allergic airway inflammation in pregnant heterozygous ($Adam33^{+/-}$) mice through exposure to house dust mite (HDM) through intranasal challenges during pregnancy. Control mice were challenged with saline. WT ($Adam33^{+/+}$) and KO ($Adam33^{-/-}$) offspring from the same litters were studied on embryonic day (ED)17.5 and 2 or 4 weeks post partum(pp). Lung function was measured in response to increasing doses of methacholine, bronchoalveolar lavage fluid (BALF) was collected for differential cell counts and ELISA, and lung tissue for RTqPCR, Western blot and immunohistochemistry.

Results Adam33 mRNA expression was significantly enhanced in WT lungs of HDM challenged mothers at ED17.5, but unchanged pp. However, at 4 weeks pp, WT offspring of HDM challenged mothers showed significantly enhanced AHR compared to the offspring of saline challenged mothers. KO offspring of HDM challenged mothers were protected against development of AHR. mRNA expression of typical inflammatory mediators and remodelling genes were not affected at any time point studied; moreover, no inflammatory cells were present in the BALF of any of the offspring. In contrast, Cholinergic Receptor Muscarinic 1 (Chrm1) mRNA was increased at 4 weeks in WT offspring of HDM challenged mothers.

Conclusions This study identifies an important in utero gene-environment interaction involving Adam33 that has implications for the subsequent development of AHR in early life. Further studies are needed to elucidate the precise mechanism(s) whereby ADAM33 mediates its effects. Our data suggest modulation of the contractility of the airways, possibly involving muscarinic receptors.

105.

House dust mite sensitization and challenge protects against a lethal paramyxoviral respiratory infection.

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Atopic diseases are increasing in the westernized world; the cause of this increase is unknown. Interestingly, in the 2009 influenza pandemic hospitalized asthma patients had lower mortality (4%) than those without asthma (10%; p=0.04; BMC Infectious Diseases 2013,13:57). This suggested atopic disease might provide a survival advantage with severe respiratory infections. Using our well-characterized mouse model of severe respiratory viral infection, we examined whether atopy protects mice from lethal paramyxoviral infections. C57BL6 wild-type (WT) and $IgE^{-/-}$ mice were sensitized intranasally (i.n.) with 1µg house dust mite extract (HDM) or PBS on day 0, challenged i.n. with HDM (10µg) or PBS daily from day 7-11, and inoculated with a normally lethal dose ($2x10^6$ pfu) of Sendai virus (SeV) on day 14. Surprisingly, all WT and $IgE^{-/-}$ mice sensitized and challenged with HDM survived the infection, while

all mice challenged with PBS (regardless of sensitization) died by day 10 post inoculation (PI) SeV ($p<0.0001$; n=5 mice/group). Thus, atopy (in an IgE independent fashion) provided a survival advantage. To determine how atopy altered the antiviral immune response, we performed similar experiments using a non-lethal SeV dose ($2x10^5$ pfu) in WT mice. Weight loss was significantly attenuated in SeV infected HDM sensitized/challenged mice (95.7+/-2.5% of baseline, mean+/-SEM day 8 PI SeV, n=5) compared to SeV infected HDM sensitized/PBS challenged mice (80.0+/-1.1%, n=5; p=0.001). Fewer CD49d expressing BAL neutrophils were found at day 5 PI SeV (32.6+/-3.0% versus 54.9+/-2.0% of PMN expressing CD49d; n=3; p=0.005). The frequency of day 5 PI SeV FceRI expressing lung conventional dendritic cells was reduced in HDM versus PBS challenged mice (69.2+/-3.7% versus 90.7+/-1.9%; n=3; p=0.014). SeV specific CD8 T cells were reduced at day 10 PI SeV in atopic mice (frequency SeV tetramer positive CD8 T cells 4.9+/-0.8% versus 10.7+/-1.7%; n=3; p=0.06), and Muc5ac was reduced 47.2% in SeV infected atopic compared to non-atopic mice (n=3-5; p=0.033). Our model suggests HDM sensitization and challenge protects mice from severe paramyxoviral infections by dampening immune responses in an IgE independent fashion, suggesting a survival advantage for atopy. Further studies will explore potential mechanisms underlying this apparent protection.

107.

Immune cell phenotype and functional defects in Netherton syndrome

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Netherton syndrome (NS) is a rare life-threatening syndrome caused by SPINK5 mutations leading to a skin barrier defect and a severe atopic diathesis. NS patients are prone to bacterial infections, but the understanding of the immune deficiency is incomplete.

We analysed blood lymphocyte phenotypes and function in relation to clinical infections in a group of 11 Finnish NS patients, aged 3 to 17 years, and healthy age-matched controls.

The proportion of B cells ($CD19^+$) and naïve B cells ($CD27^-$, IgD^+) were high while memory B cells ($CD27^+$) and switched memory B cells (SM, $CD27^+IgM^-IgD^-$), crucial for the secondary response to pathogens, were below the reference values in 8/11 and 7/11 patients, respectively. The proportion of activated B cells ($CD21^{low}$, $CD38^{low}$) was below the reference value or in the lowest quartile in 10/11 (91%). Despite normal T cell counts, the proportion of naïve $CD4^+$ T cells was reduced significantly and the proportion of $CD8^+$ T central memory (TCM) significantly elevated. An increased proportion of $CD57^+$ $CD8^+$ T cells and a decline in the proportion of $CD27^+CD8^+$ T cells indicated increased differentiation potential of the T cells. In functional tests the cytokine (IFNγ and TNFα) production by both T and NK cells of NS patients was increased compared with matched healthy individuals. The proportion of mature NK cells was elevated in NS patients but NK cell cytotoxicity and activation were impaired based on the reduced proportion of $CD27^+$ NK cells and the decreased expression of CD107a/b, both significant. The frequency of skin infections correlated with the proportion of $CD62L^+$ T cells, naïve $CD4^+$ and $CD27^+$ $CD8^+$ cells and with activated B cells. Clinically beneficial IVIG therapy increased naïve T cells and TEMRA CD8 cells and decreased the proportion of activated B cells and plasmablasts in three patients studied.

This study shows novel quantitative and functional aberrations in several lymphocyte subpopulations, which correlate with the frequency of infections in patients with Netherton syndrome. IVIG therapy normalized some dysbalancies and was clinically beneficial.

108.

Ascaris lumbricoides cystatin has strong anti-inflammatory effects on mice respiratory allergic inflammation

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Chronic noninfectious inflammatory disorders are increasing worldwide and novel preventive or therapeutic approaches are needed. Severe helminth infections are negatively associated to allergic diseases including asthma and the immunomodulatory properties of parasite-derived components have been increasingly analyzed, raising the possibility of their use as anti-inflammatory molecules. In our current studies of human and mouse immune responses to Ascaris lumbricoides components, we evaluated the immunomodulatory properties of the recombinant cystatin (rAl-CPI) in a mouse model of asthma induced by the house dust mite Blomia tropicalis and also its effects on monocyte derived human dendritic cells. rAl-CPI was obtained from an A. lumbricoides cDNA library, cloned into pQE30 and expressed in E. coli system as a histidine label protein. Healthy 6 to 8 weeks old female BALB/c mice were used. The B. tropicalis sensitized and challenged mice developed an extensive cellular airway inflammatory response, which was significantly reduced when treated with rAl-CPI before sensitization. The changes were particularly evident in the perivascular/peribronchial infiltrate cells, eosinophils, neutrophils and goblet cells numbers. Also, high levels of IL-5 and IL-13 were detected in the bronchoalveolar lavage (BAL). A significant decrease of Th2 cytokines, total and specific IgE antibodies to B. tropicalis was also found in rAl-CPI treated mice, being the IgG2a antibody response significantly increased. The rAl-CPI treated groups (alone or before allergenic sensitization) showed significant increase of Tregs number in spleen and elevated levels of IL-10 in both BAL and splenocyte culture supernatants. The use of anti-IL10R did not affect the anti-allergic effects; even it significantly lowered the Treg number. In vitro rAl-CPI showed a strong effect on human monocyte-derived dendritic cells (HmoDCs), diminishing the expression of HLA-DR and CD83 and CD86 markers, while inducing IL-10 and IL-6, which suggest an inhibition of their maturation and a possible relationship with the inhibition of the allergic response observed in the murine model. Our results support previous studies on cystatin and, for the first time, suggest that A. lumbricoides cystatin has potential anti-inflammatory properties at the respiratory level. More studies on the mechanisms underlying these promising effects are warranted.

109.

Modulation of human T cell responses to peanut allergen through treatment of dendritic cells with STAT6-inhibitory peptide. A management approach for food allergies.

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Food allergies affect up to 6% of children and 4% of adults and the burden of illness for patients and families is significant. Food allergies develop when TH2-type immune responses are activated by food proteins leading to formation of allergen-specific IgE. Upon re-exposure the allergic cascade is activated. In this study we targeted STAT6, a key transcription factor mediating TH2 responses.

Human peripheral blood mononuclear cells were obtained from patients with peanut allergy or non-allergic volunteers. Lymphocytes were isolated and frozen. Mononuclear cells were differentiated into immature CD11c+ve dendritic cells (DC). Immature DCs were treated with STAT6-inhibitory peptide (STAT6-IP) or appropriate control and matured. Mature DCs were then co-cultured with T cells plus peanut extract. Proliferation and cytokine expression/ production were assessed. To determine the localization of STAT6-IP, FAM-labelled peptides were also used.

Co-cultures from peanut allergic donors proliferated with peanut allergen and TH2 cytokines, IL4 and IL13 were increased. Cells from non allergic donors also proliferated albeit to a lesser extent. Treatment of DCs with STAT6-IP significantly reduced proliferation while control peptide had no effect. IL4 and IL13 were also significantly decreased in STAT6-IP cultures while TGF-β was increased. When FAM-labelled peptide was used there was uptake demonstrable in 73% of DCs after 48 hours. Following co-culture of labelled DCs with T cells, T cells were also examined for FAM expression. Interestingly, at 24 hours of co-culture 45% of CD4+ve T cells were FAM positive and at 48 hrs 10% remained FAM positive.

These data suggest that STAT6-IP is taken up by human DCS. Co-culture with cells from allergic donors results in reduced proliferation and expression of TH2 cytokines as well as increases in TGFβ. The transfer of FAM from DCs to T cells suggest that STAT6-IP may be delivered directly to the T cell by DCs suggesting a mechanism by which the DC modulates the T cell phenotype. STAT6-IP is a targeted immunotherapy with significant potential for therapeutic translation in food allergy.

110.

Development of trans-culturized National Asthma Guidelines, broadly accepted by local physicians, using the ADAPTE approach

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Introduction: Even though several high-quality (Q), international asthma guidelines (GLs) exist in English, in Mexico there is a need for a national asthma clinical practice GL, to unify several different concepts of the disease among groups of Primary Care physicians and specialists of both, the public and private sectors.

Purpose: to develop GUIMA (Guía Mexicana del Asma), based on the evidence of the 3 highest Q international asthma GLs, trans-culturized to the Mexican reality, with the cooperation of 12 national societies of specialists and Primary Healthcare physicians, and stakeholders from the public and private sector, using the guideline adaptation resource toolkit, ADAPTE.

Methods: The project was started by the director of the National Institute of Respiratory Diseases (INER). The core-GL-development group (see authors) developed the draft documents of clinical questions and their replies (see below), using online 1-2 weekly meetings. 46 Physicians, assigned by the presidents of 12 national societies (pediatric and adult pulmonologists and allergists, ENTs, paediatricians, family doctors, GPs and respiratory therapists), adjusted the draft texts in several rounds of a Delphi process led by methodologists. Finally, the concepts of the clinical questions were fused into one fluent GL document, corrected in a final face-to-face meeting by the complete GL-development group.

Results: after defining the scope of the GL with SCOPE, a literature search using 3 search engines a total of 40 asthma GL documents were found. With AGREE-II evaluation the 3 highest Q GLs were selected: BTS, GEMA and GINA (= 'mother GLs'). Clinical questions were formulated according to all steps of the process of the diagnosis and treatment of asthma and asthma exacerbations, for patients of different age-groups and at the 3 levels of Health Care. Clinical questions were replied fusing the evidence from the mother GLs, and taking into account the availability and costs of diagnostic and treatment procedures in Mexico.

Conclusion: The here presented project could be of use for low-middle income countries. In the settings of a large country with limited resources, a high-Q national asthma GL with a broad basis among national specialists and Primary Care physicians could be developed in the course of 18 months, using online communication tools, SCOPE, AGREE-II and ADAPTE.

111.

Distinct immune cell phenotypes are associated with response to therapy in rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease mediated through complex immunologic pathways. The inflammation can lead to irreversible articular damage and loss of joint function and mobility. Some patients respond to first line therapy with methotrexate (MTX) and the inflammation resolves. However, many fail to improve and can require long term treatment with expensive biologic agents, such as TNF inhibitors, IL-6 receptor antagonists, B cell depletion and JAK inhibitors. We hypothesized that by examining comprehensive immune phenotypes of these patients, we can identify specific immunologic pathways associated with response to MTX and resolution of joint inflammation.

Methods: Patients with early RA were treated with standard of care MTX therapy. Disease activity was determined and comprehensive blood T cell immunophenotyping was performed on these patients at baseline and at 6 months. Multi-parameter immunofluorescent flow cytometry was used to assess immune phenotypes of T cell subsets.

Results: Ninety six patients with RA and healthy controls were studied. RA patient Disease Activity Scores (DAS-28) ranged from 4.7 to 6.6 at baseline. Fifty percent of the patients responded to MTX and had a major drop in DAS and inflammatory markers. Baseline measures of disease activity (DAS-28 scores, CRP, ESR, RF) did not differ between individuals who

responded to therapy and those who did not respond after 6 months of MTX. RA patients had a decrease in naïve T cells ($p<0.05$) and an increase in memory effector CD8 cells compared to normals ($p<0.01$). CD8 T cells were significantly lower in MTX responders, but there was an increase in the interferon-γ producing CD8 subsets ($p<0.04$). CD4 Th2 subsets were increased in cells from non-responders compared to responders ($p<0.02$). Surface expression of checkpoint inhibitor markers (PD-1, Tim-3) also differed significantly between responders and non-responders at baseline and 6 months.

Conclusions: Baseline immune phenotypes, but not clinical measures of disease activity, differ between responders vs non-responders and may be useful in prediction of optimal therapy.

112.

Anaphylaxis to monoclonal antibodies: new classification of symptoms and desensitization approach

Marlene Garcia-Neuer, Donna-Marie Lynch, Mariana Castells

Background: Due to the increased usage of monoclonal antibodies (mAbs), a rise in severe hypersensitivity reactions (HSRs) and anaphylaxis has caused the discontinuation of first-line therapy in many patients. Current, anaphylactic symptoms, diagnostic tools and management approaches have not been standardized.We propose a novel evidence-based classification for anaphylaxis to mAbs, based on the clinical phenotypes, underlying endotypes and biomarkers; as well as their management with desensitization. HSRs to mAbs were defined as Type I, Cytokine-Release, Mixed (Type I/Cytokine-Release) and Type IV reactions, by skin testing, tryptase, and IL-6 as biomarkers (Isabwe et al). Identifying these phenotypes assists in providing personalized and precision medicine for desensitization allowing the patient to be maintained on first line therapy despite developing severe HSRs.

Methods: We reviewed the phenotypes, endotypes and biomarkers of anaphylaxis to 9 mAbs (cetuximab, golimumab, infliximab, nivolimumab, obinituzumab, omalizumab, rituximab, tocilizumab, trastuzumab) in 54 patients who presented grade III reactions to mAbs and underwent evaluation and desensitization from 2011-2017 at Brigham and Women's Hospital Drug Hypersensitivity and Desensitization Center.

Results: 51 patients with anaphylactic reactions were desensitized: 57% (29) patients had no reaction and 98% (50 patients) completed their first desensitization. Of the 43% (22) of patients who developed a reaction; 63% (14 patients) were grade I, 23% (4 patients) were grade II, 14% (3 patients) were grade III. Of the 51 protocols, 59% (30) used a 12 step 3 bags protocol, while 31% (16) used a 16 step 4 bags protocol. A 7-step subcutaneous protocol was administered in 12% (6) of patients. 39 patients completed skin testing to rituximab, infliximab, trastuzumab, tocilizumab, cetuximab and 56 % (22) had positive results. The most common reaction phenotype was Type-1 during both initial and desensitization reactions (66% (34) and 63% (14) respectively). Tryptase was elevated in Type 1 reactions and IL-6 was elevated in cytokine release reactions.

Conclusion: Desensitization is an effective treatment mode for patients with severe HSRs. Phenotyping and endotyping the reactions to mAbs allows for a personalized approach and the reintroduction of first line therapy in patients presenting with anaphylaxis.

113.

Elevated plasma S1P levels are associated with protection from anaphylaxis during food challenges in food allergic pediatric patients

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Introduction: Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator playing a major role in lymphocyte trafficking and immune responses. We have previously demonstrated that modulation of the S1P homeostasis alters the sensitization and effector phase of food allergy in an experimental model indicating its essential role in food allergy. Moreover, S1P metabolism and its receptor S1P2 were reported to substantially influence histamine clearance and recovery from anaphylactic shock in mice. Thus, the aim of this study was to translate these experimental data into clinics and to determine systemic S1P levels in food allergic patients in comparison with severity of clinical responses.

Materials and Methods: Plasma was collected from 70 pediatric patients with suspected food allergy undergoing diagnostic food challenges and 10 non-allergic, age-matched healthy controls with a defined standard operation procedure to impede unspecific S1P release from blood cells. S1P levels in samples collected before food challenges were measured by mass spectrometry and compared to the mast cell activation markers histamine. Furthermore, S1P levels were compared with sensitization status and clinical responses observed during food challenges.

Results: Even though histamine levels were significantly lower in non-sensitized children compared to allergic or sensitized patients, we observed no statistically significant differences of systemic S1P levels between these groups. However, when plasma S1P levels before food challenges were compared between severity outcome groups, significantly higher levels of the sphingolipid S1P were detected in samples of food allergic patients being protected against documented anaphylaxis compared to patients with a documented anaphylactic response. Differences in S1P levels were associated with sensitization pattern as significantly lower levels were measured in oligosensitized compared to non-sensitized children.

Conclusions: To the best of our knowledge this is the first study translating the experimental data on the role of S1P in severe food allergies into clinics. Due to its supposed beneficial role in anaphylaxis prevention, modulation of systemic S1P levels might be an interesting application for future therapeutic interventions preventing severe systemic reactions in food allergy.

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114.

A subset of patients with Mast Cell Activation Syndrome (MCAS) associated with POTS and EDS have classic clinical MCAS features

Raied T Hufdhi, Matthew P Giannetti, Peter Novak, Matthew J Hamilton; Mariana Castells

Background: MCAS refers to a group of disorders defined by episodic and/or chronic multisystem symptoms, detection of increased serum and/or urinary mast cell (MC) mediators (Tryptase, Histamine, Prostaglandins) and response to anti-MC mediator medications. More recently, a subset of MCAS associated with postural orthostatic tachycardia syndrome (POTS) and Ehlers-Danlos syndrome (EDS) is recognized. The MCAS profile of symptoms, mediators and response to anti-MC mediator therapy in this subset has not been described.

Materials and Methods: We studied twenty patients referred to Brigham and Women`s Hospital Mastocytosis Center for MC mediator-related symptoms who carried the diagnosis of POTS and/or EDS. We examined the results of MC mediator tests, autonomic testing, and response to anti-MC mediator medications.

Results: Twenty patients (19 female, 1 male; 18 white, 2 African- American) had a diagnosis of POTS and 12 (60%) had EDS. The most common presenting MC symptoms included flushing (13, 65%), pruritus (7, 35%), urticaria (5,25%), dermatographism (3, 15%); abdominal pain/cramping (10, 50%), diarrhea (10, 50%) nausea (6, 30%), acid reflux (6, 30%), headache (10, 50%), brain fog (8, 40%), depression (7, 35%), anxiety (5, 25%), dyspnea (12, 60%), chest tightness (8, 40%), and cough (6, 30%). Tilt table test confirmed POTS in 12 patients (60.0%). Baseline serum Tryptase was elevated (>11.4ng/ml) in 2/20; 24 hour urine histamine and N-Methylhistamine (> 200ug/g Cr) was elevated in 6/20 (30%); urine 11 beta-Prostaglandin F2(2,3 BPG) was elevated in 5/10 (50%); and total IgE was elevated (> 100kU/L) in 5/17 (29%). Peripheral blood test for KIT mutation D816V was negative in 14/15. Significant symptom improvement occurred with standard medical therapy in 15/20 subjects (75%).

Conclusion: A subset of patients with MCAS and associated POTS and EDS may be characterized by classic MC mediator-related symptoms, atopy, and significant response to anti-MC mediator medications. Despite the association with POTS and EDS, this MCAS subtype appears to be similar to other types of MCAS with regards to current diagnostic evaluations and response to treatment. A smaller number of patients in our cohort had an elevated Tryptase, but further studies with a larger population will need to confirm this observation.

115.

he effect of Omalizumab in mastocytosis patients. Prospective double-blind, placebo-controlled multicentre study

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Introduction: Patients with mastocytosis often suffer from a variety of symptoms caused by mast cell mediators. Besides H1-blockers, treatment remains difficult. Omalizumab, a monoclonal anti-IgE-antibody has been postulated to have a positive impact on mastocytosis-associated symptoms such as flush, vertigo, GI troubles or anaphylaxis..

Methods: In a multicenter trial the effect of omalizumab 17 patients with various forms of mastocytosis were investigated in a prospective double-blind placebo-controlled study.7 patients were randomised to omalizumab group, 9 to placebo. Omalizumab was dosed on total serum IgE and body weight as in allergic asthma. Primary endpoint of the study was the change in the AFIRMM after 6 months of treatment. Groups were age-balanced (45.4 y ± 8.8 in the placebo versus 47.7 ± 13.8 in the verum), whereas 66,6% in the Omalizumab and 85.7% in placebo group were female. Median disease duration was 4.5 y ± 2.9 in the placebo and 10.0y ± 5.1 in the verum group. A variety of laboratory parameters were also analysed.

Result: After 6 months the median AFIRMM score improved from 104.0 to 102.0 for the placebo and from 52.0 to 26.0 for the Omalizumab group, respectively (p=0.286). The amount of reduction was not significantly different in the treatment groups (p=0.941). Regarding the secondary endpoints – including changes in the AFIRMM score at the end of the study, the number of allergic reactions, changes in VAS for major complaints, pressure-induced wheale and flare and the frequency of the use of mastocytosis-specific drugs such as antihistamines or cromoglycates showed a strong, but again not significant improvement in the Xolair group. Adverse events (AE) events like urticaria, bronchospasm, anaphylactic shock showed no significant difference between both groups. Expression of FceRI on basophils reduced in patients receiving Omalizumab but not with placebo.

Disscusion: Omalizumab seems to improve mastocytosis symptoms in the omalizumab corresponding to effects described in the literature, involving mainly diarrhea, dizziness and flush and reducing anaphylactic reactions. An improvement in the AFIRMM score and in the secondary endpoints was seen. AE were few in number and equally distributed. To our knowledge, this is the first double-blind placebo controlled study for omalizumab in mastocytosis patients. Further larger studies would be advisable to confirm our findings in a small group of patients.

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116.

Children with limited cutaneous Mastocytosis often present with systemic symptoms.

Raied T Hufdhi, Matthew P Giannetti, Matthew J Hamilton; Mariana Castells

Background: Mastocytosis is a disease characterized by a pathologic increase in mast cells which can occur in children and adults. Pediatric-onset Mastocytosis is frequently diagnosed

prior to age 2 and is typically limited to cutaneous disease. Children with Cutaneous Mastocytosis (CM) without organomegaly or elevated serum tryptase have bone marrow biopsies without evidence of systemic involvement (Carter JACI 2015). The aim of the study was to characterize systemic symptoms present in children with isolated cutaneous disease and tryptase <20 ng/ml. Methods: We studied 78 subjects born after January 1st 2000, who were referred to Brigham and Women`s Hospital Mastocytosis Center for evaluation of CM. All patients underwent a comprehensive evaluation including level of skin involvement, type of skin lesions, presence of KIT mutation D816V in peripheral blood, serum tryptase level, and systemic symptoms.

Results: There were 32 female (41%) and 46 male (59%). White (65, 83%), Hispanic (6, 7%), African American (3, 3%) and Asian (3, 3%). The polymorphic maculopapular cutaneous mastocytosis variant (Urticaria Pigmentosa) was the most common presentation in 66 (84%) followed by mastocytoma (17, 21%), Telangiectasia Macularis Eruptiva Perstans (TMEP) (3, 3%), and diffuse cutaneous mastocytosis (DCM) (2, 2%). Darier`s sign was positive in 35/53 (66%) negative in 6/53, 11%. Peripheral blood test for KIT mutation (D- 816V) was negative in 6/7, positive in 1/7 and Tryptase was >11.4ng/mL in 15/65 (23%) Clinical manifestations included urticaria in 33 patients (42%), flushing in (32 patients, 41%), pruritus (29, 37%), diarrhea (33, 42%), abdominal pain (23, 29%), nausea/vomiting (22, 28%) headache (6, 7%), anxiety (5, 6%), and poor concentration (5, 6%). Atopic symptoms such as nasal congestion (10, 12%), ocular pruritus (10, 12%) and asthma (9, 11%) were also present. Anaphylaxis was reported in 15/78, (19%). Few patients had fever (6, 7%) and fatigue (3, 3%).

Conclusion: Children with limited cutaneous mastocytosis have a predominant presentation as the polymorphic maculopapular mastocytosis and present with systemic symptoms mostly gastrointestinal and skin symptoms and anaphylaxis. Surprisingly neuropsychiatric symptoms have not been recognized up to now as part of the mast cell symptomatic complex and require future studies.

117.

Management of pediatric cases of anaphylaxis at school and daycare

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Background: Anaphylaxis is a life-threatening allergic reaction and recent studies suggest increased prevalence. However, there is little data regarding the management of anaphylaxis in school/ daycare. We aimed to characterize anaphylaxis among children at school/ daycare and determine factors associated with epinephrine administration, the first line treatment for anaphylaxis.

Methods: The Cross-Canada Anaphylaxis Registry (C-CARE) enrols anaphylaxis cases presenting to emergency departments (EDs) across Canada. ED physicians documented characteristics, triggers, co-morbidities, and management prior and after arrival to the ED using standardized forms. We utilized C-CARE to assess reactions occurring at school/daycare in children between April 2011 and December 2017 in six EDs in 4 provinces: Quebec, Ontario, British Columbia and Newfoundland. Multivariate logistic regression was used to identify factors associated with epinephrine treatment. Independent variables included age, sex, reaction severity, presence of asthma/known food allergy and province of residence.

Results: Among 2528 pediatric cases of anaphylaxis presenting to the EDs, 326 (12.9%) reactions occurred at school/daycare. Reactions at school/daycare occurred at a median age of 7.5 years (interquartile range: 3.4, 13.7), the majority were females, and most cases were from Quebec. Almost 80% of cases at school/daycare were triggered by food, mainly peanut and milk, and in 62% of all cases there was a known food allergy. Severe reactions (cyanosis/hypoxia/respiratory arrest/hypotension/dysrhythmia/confusion/ loss of consciousness) occurred in 22 (7%) cases. Among all cases, 60% were treated with epinephrine, but 20% of severe anaphylaxis cases were not treated with epinephrine. Older children [adjusted odds ratio (aOR):1.05(95%CI:1.00,1.10)], those with known food allergy [aOR:4.28(2.58,7,08), and those with severe reactions [aOR:3.62(1.13,11.63) were more likely treated with epinephrine at school/daycare. However, children in BC were less likely [aOR:0.37(0.17,0.83)] treated with epinephrine.

Conclusion: Anaphylaxis at school/daycare is principally triggered by food. There are disparities is epinephrine utilization between Canadian provinces at school/daycare. Policies and regulations promoting increased epinephrine use at school/daycare in all cases of anaphylaxis are required.

118.

Risk factors for severe systemic sting reactions in wasp and honeybee venom allergic patients

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Background: Hymenoptera stings are a major cause of anaphylaxis. Various risk factors are discussed in literature.

Objective: This study aims to investigate potential risk factors for severe sting reactions in wasp and honeybee venom allergic patients and analyses the correlation between diagnostic test results and the severity of the allergic reaction.

Methods: 480 patients suffering from wasp or honeybee venom allergy were included in this retrospective case series. The severity of their systemic field sting reaction was analysed with regard to the amount of specific IgE antibodies to whole venom extracts and to major allergens of honeybee respectively wasp venom. Furthermore, the following potential risk factors for severe sting reactions were examined: age, sex, latency time, skin symptoms, baseline serum tryptase levels and the concentration of venom inducing a positive intracutaneous test.

Results: The two following indicators for severe systemic sting reactions in honeybee and wasp venom allergic patients have been identified: a short latency time and the absence of skin symptoms. The patient's age and baseline serum tryptase levels have been found to positively correlate with the grade of the sting reaction only in individuals allergic to wasp venom. No correlation could be found between the degree of sensitisation and the severity of the allergic reaction. Neither the amount of specific IgE antibodies to whole venom extracts nor to major allergens were significantly associated with the severity of the sting reaction.

Conclusion: The clinical history is essential for the allergological workup and therapeutic decision on Hymenoptera venom allergies. A short latency time and the absence of skin symptoms are indicators of severe sting reactions, followed by patients age and serum tryptase levels.

119.

Alcohol hyper-responsiveness in chronic rhinosinusitis with nasal polyps

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Introduction and aim: Patients diagnosed with chronic airway disease frequently report alcohol-induced worsening of their symptoms. Literature about this topic is scarce.

Our aim was to estimate the prevalence and characteristics of alcohol hyper-responsiveness in chronic airway disease and to investigate the mechanism of this phenomenon in chronic rhinosinusitis with nasal polyps (CRSwNP).

Material and methods: This study encompassed 3 parts. First 1281 subjects were approached by means of a questionnaire. Nasal polyp patients with and without NSAID exacerbated respiratory disease (NERD), chronic rhinosinusitis patients without nasal polyps (CRSsNP), allergic rhinitis (AR) patients and healthy controls were included. Then, inflammatory markers (ECP, IL-5, IgE, SAE specific IgE, IL-17, TNFα and IFNγ) in tissue were compared between alcohol hyper-responsive and non-hyper-responsive CRSwNP patients. Finally, 34 CRSwNP patients and 14 controls underwent an oral challenge test with of 15 vol% alcohol. Results were based on anterior rhinomanometry and symptoms.

Results:

The highest prevalence of alcohol hyper-responsiveness was observed in patients with NERD, followed by CRSwNP. Alcohol hyper-responsiveness is significantly more prevalent in CRSwNP patients suffering from recurrent disease and severe symptoms. In nasal tissue of the hyper-responsive CRSwNP group we observed significantly higher nasal levels of the eosinophilic biomarker ECP. The oral challenge test was significantly more positive in CRSwNP as compared to healthy controls.

Conclusion: Nasal hyper-responsiveness to alcohol is significantly more prevalent in severe eosinophilic respiratory disease and in more aggressive subgroups of CRSwNP. In at least a subgroup of these patients alcohol itself is responsible for respiratory reactions.

120.

Burden of disease and individual suffering in adults with severe atopic eczema (AE)

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Background: Atopic eczema (AE) is one of the most common non-communicable inflammatory skin diseases and often regarded as a childhood disease. Much less is known about adult AE which becomes more and more common. This study investigated the burden of disease and individual suffering in adults with severe AE.

Methods: From October 2017 to march 2018 a survey was carried out as computer-assisted telephone interview (CATI) in 1189 patients with moderate to severe AE from 9 European countries. A questionnaire was used containing validated instruments for severity of AE, anxiety and depression symptoms and quality of life as well as a newly developed instrument measuring the individual suffering and emotional consequences of AE.

Results: Despite normal specialist management including actual phototherapy in 32 % and systemic immunosuppression in 23 %, 55 % of the patients were currently suffering from moderate to very severe eczema with large impairment in quality of life. 26 % missed more than 1 week of work in the last year. Indirect additional health care expenses to be paid out of the pocket were calculated as ca 900,- Euro per year. 10 % reported symptoms of depression. Patients reported marked emotional consequences: 57 % said the itch would drive them crazy, 51 % were hiding their eczema, 43 % said the eczema made them angry, 39 % had problems with intimacy or felt guilty about their condition. 88 % of the currently (very) severe AE patients felt compromised in their ability to face life.

Conclusions: Adult patients with severe AE are suffering more than seems acceptable for a modern health care system. There is a need for better management providing improved treatment and access to affordable health care. Efforts should be undertaken to increase awareness of this problem among health care professionals and policy makers to support research, education and improved clinical care in this field.

121.

Chronic pruritus in skin diseases - more common and more severe than we thought

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Many dermatological disorders are associated with pruritus. While in some diseases pruritus is a hallmark symptom, i.e. urticaria, atopic dermatitis or lichen planus, the prevalence and intensity of pruritus in other diseases and the impact of pruritus on the general well-being and quality of life is less well known. In recent years, there have been various reports on the prevalence and burden of itch in some selected skin diseases; a detailed characterization of the presence, intensity, localization, quality and impact of pruritus in different skin diseases was, as of yet, missing. We have therefore contacted more than 1,300 unselected, consecutive in- and outpatients with active dermatological disorders and have collected data from 880 patients with 19 different dermatological diagnoses (including chronic spontaneous urticaria [n=143], psoriasis [n=138], atopic dermatitis [n=129], chronic prurigo [n=75], cutaneous T and B cell lymphoma [n=68, 26], mastocytosis [n=54], and bullous pemphigoid [n=15]). Using validated questionnaires we have collected information on the on the presence, intensity, quality and localization of pruritus, and have analyzed the impact of pruritus on the quality of life, the effects on sleep and activity and work productivity and the impact of pruritus on suicidal ideation.

Our data indicate that chronic pruritus is more common and more severe than previously anticipated in most skin conditions and that pruritus in these diseases is often associated with an impairment of quality of life, sleep, and work productivity, and that pruritus is linked to depression and suicidal ideations. Body maps representing the localization of pruritus additionally show distinct bodily distribution patterns of pruritus, which, together with information on quality of pruritus and scratching behavior, indicate disease specific pruritus characteristics in different dermatoses. Consequently, the results can lead to a better understanding of the pathophysiology of itch in these diseases, help in the development of better treatment options and can lead to a better management of our patients.

122.

Hypersensitivity to NSAIDs: results of an Austrian cohort study

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Introduction: Hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) is the second most common cause of drug hypersensitivity. Despite the importance of NSAIDs in routine analgesia only few studies have systematically addressed the question of tolerability in hypersensitive patients. In this study, we included 398 patients with a hypersensitivity to NSAIDs in their past medical history, and subsequently conducted skin prick and oral provocation tests to assess the most tolerable anti-inflammatory drugs.

Methods: We analyzed 398 patients, who have been treated at the Department of Dermatology, University Hospital Linz, Austria, retrospectively from 2012 to 2016 with a clinical history of NSAIDs hypersensitivity. We performed skin prick tests (SPT) to the common NSAIDs and assessed total IgE and serum tryptase in patients. Furthermore, patients were subjected to oral provocation testing with the culprit drug or an alternative NSAIDs.

Results: Out of the 398 patients, 153 patients had a medical history to diclofenac intolerance. Other culprit drugs included acetylsalicylic acid (121 patients), acetaminophen (106 patients), mefenamic acid (80 patients), ibuprofen (72 patients), and metamizole (68 patients). Out of 361 patients (90.7%), who were subjected to SPT, 25 patients (6.3%) tested positive against the culprit drug in skin tests. Regarding OPT, 342 (85.9%) patients were tested against the culprit drug and/or subsequently alternative NSAIDs according to common protocols. Seventy-nine patients were exposed orally to the culprit drug, of which 12 displayed a hypersensitivity reaction (reaction rate of 15.2%). In terms of alternative NSAIDs testing, the three most common tested drugs were acetaminophen (206 times), lornoxicam (202 times), and celecoxib (135 times) with patients' hypersensitivity reactions of 6.8%, 13.9%, and 8.9%, respectively. The highest reaction rate was observed for diclofenac which was tested 24 times leading to a positive reactions in 4 cases (reaction rate 16.7%).

Conclusion: Hypersensitivity to NSAIDs is common and tolerability varies greatly between specific drugs.

123.

Patient education for adults and children with atopic dermatitis in Switzerland

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Background. Atopic dermatitis (AD) is a chronic inflammatory skin disease. Recent studies corroborated the importance of patient education for treatment adherence and the improvement of AD severity. However, AD patients in Switzerland rarely undergo patient education because it is rarely offered and not covered by insurances.

Objective. To assess the feasibility of patient education and its acceptance by children and adults with AD in Switzerland.

Methods. Education for AD patients is performed in three centers: Since 2014 at the Department of Dermatology, University Hospital of Zurich and since 2016 at the Dermatology Unit at the Children's Hospital Zurich and at the Department of Dermatology, University Hospital Lausanne. Patients received a personalized education for 50 minutes with an health care professional AD expert, covering topics such as topical treatment, itch management or coping strategies. Patients or their parents received a questionnaire to assess the organization of the

education, satisfaction with trained staff, patient's feelings concerning the consultation, the relevance and practicability of information, and general satisfaction.

Results. Between 2014 and 2017, 416 patients underwent education. Of these, 141 (33.9%) returned a questionnaire. Most patients (n=65, 46.1%) underwent education at the University Hospital of Lausanne, followed by the University Hospital of Zürich (n=61, 43.3%) and the Children's Hospital of Zurich (n=15, 10.6%). Most patients were children with AD together with their parents (n=96, 68%), while 42 individuals (30%) were adults with AD. Three individuals (2%) did not report their age. The number of patients undergoing education increased from 38 in 2014 (9.1%) and 20 in 2015 (4.8%) to 153 in 2016 (36.8%) and 205 (49.3%) in 2017. The overall satisfaction with the education was high in 118 patients (84.3%), but low in only 5 patients (3.6%). Of importance, most patients (n=137, 98.6%) would recommend this education to other AD patients.

Conclusions. Patient education for AD patients is feasible in Switzerland. A clear majority of AD patients is very satisfied with education. The interest of AD patients in patient education is increasing. While the common AD patient undergoing education is a child with its parents, also adults with AD should be motivated to undergo education.

124.

A case of type-1 allergy to polyethylenglykols in vaginal suppository and cream

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Background: Polyethylenglycols (also macrogols) are nontoxic and water-soluable polymers. Polyoxyethylene sorbitan esters are synthesized by the addition of ethylene oxide to sorbitan fatty acid esters and are widely known as polysorbates. Macrogols and polysorbats are used in pharmacy and cosmetics as emulsifiers and carriers.

Case report: A 26-year old non-atopic woman presented with history of generalised urticaria, pruritus and dyspnoea having developed 15 minutes after using a vaginal suppository with macrogol 1500 and 6000 as well as a vaginal cream containing polysorbate 60. She recovered under adrenaline intramuscular and intravenous antihistamines. She reported on multiple previous immediate type reactions of varying severity after using cosmetical products, i.e. tooth pastes, hair conditioner, shower gels and skin creams.

Diagnostic: Total IgE was 53,7 kU/l (normal < 114), serum tryptase 7,2 µg/l (< 11,0). Skin prick test was positive for vaginal cream, polysorbate 20, 40, 60 and 80 as well as for macrogol 1500 and 3000 but negative for macrogol 300 and 400. Macrogol 6000 was not available. Basophil activation test was positive for polysorbate 80 (polaysorbate 60 not available) and macrogol 4000, negative for polysorbate 20 as well as for macrogol 400. The patient only consented to oral provocation test (OPT) with substances tested negative at BAT. Single blind placebo-controlled OPT with macrogol 400 (cumulative dose [CD] 1,1g) was well tolerated. OPT with polysorbate 20 (CD 0,95g) induced generalized urticaria. Further OPT was denied. Topical cream containing polysorbate 60, applied accidently, induced contact urticaria.

Comment: We diagnosed type-1-allergy to polysorbate 20 (E432), polysorbate 60 (E 435) and probably to macrogol 1500 as well as sensitization to different concentrations and chain lengths of macrogols and polysorbates. The patient was told to avoid the intake of these substances with exception of macrogol 400 which was well tolerated at OPT. She received additive free emergency drugs and was informed about the widespread use of the substances. In conclusion, macrogols and polysorbates have to be considered as a rare causes of local or systemic allergic reaction in patients with reactions to multiple drugs and/or cosmetics.

125.

A novel handheld digital device for objective assessment of skin lesions in atopic dermatitis and urticaria

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Background: Currently, assessment of cutaneous symptoms in allergic diseases, e.g. atopic dermatitis or urticaria is mainly based on scoring by investigators and/or patients and thus subjective. Currently in atopic dermatitis assessing disease severity is based on complicated scoring systems like SCORAD or IGA but objective parameters, which can be measured as biomarker or standardized skin measurement technologies do not exist likewise in urticaria where the urticaria activity score is only based on patient evaluation.Recently prototype of a novel handheld digital camera based electronic instrument of the size of a dermatoscope linked to a tablet by wifi has been developed for a more objective assessment of inflammatory skin lesions in clinical trials. In further development, a smartphone based solution is also aimed at for patient self-assessment.

Methods: The primary goal was to validate the device as physician based instrument for objective assessment of severity in atopic dermatitis and urticaria. The digital device can take overview pictures and dermatoscopic close ups. Augmented readings include multispectral measurements, including UV, IR, and various polarizations.The pilot-study included the primary measurement of the lesion counts, the lesion type, percentage of redness and inflammation as well as the sebum activity level. Secondary measurement factors were the pore size, the pore blockage, the sebum age and the sebume volume in 20 patients of different subsets of atopic dermatitits and urticaria. **Results:** In the pilot-study, the prototype proved to be a reliable device in the assessment of severity of skin lesions in atopic dermatitis and urticaria. The parameters lesion counts, the lesion type, the percentage of redness and inflammation as well as the sebum activity level, the pore size, the pore blockage, the sebum age and the sebume volume could be objectively be documented. The interassay reliability of repetitive measurements is up to 98%. In addition, the handling in the clinical setting is fast and reliable, needing on average 150 seconds.

Conclusion: The prototype is a reliable, safe and fast device for analysing severity of atopic dermatitis and urticaria. This offers an improvement both in patients´care as well as in clinical trials

126.

Duration of eczema from its onset and the severity were the risk factor of food allergy at 2 years of age.

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Background Early onset eczema was reported to be a strong risk factor of food allergy (FA), however, it remains to be explored whether onset age of eczema or duration of eczema before starting proactive therapy to clear eczema would be more significant.

Methods A retrospective cohort study was performed to analyze the data obtained from the electronic medical records of patients who visited the National Center for Child Health and Development for the first time to receive eczema treatment in their infancy (age < 12 months) between April 2011 and December 2014. All caregivers of the patients were educated to treat their children's skin with proactive therapy resulting in sustained skin clearness thereafter.

They were divided into two groups of the early intervention group (EI) who started proactive therapy within 4months from eczema onset, and the late intervention group (LI) who started it after 4 months from eczema onset.

Logistic regression analysis was performed to explore the risk factors to influence onset of food allergy at 24 months of age.

Results Out of 177 patients who visited the hospital for the first time, 77 patients in EI and 70 in LI could be followed up and were evaluated at 24 months of age. SCORAD (median: IQR) of EI at the first visit was higher than that of LI (42.6: 24.0-61.0 vs 23.5: 11.9-40.0, p<0.01). The median age at first visit was 5 months of age in EI and 9 months of age in LI (p<0.01). At 24 months of age, incidence of FA in EI was lower than that of LI (24.7% vs 45.7%, p<0.01). Logistic regression analysis revealed that adjusted odd ratio (aOR) of onset age of eczema(> 2 months) was 1.8 (95%CI:0.8-3.9), aOR of high SCORAD was 1.3 (95%CI:1.1-1.5) and aOR of longer duration of eczema was 4.1 (95%CI:1.8-9.1).

Conclusions The longer and the more severe infantile eczema was, the more it developed food allergy.

127.

Early detection of gastroduodenal disorders in patients with common variable immunodeficiency

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Background: Common Variable Immunodeficiency (CVID) is the most frequent symptomatic primary immunodeficiency in adulthood. Clinical manifestations include recurrent infections, inflammatory and autoimmune diseases, and malignancies. Gastrointestinal disorders are frequently associated with symptomatic CVID. In this cross-sectional study we have assessed the prevalence of gastric and duodenal pathological findings in a cohort of CVID patients with mild gastrointestinal symptoms.

Methods: 58 adult patients (26 males; mean age 46.2±14.9 years) diagnosed with CVID according to the European Society for Immunodeficiencies (ESID) diagnostic criteria underwent upper endoscopic examination. 43 patients were treated with immunoglobulins intravenously (IVIG) and 15 subcutaneously (SCIG). At least two biopsies of the gastric antrum and descending duodenum were performed in each patient. A cut-off level of 25 lymphocytes per 100 enterocytes defined increased intraepithelial lymphocytes (IELs) in duodenal biopsy. Gastric and duodenal biopsies were blindly examined by two expert pathologists.

Results: The main pathological findings were: chronic active gastritis (40%), Helicobacter pylori infection (18%), chronic duodenitis with increased IELs and absence of plasma cells (36%), and autoimmune atrophic gastritis (5%). Intestinal metaplasia of the gastric antrum was reported in five patients. In two patients intestinal metaplasia was associated with autoimmune atrophic gastritis. Giardia lamblia was detected in the duodenum samples in three cases. Gastric adenocarcinoma was found in one asymptomatic woman. An overlap of different pathological findings in the stomach and the duodenum was found in 22% of patients.

Conclusions: The results of our study indicate that gastric and duodenal involvement is common and can occur early in adult CVID patients even in paucisymptomatic subjects. A strict endoscopic follow-up appears necessary in CVID patients irrespective of the gastrointestinal symptoms.

*These authors equally contributed to this study.

128.

Auto-antibodies to IgE and FceRI and the natural variability of SYK expression in basophils in the general population

MacGlashan, Donald

Background: Secretion from human basophils and mast cells requires the activity of SYK but expression of SYK is highly variable in the general population and this variability predicts the magnitude of IgE-mediated secretion. SYK expression in basophils is also a biomarker of the clinical efficacy of omalizumab in both food allergy and asthma. One known mechanism of modulating SYK expression in human basophils is aggregation of FceRI. This study examines the possibility that functional auto-antibodies are present in a wide variety of subjects and in particular, subjects whose basophils poorly express SYK. It also tests whether any found antibodies could modulate SYK expression in maturing basophils and whether interaction with CD32b modulates the effect.

Methods: An experimental algorithm for classifying the nature of histamine release induced by serum from 3 classes of subjects was developed. Sera were tested for the presence of auto-antibodies to FceRI or IgE and sera with the correct properties further tested for whether they modulated SYK expression in mature or maturing basophils (derived from CD34+ progenitors).

Results: The frequency of functional auto-antibodies that produce characteristics concordant with FceRI-mediated secretion was zero in 34 subjects without chronic spontaneous urticaria (CSU). In subjects with CSU, the frequency was lower than expected, approximately 7%. For the 5/68 unique CSU sera tested that contained anti-FceRI or anti-IgE Abs, these antibodies were found to induce down-regulation of SYK in both peripheral blood basophils and basophils developed from CD34+ progenitors. Blocking interaction of these antibodies with CD32b did not alter their ability to down-regulate SYK expression.

Conclusions: This study establishes that functional auto-antibodies to IgE/FceRI do not provide a good explanation for the variability in SYK expression in basophils in the general population. However, they do show that if antibodies with these characteristics are present, they are capable of modulating SYK expression in developing and mature basophils.

129.

Specific expression profiles of microRNA in human mast cell-derived exosomes in innate and acquired immunity

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Background: Exosomes are cell-derived extracellular vesicles that function as intercellular delivery carriers. Exosomes contain bioactive compounds such as microRNA (miRNA), mRNA, protein and lipids. Mast cells (MCs) are key effector cells in innate and acquired immunity. MC exosomes reportedly have an influence on cell phenotypes and differentiation of immune cells. However, the role of human MC exosomes in innate and acquired immunity remain unknown. Herein, to address this issue, we compared expression profiles of exosomal miRNA derived from human MCs in response to IL-33 and anti-IgE stimulation.

Method: Human cultured synovium-derived MCs were generated by culturing synovial cells with stem cell factor for 12 weeks. MCs were incubated with IL-33, human myeloma IgE or human myeloma IgE plus anti-IgE for 24 hours. Exosomes in the MC supernatants were purified using ExoQuick-TC. Exosomal miRNA expression profiling was performed with miRNA microarrays, and results were confirmed by quantitative PCR.

Results: The number of MC-derived exosomes increased more than tenfold by anti-IgE stimulation compared with non-stimulation. Microarray-based screening of miRNA showed that ~300 miRNAs were identified in MC exsosomes before stimulation. Following stimulation with IL-33 or anti-IgE, ~350 miRNAs were expressed in human MC exosomes and expression levels of ~60 miRNAs were upregulated more than 2-fold. The expression profiles of miRNA in MC exosomes stimulated with IL-33 were different from those stimulated with anti-IgE. The profiles were donor-dependent, but common miRNA expression in three different donors was observed. IL-33 stimulation specifically upregulated four miRNA expression in 3-donors' MC exosomes. They included miR-1295a and 155-5p. Anti-IgE stimulation specifically upregulated seven miRNA in 3-donors' MC exosomes, including miR-134-5p and 23b-3p. The upregulation of these miRNA expression was confirmed by quantitative PCR. The miR-155-5p reportedly promotes proliferation of group 2 innate lymphoid cells and the miR-134-5p induces apoptosis of eosinophils.

Conclusions: These results suggest that exosomal miRNA derived from MCs may have different regulatory functions in innate and acquired immunity.

130.

Interleukin-33 induces histidine decarboxylase transcription and histamine accumulation in skin-derived mast cells by a mechanism involving p38 mitogen-activated protein kinase, but not c-Jun N-terminal kinase

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Background: Mast cells (MCs) are highly susceptible to IL-33. Depending on the MC subset and conditions, distinct biological processes are modulated by this IL-1 family cytokine. We previously observed that human skin MCs cultured in the presence of IL-33 change their phenotype, including upregulation of histidine decarboxylase (HDC) and accordingly histamine. Here, we set out to elucidate the mechanism underlying this heightened histamine production.

Methods: MCs were purified to homogeneity (>98%) from human skin tissue and expanded in the presence of SCF (without IL-33). Growth factor-deprived cells were stimulated with IL-33 for 2-24 h. Gene expression was quantified by RT-qPCR, and histamine by an autoanalyzer-based technique. Immunoblot and flow-cytometry were utilized to discern signaling cascades. Kinase inhibitors and RNA-interference (RNAi) were employed to pinpoint the pathway(s) underlying HDC/histamine overexpression following IL-33.

Results: HDC mRNA upregulation was rapid and associated with enhanced histamine levels. Immunoblotting revealed rapid phosphorylation of JNK and p38 by IL-33, but no p-ERK1/2 or p-AKT, resulting in a complementary pattern vis-à-vis SCF. This pattern was confirmed by flow-cytometry. While pharmacological inhibition of JNK (SP600125) did not affect IL-33-augmented HDC or histamine, the p38-specific inhibitor SB203580 reversed HDC upregulation ($p < 0.01$) and histamine accumulation ($p < 0.01$). To confirm the results with an independent approach, Accell® supported RNAi of JNK and p38 was used. Both siRNAs proved highly efficient at reducing their targets ($p < 0.0001$). Using this system, we confirmed the participation of p38, but not JNK, in the induction of HDC transcription ($p < 0.01$) and associated histamine production ($p < 0.05$). In addition to HDC, IL-33 induced anti-apoptotic Bcl-xl ($p < 0.01$), in accordance with previous reports, but contrary to HDC, augmentation of this responder gene was independent of either p38 or JNK.

Conclusions: We conclude that IL-33 triggers at least three separate signaling routes in skin-derived MCs: p38, JNK and a yet-to-be identified pathway underlying Bcl-xl induction. Our study sheds light on the mechanisms regulating histamine quantity, which is as decisive for the extent of clinical symptoms during allergic reactions as the percentage of histamine released on MC stimulation.

I31.

Interleukin-33 enhances the permissiveness of mast cells for rhinovirus replication via increased ICAM1 expression

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Background: Mast cells (MCs) are tissue-resident immune cells that are classically associated with the early phase reaction in allergic asthma. Their numbers and location increase with disease severity where they lie in close association with the bronchial epithelium. Rhinovirus (RV) is a major risk factor for asthma development in early life and is the major cause of viral-induced exacerbations of asthma. We recently demonstrated that MCs support the replication and release of infectious viral particles, a quality unique among immune cells but their role in viral-induced exacerbations of asthma is unclear. IL-33 is an epithelial-derived cytokine which induces Th2 cytokine release from target cells with MCs being the major target of IL-33 in allergic asthma. Therefore we hypothesised that IL-33 would enhance MC responses to RV16 infection.

Methods: The LAD2 MC line or primary human cord blood-derived MCs (CMBCs) was treated with IL-33 (1 - 10 ng/ml) in the absence or presence of RV16 or UV-irradiated RV16 (control). After 24 hours, responses were assessed by RT-qPCR, MSD, ELISA and flow cytometry. Viral replication was determined by RT-qPCR and virion release by TCID50 assay.

Results: IL-33 treatment of LAD2 MCs induced a concentration-dependent increase in the release of IL-5 and IL-13 but this was not enhanced by RV16 infection. However, IL-33 increased RV16 replication and release of infectious RV particles. Innate immune responses including RV-dependent type I and III interferons and the IFN-stimulated genes MDA5 and OAS1 were also enhanced by IL-33 pre-treatment. Similar findings were observed with CBMCs. The mechanism of IL-33-dependent enhancement of RV16-dependent MC responses was next investigated. Flow cytometric analysis revealed that ICAM-1, the receptor for RV16, was increased following exposure of MCs to IL-33. Furthermore, neutralisation of ICAM-1 reduced the IL-33-dependent enhancement in RV16 replication and release of infectious RV particles and IFNβ in both LAD2 MCs and CBMCs.

Conclusion: These findings show for the first time that IL-33 enhances the permissiveness of MCs for RV16 infection and further implicate MCs in HRV-induced asthma exacerbations.

I32.

Divergent effects of acute or prolonged Interleukin-33 exposure on mast cell IgE-mediated functions

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Background: Epithelial cytokines, including IL-33 and thymic stromal lymphopoietin (TSLP), have gained a lot of interest for their role in the initiation and perpetuation of chronic allergic inflammations such as asthma. Mast cells are one of the major target of IL-33 and respond by secreting inflammatory cytokines and chemokines. Most studies have so far investigated the acute effect of IL-33 on mast cells, which correspond to the function of IL-33 as an alarmin.

Objective: In the current study we have investigated how prolonged exposure of human mast cells to IL-33 affects mediator synthesis and responses to IgE-mediated activation.

Methods: Human lung mast cells (HLMC), primary cord blood derived mast cells (CBMC), and the mast cell lines ROSA and LAD-2, were used for the study. Expression of FceRI, CD63, and receptors for IL-33 and TSLP, was measured by flow cytometry. Mediators were detected by ELISA, Luminex or flow cytometry.

Results: All mast cell populations analyzed express the ST2 receptor, IL-33R, whereas the TSLPR was only expressed on the cell lines ROSA and LAD-2, and IL-7Rα was low in all cells analyzed. IL-33 treatment further upregulated TSLPR on ROSA cells. As previously described, neither TSLP nor IL-33 induce degranulation and histamine release, but during a four day IL-33 treament an increase in histamine in the culture supernatant could be measured. Acute IL-33 treatment strongly potentiates IgE-mediated mast cell activation measured as histamine release and CD63 expression. In contrast, four days exposure to IL-33 decreases IgE-induced CD63 expression and histamine release. Analysis of FceRI revealed a strong reduction of FceRI on both CBMC and HLMC.

Conclusion: We describe a dual role of IL-33 on mast cell functions where the acute effects is a potentiation of IgE-mediated degranulation, whereas a prolonged exposure to IL-33 down-regulates the expression of FceRI accompanied with a dampening of IgE-mediated release of histamine. We conclude that mast cells act quickly as a sentinel to the alarmin IL-33 to initiate an acute inflammatory response, whereas extended exposure during an prolonged inflammation, IL-33 dampens IgE-mediated responses. This negative feedback appears to be a novel regulatory pathway to regulate IgE-mediated mast cell responses.

I33.

Exosome-mediated uptake of mast cell tryptase into the nucleus of melanoma cells: a novel mechanistic axis regulating proliferation and gene expression in tumor cells

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Background Mast cells are implicated in various malignancies but how they impact on cancer cells is not well understood. Here we addressed this issue.

Methods Bone marrow derived mast cells from wild type or tryptase (Mcpt6)-deficient mice were incubated with mouse melanoma cells. In addition, human recombinant tryptase was incubated with human melanoma cell lines. Effects of mast cells/tryptase on melanoma cells was studied by a panel of methods, including confocal/super resolution microscopy, Western

blot analysis, proliferation/apoptosis measurements, gene expression analysis and proteomics.

Results We show that mouse bone marrow-derived mast cells physically interact with melanoma cells and that they reduce their growth rate. In contrast, mast lacking the expression of tryptase (Mcpt6), a major protease localized in the mast cells secretory granules, did not affect the melanoma cell growth. Tryptase was shown to be delivered from mast cells through synapse-like connections into the interior of the melanoma cells. Uptake of tryptase into melanoma cells was verified after incubation of human melanoma cells with human recombinant tryptase, and it was also demonstrated that tryptase enters the nucleus of the melanoma cells. EdU labeling experiments revealed that tryptase reduced melanoma cell growth by blocking of proliferation, whereas no induction of apoptosis was seen. Further, tryptase was found to cause truncation of nucleosomal core histone 3 and nuclear lamin B1, and to cause extensive remodeling of the nuclear architecture in the melanoma cells. It was also shown that tryptase caused major effects on the gene expression profile in melanoma cells, in particular by affecting the expression of several microRNAs implicated in the regulation of tumor cell growth. The mechanism of tryptase uptake involved binding of tryptase to exosomes released from the melanoma cells, followed by dynamin-dependent endocytosis. Intriguingly, tryptase was found to bind to DNA present on the surface of the melanoma cell-derived exosomes and it was demonstrated that DNA had the ability to preserve tryptase activity.

Conclusion Altogether, this study reveals a novel mechanism for how mast cells can affect tumor cells. Moreover, on a more general level, these findings define a novel mechanism for regulation of tumor cell proliferation.

134.

Pollen exposure weakens innate defense against respiratory viruses

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Background: Individuals with respiratory allergies have more frequent and severe respiratory viral infections due to limited antiviral interferon responses. Pollen grains are among the most relevant airborne allergens, and maximal environmental presence co-occurs with that of respiratory viruses during spring.

Method: In a comprehensive translational study answering the question whether pollen impair the mucosal antiviral response. First, we used complex in vitro 2D and 3D mucosa models. A mouse model of ambrosia exposure, without sensitizing the mouse, the outcome of co-exposure will virus intranasal was analyzed. Furthermore, patients were exposed to pollen and transcriptome analysis of nasal mucosa was performed. To complete the translational approach, a large patient cohort (n = 5,782) of individuals presenting in hospitals with respiratory infections rhinovirus-positive cases were correlated with birch pollen data in sophisticated bioinformatics algorithms.

Results: Here we report a series of translational studies consistently showing that pollen directly impair cellular antiviral defense mechanisms, thereby promoting respiratory virus infections. Specifically, pollen significantly diminished interferon-λ responses of airway epithelia to rhinovirus and viral mimics, and decreased nuclear translocation of interferon regulatory factors in vitro. This effect was independent of donor allergy status. In mice infected with respiratory syncytial virus, co-exposure to pollen caused an attenuated interferon-λ response and increased pulmonary viral titers. In non-allergic human volunteers, nasal symptoms strongly correlated with airborne birch pollen abundance, and nasal birch pollen challenge suppressed interferon production in nasal mucosa. In a large patient cohort (n = 5,782) of individuals presenting in hospitals with respiratory infections rhinovirus-positive cases correlated with respective airborne birch pollen concentrations.

Conclusion: Pollen release immune-modulatory substance(s). We suggest that pollen exposure can render allergic and non-allergic people more susceptible to respiratory viral infections. The ability of pollen to suppress innate antiviral immunity suggests that high-risk population groups should avoid extensive outdoor activities when pollen and respiratory virus seasons coincide.

135.

Transcriptomic and proteomic signature of allergen-specific CD4+T and regulatory T cells during allergen-specific immunotherapy

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Background: Allergic patients display abnormal induction of type 2 immunity resulting in development of allergy instead of tolerance, which can be targeted by allergen-specific immunotherapy (AIT). Profound gene expression profiles of allergenspecific T cells and Treg cells and their potential change upon AIT have not been studied in detail, due to the extremely low frequency of these cells in the peripheral blood.

Methods: We investigated whole-genome transcriptomic changes of circulating birch (Bet v 1)- and grass pollen (Phl p 5a)-specific MHC class II tertramer+ CD4+ T cells and Treg cells, together with serum and nasal proteomics, and serum specific IgE and IgG4 in allergic patients before and 3, 6, and 12 months after pre-seasonal 3 months' birch and grass polen allergoid AIT. Non-allergic healthy controls were studied for comparison in corresponding seasonal time points.

Results: At baseline, before immunotherapy, outside of pollen season, allergenspecific CD4+ T cells were more frequent in patients compared to controls, but displayed profound dysregulation of innate and adaptive immune pathways, lipid and glucose metabolism and oxidative phosphorylation. We observed an increase in allergen-specific CD4+T cells parallel to substantial decrease of total and allergenspecific CRTH2+ CD4+T cells, starting already after 3 months of AIT. AIT induced extensive and precise changes in gene expression of several previously dysregulated immune and metabolic processes and led to induction of tolerance programs in allergenspecific CD4+ T cells, some persisting until 12 months of the therapy. AIT-induced gene expression changes differed between clinical responders and non-responders. At early time points, allergen-specific Treg cells of allergic patients displayed profiles suggesting dysregulated suppressive functions. The increase in the frequency of these cells observed after AIT, correlated with the upregulation of survival programs and correction of immune regulatory functions.

Conclusions: AIT causes profound changes in the frequency, gene, and protein expression profiles of allergen-specific T and Treg cells. These profiles are abnormal in allergic patients, but AIT is skewing them towards the levels of immune tolerant controls.

136.

Epigenetic changes in SATB1 gene in FoxP3+ regulatory T cells reflect Immunetolerance status during grass pollen subcutaneous and sublingual immunotherapy

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Rationale: Regulatory T cells (Tregs) play an indispensable role in immune tolerance induction during allergen immunotherapy (AIT) which can be administered either subcutaneously (SCIT) or sublingually (SLIT). Recently, a T lineage-enriched transcription factor, special AT-rich sequence binding protein-1 (SATB1), has been reported to be a functional marker of Tregs. We hypothesised that Foxp3+Treg cells are dysregulated in grass pollen allergic subjects (SAR) and their functional activity following SCIT and SLIT is restored when SATB1 is repressed in Tregs. Furthermore, SATB1 gene is differentially methylated between the SAR and AIT-treated groups.

Method: In a prospective, controlled study of AIT conducted during the pollen season, peripheral blood mononuclear cells were obtained from SCIT (n=12), SLIT (n=12), those who completed 3 years of SLIT (SLIT-TOL; n= 6), SAR (n= 24), and non-atopic controls (NA) (n=24). FoxP3+ and SATB1+FoxP3+ Tregs were enumerated by flow cytometry and confirmed by qRT-PCR. In addition, genome-wide DNA methylation was performed in FoxP3+ Tregs.

Results: SCIT, SLIT and SLIT-TOL groups had lower rhinoconjucntivitis symptom scores compared to SAR (all, $P<0.05$). The proportion of FoxP3+Tregs were significantly lower in SAR compared to NA ($P<0.001$). However, there were no difference in FoxP3+Tregs in SCIT, SLIT and SLIT-TOL groups when compared SAR. In contrast, a higher proportion of SATB1+FoxP3+Tregs were observed in SAR compared to NA ($P<0.001$) and a significant lower proportion in SCIT, SLIT and SLIT-TOL groups (all, $P<0.001$). SATB1 mRNA expression was downregulated in SCIT, SLIT and SLIT-TOL (all, $P<0.001$) when compared to SAR group. Functional analysis demonstrated a significant reduction in the suppressive capacity of FoxP3+Tregs in SAR compared to SCIT, SLIT and SLIT-TOL groups (all, $P<0.05$). FoxP3 methylation status did not reveal any differential methylation between patient groups. Interestingly, SATB1 gene was significantly unmethylated SAR compare NA and methylated in AIT-treated groups ($P<0.05$).

Conclusion: For the first time, we report that SATB1 expression is reduced in FoxP3+ Tregs following AIT and that SATB1 is differentially methylated between SAR and AIT-treated groups. Furthermore, SATB1 expression was associated with successful AIT, highlighting its potential role as a novel molecular biomarker of AIT.

137.

Sublingual grass pollen tablet immunotherapy: local and systemic allergen-specific IgA represents a distinct mechanism of long-term tolerance

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Background: Sublingual or subcutaneous immunotherapy for 2 years was effective in suppressing the clinical response to nasal allergen challenge but this was not sustained at 3 years, 12 months after discontinuation (Scadding G et al., JAMA 2017). For both routes reductions in allergen-specific Th2 cells most closely paralleled the clinical response with rebound at 3 years, whereas serum allergen-specific (s) IgG4 persisted, at least in part, for 3 years (Renand A et al., JACI 2018). SCIT elicited 10-30-fold higher levels of allergen-specific IgG4 compared to SLIT, whereas serum inhibitory activity for IgE-facilitated allergen binding to B cells (IgE-FAB) was nearly identical between SLIT and SCIT. These data suggested that IgE-blocking activity after SLIT could reside within an alternative antibody compartment.

Methods: Sera and nasal lining fluid samples were obtained at yearly intervals (per protocol population, n=84 of 92 completers). Allergen-specific total IgG and IgG1 were measured by ImmunoCAP system and specific IgA1 and IgA2 by ELISA.

Results: After both SCIT and SLIT, specific IgG and IgG1 levels increased ($p<0.01$) to a similar degree in both serum and nasal fluid at 1, 2 and 3 years. Serum allergen-specific IgA1 was markedly elevated at 2years after SLIT compared to SCIT and compared to placebo (respectively 436.2 v 96.7 and v 40 Arbitrary Units (AU)/mL, both $p<0.01$) and at 3 years (178.7 v 43 and v 42.8AU/mL, both $p<0.01$). Specific IgA1 levels in nasal fluid were elevated after SLIT compared to SCIT and to placebo at year 2 (78.3 v 15.9 and v 11.9 AU/ml, both $p<0.01$) and at year 3 (71.8 v 20.9 and v 16.7AU/mL, both $p<0.01$). Similarly nasal specific IgA2 levels were increased after SLIT compared to SCIT and placebo at year 2 (94.7 v 57.4 and v 34.2 AU/ml, both $p<0.01$) and at year 3 (103.4 v 65.2 and v 39.8 AU/mL, both $p<0.01$).

Conclusion: Whereas SCIT demonstrated higher specific IgG4 levels than SLIT, SLIT demonstrated equivalent specific IgG1 levels and much higher IgA levels both in serum and in local nasal fluid that persisted after discontinuation. Specific IgA may represent a distinct mechanism for long-term tolerance after SLIT.

138.

Nanoparticles as an effective adjuvant for oral peanut immunotherapy

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Introduction: There is a great need to find safer and effective treatment for peanut allergy.

Objective: To evaluate the potential application of poly(anhydride) nanoparticles (NPs) as immunoadjuvants for peanut oral immunotherapy.

Material and Methods: Poly(methyl vinyl ether-co-maleic anhydride) nanoparticles

(NP) were prepared following a solvent displacement method. After purifycating, NP were dried by lyophilization (PE-NP-LF) or spray-drying (PE-NP-SD) and loaded with roasted peanut protein. We then selected an animal model among CD1, C57BL/6 and BALB/c strains, analyzed the sensitization profile and checked the NP protection.

Results: After intragastric sensitization, CD1 mice produced the highest levels of peanut-specific IgE and IgG2a levels. CD1 and BALB/c strains produced higher IgG1 levels. The CD1 and C57BL/6 strains had higher levels of IgG2a, being very low in the BALB/c strain. CD1 showed a strong mucosal antibody response with high fecal IgA levels.

At 2 and 4 weeks mice were challenged intraperitoneally with 2 mg of a hydrophilic fraction of roasted peanut. Systemic anaphylactic symptoms were only verified in the sensitized CD1 mice with a mortality rate of up to 100% at week 2 and 50% at week 4. Neither the BALB/c nor the C57BL/6 mice experienced temperature body drop.

Cytokine production after spleen cells stimulation with peanut showed in BALB/c and C57BL/6 mice an increase in Th1 and Th17 cytokines. However, sensitized CD1 mice experienced an increase in Th2 cytokines. Spleen cells from mice who had died from an anaphylactic shock expressed high levels of IL-10 and IL-17.

Once the strain selected, CD1 mice was treated with PE-NP-LF or PE-NP-SD. Total IgE levels decreased following NP immunization, with the largest drop in the PE-NP-SD treated group. Sensitized mice, either untreated or treated with whole peanut, had a similar mortality rate (67%), whereas the mortality decreased to 33% in the PE-NP-SD group. Mice alive had high levels of IFN-gamma, IL-17 and IL-10. In contrast, dead mice produced more TNF-α. Oral immunization PE loaded NP was associated with a significant decrease in IL4, IL5, IL6 and an increase in INF-gamma and IL10, particularly the PE-NP-SD formulation.

Conclusion: Polymeric nanoparticles show promising results for oral peanut immunotherapy.

139.

Efficacy and Safety of AR101: Results of the Phase 3 Peanut Allergy Oral Immunotherapy Study for Desensitization (PALISADE) Trial

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Background: Peanut allergy is a common and serious immunological disorder characterized by high unmet medical need. AR101 is a novel, investigational oral biologic drug product designed to address this unmet need by reducing the risk of allergic reactions following accidental peanut exposures.

Methods: PALISADE was a phase 3 randomized, double-blind, placebo-controlled trial of oral immunotherapy with AR101 in peanut-allergic participants aged 4-55 years conducted in the United States, Canada, and Europe. Eligible participants reacted at ≤100mg of peanut protein during double-blind, placebo-controlled food challenge (DBPCFC) at screening. Participants completed initial escalation and up-dosing phases, approximately 6 months of 300mg/day maintenance treatment, and an exit DBPCFC. Participants aged 4-17 years were the primary analysis population.

Results: Of 750 participants aged 4-17 who entered screening, 496 were randomized (AR101 n=372, placebo n=124). Of randomized participants, 66% were aged 4-11 years, 34% aged 12-17 years; 57% were male; 78% Caucasian; 72% had a history of peanut anaphylaxis; 53% had a history of asthma; and 66% reported multiple food allergies. At baseline, the median (IQR) peanut skin prick wheal diameter was 11 mm (9, 15), median peanut-specific IgE was 71 (20, 202) kUA/L, and median (range) maximum tolerated DBPCFC peanut protein dose was 10mg (3, 30). Overall, 296 (80%) AR101 and 116 (94%) placebo participants completed the study. Percentages of ITT participants able to tolerate a single highest dose of 300, 600 (primary endpoint), and 1000mg peanut protein at exit DBPCFC were 77%, 67%, and 50% for AR101 participants versus 8%, 4%, and 2% for placebo participants, respectively (P<0.00001 for all comparisons). No deaths, life-threatening adverse events, or unexpected serious adverse reactions (SAEs) were reported; 9 SAEs (4 study drug-related, 5 leading to discontinuation) occurred in 8 (2.2%) AR101 participants versus 1 SAE in 1 placebo subject. Study discontinuation due to systemic hypersensitivity and gastrointestinal-related adverse events occurred in 2.7% versus 0% and 6.7% versus 0% AR101 and placebo participants, respectively.

Conclusions: These data, from the largest peanut allergy trial ever conducted, suggest AR101 could be useful in the treatment of peanut allergy in a highly sensitive pediatric population.

140.

A randomized clinical trial of passive immunotherapy With single-dose Anti-Fel D 1 monoclonal antibodies R1908-1909 In cat-induced rhinoconjunctivitis: Clinical efficacy endpoints and biomarkers

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Background: Cat allergens are major indoor allergens that cause IgE-mediated allergic disease that contribute to allergic rhinitis and asthma worldwide. The aim of this study was to evaluate the clinical and immunologic effects of a passive administered mixture of R1908 and R1909 (R1908/9), high-affinity monoclonal antibodies binding distinct Fel d 1 epitopes in cat allergic individuals.

Method: In a prospective placebo-controlled study, subjects were randomized 1:1 to a single-dose subcutaneous R1908/9 (n=36) or placebo (n=37). Nasal allergen challenge was performed at baseline and days 8, 29, 57, and 85; patient-reported nasal symptom scores, Visual Analog Scale (VAS;0-10 cm) at 0-60 min was recorded. Blood and nasal fluids samples were collected after cat allergen challenge at predefined time points for pharmacokinetics (PK) and biomarker analyses, respectively.

Results: Baseline VAS AUC0-1h was 49.8±16.1 for R1908/9 and 51.0±15.1 for placebo with percent change at Day 8 of -58.7%±44.5% and -34.4%±34.9% , respectively; least square mean difference was -24.1% (95% CI -43.2%, -4.9%; p<0.05). Percent change from baseline in VAS AUC01h was significant for R1908/9 vs placebo at Day 29 (p<0.05). Percent change in peak VAS scores was significant for R1908/9 vs placebo at all time points except Day 57 (p<0.05). Cat-allergen-induced basophil responsiveness as measured by CD63 expression on CRTh2+ basophils were decreased at day 8, 29 and 85 in subjects treated with R1908/9 compared to placebo. Type 2 cytokines in nasal fluid were significantly reduced at 6 hours after cat allergen challenge on day 8 (IL-4, IL-5 and IL-13; all p<0.05) in R1908/9 compared to placebo-treated group. TARC levels in the nasal fluid were reduced in R1908/9-treated group at 6 hour on day 8 compared to placebo (p<0.05). Nasal and serum inhibitory activity for CD23-mediated IgE-facilitated allergen binding (IgE-FAB) to B cells were inhibited in R1908/9 group (all, p<0.05 [nasal:day8,29,57;serum: day 8,29,57,85]) compared to placebo.

Conclusions: For the first time, we show that sustained clinical effect of passive administration of Feld 1 monocloanal antibodies (R1908/9) was associated with basophil hyporesponsiveness, sustained nasal (local) and serum (systemic) inhibitory activity for CD23-mediated IgE-FAB and suppression of Th2 cytokines in nasal fluid at day 8.

141.

Impact of allergen specific immunotherapy on cells of the lower airways

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Background: Allergic rhinitis is characterized by inflammation of the upper airways that frequently proceeds to the lower airways resulting in allergic asthma. Allergen-specific immunotherapy (ASIT) has been shown to prevent or delay this transition. However, the understanding of therapy effects on the lower airways is limited.

Method: In this study, we investigated for the first time the effect of ASIT on sputum cells from the from non-asthmatic rhinitis patients. We were interested whether the tolerogenic therapy induces an anti-inflammatory footprint that exceeds the absence of inflammatory marker and hypothesized that this footprint should be detectable not only in-season, but also off-season. We therefore analyzed sputum transcriptomes of 40 grass-pollen allergic rhinitis patients with or without asthma both during pollen flight and in winter. Twenty of these patients received ASIT.

Results: Consistent with previous studies, we observed season-dependent inhibition of prototypic type-2 markers in season such as CCL-26, IL-24, but also other general pro-inflammatory indicators such as IL-8 and IL-17. In contrast off-season differences were restricted to the decrease of IL-24 and IL-17. Interestingly, we could identify ASIT-induced and potentially anti-inflammatory genes such as CCL-20, Secretoglobin-1A1 and the Androgen receptor. Of these genes only CCL-20 and Secretoglobin-1A1 were significantly increased off-season. Inhibin A was only observed in asthma patients both in- and off-season. Both CCL-20 and Secretoglobin-1A1 are primarily expressed by macrophages and epithelial cells.

Conclusions: Taken together the current study reveals a novel therapy-induced footprint of anti-inflammatory mediators. These genes could be relevant for diagnostic purposes as well as for the mechanistic understanding of ASIT.

142.

Predictors of severe adverse events during venom immunotherapy (VIT), and the use of omalizumab as adjunct to VIT in patients with systemic adverse events

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Background: Subcutaneous VIT is the only effective treatment for patients with severe hymenoptera sting-induced allergic reactions. However, in some patients, the treatment has to be discontinued because of recurrent severe systemic adverse events (SAEs). We sought to investigate the predictors of SAEs to VIT, and whether the use of omalizumab as adjunct to VIT reduces SAEs and enables safe continuation of the treatment.

Methods: We evaluated SAEs in venom allergic patients undergoing VIT in the University Hospital Golnik, Slovenia, between 2005 and 2016. We measured sIgE (complete extract and components), baseline tryptase levels, and basophil CD63 expression. We ascertained predictors of SAEs and VIT failure using penalized logistic regression. Patients in whom VIT had to be withdrawn due to treatment-related SAEs were then invited to participate in the open-label prospective trial of omalizumab as adjunct to VIT.

Results: 1820 patients (60.2% male, age 15-84 years) commenced VIT (620 honeybee, 1068 yellow jacket, 132 both venoms). Of those, 172 patients receiving honeybee (23.1%) and 22 receiving wasp (1.8%) VIT had SAEs during treatment In all patients with wasp allergy, and in 151/172 with bee allergy, the use of a modified protocols (more gradual dose increase etc.) allowed continuation of VIT. However, in 23 honeybee allergic patients (3.1%) we had to stop VIT due to recurrent and severe SAEs. The levels of sIgE to venom, rApi m 1 and rApi m 10 were comparable between subjects with and without SAEs. Independent predictors of VIT failure due to SAEs were basophil response at 0.1 mcg/ml ($P<0.001$; log OR [95% CI]: 0.04 [0.02-0.07]) and baseline serum tryptase level ($P<0.05$; log OR [95% CI]: 0.04 [0.005-0.07]). Eleven patients who failed VIT were recruited into the open-label of omalizumab as adjunct to VIT. Optimized omalizumab pretreatment allowed rapid and safe VIT build-up in all these patients, and the reduction of SAEs correlated strongly with the basophil suppression.

Conclusions: Baseline tryptase levels and the basophil response are independent risk factors of SAEs to VIT. Adjunctive treatment with omalizumab significantly reduce SAEs, improves safety, and enables continuation of VIT in high-risk Hymenoptera venom allergic patients.

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Group 2 innate lymphoid cells (ILC2) and surfactant protein D (SP-D) in asthma and air pollution-induced airway inflammation

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We previously showed that ILC2 induce airway inflammation and airway hyperresponsiveness (AHR) in allergen or ozone (O3)-treated mice. Our group also demonstrated the protective importance of the epithelial derived SP-D in the lung. The mechanisms and significance of the ILC2 and SP-D action and whether they directly interact are not known.

Peripheral blood ILC2 (FACS) were studied from patients with severe neutrophilic asthma (n=24) healthy volunteers (n=18), asthmatic (n=12) and non-asthmatic (n=5) Rhesus macaques from the California National Primate Research Center and an O3-induced model of neutrophilic airway inflammation in wild type and SP-D-/- mice. Adoptive transfer of ILC2 was performed in RAG2γc-/- mice.

ILC2 activation was associated with increased airway obstruction in asthmatic patients, AHR in asthmatic rhesus macaques and AHR in mice upon O3 exposure. Absence of SP-D in gene

deficient mice enhanced ILC2 activation. SP-D-/- mice also had heightened and prolonged airway neutrophilia after O3 exposure. O3 induced IL-33 mRNA and protein expression. O3 also elevated ILC2 counts, increased ST2 (IL-33 receptor) and Bcl11b and induced simultaneous expression of IL-13 and IL-17A, GATA-3 and RORγc by ILC2 in the lung of mice. Lack of SP-D heightened these changes. In contrast, recombinant SP-D (1-10 μg/ml) directly suppressed activation of ILC2 in a dose dependent manner in vitro. Severe asthma patients and asthmatic macaques had increased numbers of circulating ILC2 that also co-expressed IL-13 and IL-17A, when compared with healthy subjects. IL-17A is a pro-neutrophilic cytokine previously thought to be exclusively generated by Th17 cells and ILC3. Adoptive transfer of ILC2 restored O3-induced neutrophilia in RAG2γc-/- recipient mice, but not, when these mice were treated with anti-IL-17A prior to O3 exposure.

Thus, in mice, non human primates and asthma patients we demonstrated that lung resident ILC2 can transdifferentiate into IL-17A-producing cells. We also showed that this mechanism is important in eliciting neutrophilic airway inflammation and that it is inhibited by SP-D in the lung.

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