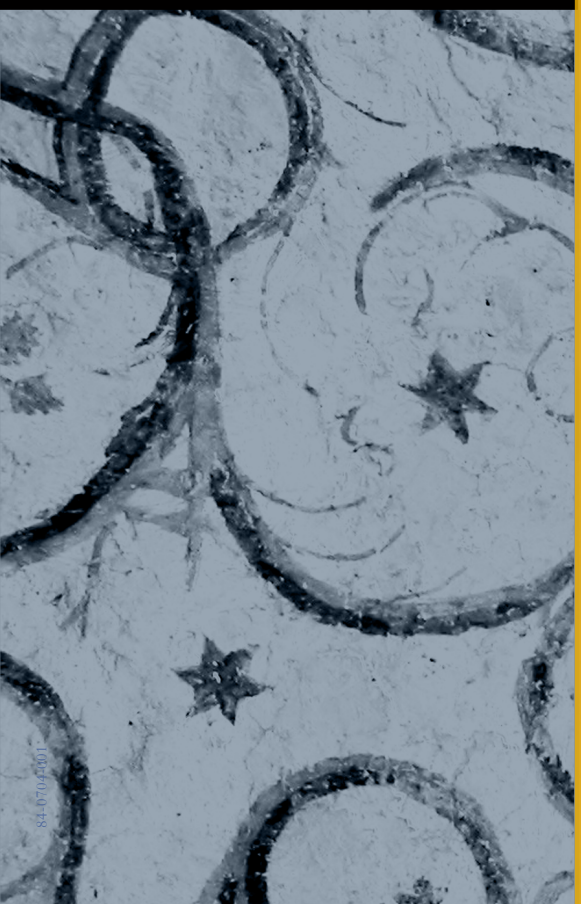
Collegium Internationale Allergologicum 25th Symposium

From Genes To Phenotypes: The Basis of Future Allergy Management

24-30 August, 2004
Bornholm, Denmark



COLLEGIUM INTERNATIONALE ALLERGOLOGICUM

CIA

50 years of Collegium Internationle Allergologicum

Collegium Internationale Allergologicum Council 2002-2004

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COLLEGIUM INTERNATIONALE CIA ALLERGOLOGICUM

President's Welcome

It is a pleasure to welcome you to the 25th CIA Symposium which happens to be the 50th anniversary of our Collegium. The "Collegium Internationale Allergologicum" was founded on the 18th of October, 1954 in London. It is the 2nd symposium taking place in Denmark after the meeting in Copenhagen 1974 (organizers: B. Diamant, N. Hjorth, P. Kallós, H. Rorsmann).

In our constitution, article No. 1 says that "the Collegium Internationale Allergologicum is an international group for the study of scientific and clinical problems in allergy and related branches of medicine and immunology. The Collegium aims to promote the humble spirit of scientific inquiry, friendly cooperation, good fellowship and professional relationships in the field of allergy." I think these aims have been achieved over the last half century in a remarkable way looking back at so many outstanding symposia, lectures and distinguished members and officers of our Collegium!

After many years with increasingly luxurious meeting places, we have decided to return a little bit to the "humble Spirit" — not only for financial reasons and to allow our young members to join more easily, but also in order to focus again vehemently on the scientific spirit!

Bornholm is the ideal place to do this. This beautiful island in the Baltic Sea allows us to meet in a friendly climate and relaxed atmosphere. Don't forget to rent a bike! I want to thank Henning Löwenstein and his wife and daughter and the local organizing committee who have worked very hard to organize this event in such a beautiful way. I also would like to take the opportunity to say thanks to all the colleagues and the staff of our secretariat contributing in the organization of the CIA to make it such a special society where over the decades excellent science, good living and — very important — good friendship are a tradition. Enjoy the program and enjoy Bornholm.

Prof. Dr. med. Dr. phil. Johannes Ring
President, Collegium Imternationale Allergologicum

Letter of Welcome

I am pleased to present to welcome you to the 25th Symposium of the Collegium Internationale Allergologicum. Bornholm offers many sights, history and art to its visitors. Many artists and craftsmen reside on the island, and have found inspiration in its vast history, small towns, rolling country sides and breathtaking sea views.

The fine art and rich history of Bornholm is the very focus of the social program. The social events include a Hans Christian Andersen evening and a view of the island's sea glass artistry in its creation. A boat ride to the historic place of Christian's Island is also planned.

Scientifically speaking, the presentations given at CIA Symposia belong to the world's best research in allergology and clinical immunology. I am very excited to be able to share this exemplary science in a friendly gathering within the relaxing and captivating atmosphere of Bornholm.

Thank you for attending.

With best regards,

Henning Löwenstein
Symposium Organizer

General Information

Venue
The 25th Symposium will be held on the quaint island of Bornholm. The landscape of the Danish island of Bornholm, isolated in the middle of the Baltic Sea, holds the landscapes of all the Scandinavian countries in a nutshell. Bornholm has wide, white beaches, dramatic cliffs, steep quarries, marshes, meadows, fields and forests and in many places you will find beech and spruce reflected in the ever-present sea. The small towns and fishing hamlets of Bornholm have their own characteristic charm and you often have the feeling that you have stumbled upon a world that stopped at least 100 years ago.

The symposium scientific program will take place at the Hotel Griffen which is located on the Baltic Sea next to the marina and close to the shopping center of Ronne. Rooms are also available for participants at the beautiful Hotel Fredensborg and the Hotel Hoffman.

Hotel Griffen
Ndr. Kystvej 34
DK-3700 Ronne
DENMARK
Tel. +45 5690 4244
Fax. +45 5690 4245

Radisson SAS Fredensborg Hotel
Strandvejen 116
DK-3700 Ronne
DENMARK
Tel. +45 5690 4444
Fax. +45 5690 4443

Hotel Hoffmann
Ndr. Kystvej 32
DK-3700 Ronne
DENMARK
Tel. +45 5695 0386
Fax. +45 5695 2515

Proceedings
The 25th Symposium presentations will be published in *Allergy and Clinical Immunology International* (Hogrefe and Huber Publishers). Authors should submit manuscripts either at the meeting or immediately thereafter. The page limit for poster presentations is 4 typewritten double-spaced pages, including tables, illustrations and references. For oral presentations the page limit is 6 typewritten double-spaced pages, including tables, illustrations and references.
There is no page charge for papers which follow the page limit instructions of the Editor. Each additional complete or partial page is charged to the author at $300 (USD).

Language
The official language of the CIA Symposium is English.

Currency
The currency in Denmark is the Danish Krone, the Euro is not always accepted. At the time of this printing, one Danish Krone is approximately $0.16 USD or ∈ 0.14.

Electricity
Electrical current in Denmark is 220 volts, 50 cycles alternating current (AC); wall outlets take Continental-type plugs, with two round prongs.

Time Zone
Denmark is in the Central European Time Zone. Central European Standard Time (CET) is 1 hour ahead of Greenwich Mean Time (GMT+1). Summer (Daylight-Saving) Time is observed in Denmark, where the time is shifted forward by 1 hour; 2 hours ahead of Greenwich Mean Time (GMT+2). Denmark is 6 hours ahead of the Eastern Time Zone in the United States. Daylight will last from 5 am to 9 pm at the time of the meeting.

Tipping
The Danish do not expect to be tipped. Service is included in bills for hotels, bars, and restaurants. Taxi drivers round up the fare to the next Krone but expect no tip. The exception is hotel porters, who receive about 5 Danish Krone per bag.

Transportation
A shuttle bus is available between the Hotel Fredensborg and the Hotel Griffen, signs with transportation times are located in the Registration Area at the Hotel Griffen.

Program at a Glance

Tuesday, 24 August	Wednesday, 25 August	Thursday, 26 August	Friday, 27 August	Saturday, 28 August	Sunday, 29 August	Monday, 30 August
14.00 to 19.30 **Registration Desk Open** Hotel Griffen Lobby	07.00 to 09.00 **Breakfast** Breakfast Rooms at Hotels	07.00 to 09.00 **Breakfast** Breakfast Rooms at Hotels	07.00 to 09.00 **Breakfast** Breakfast Rooms at Hotels	07.00 to 09.00 **Breakfast** Breakfast Rooms at Hotels	07.00 to 09.00 **Breakfast** Breakfast Rooms at Hotels	07.00 to 09.00 **Breakfast** Breakfast Rooms at Hotels
19.30 to 22.00 **Welcome Reception** Ronne Theater	07.30 to 17.30 **Registration Desk Open** Hotel Griffen Lobby	07.30 to 17.30 **Registration Desk Open** Hotel Griffen Lobby	07.30 to 13.00 **Registration Desk Open** Hotel Griffen Lobby	07.30 to 17.30 **Registration Desk Open** Hotel Griffen Lobby	07.30 to 17.30 **Registration Desk Open** Hotel Griffen Lobby	
	07.30 to 18.30 **Speaker Ready Room Open** Room L	07.30 to 18.30 **Speaker Ready Room Open** Room L	07.30 to 13.00 **Speaker Ready Room Open** Room L	07.30 to 18.30 **Speaker Ready Room Open** Room L	07.00 to 08.15 **CIA Council Meeting** Hotel Fredensborg	
	07.30 to 09.30 **Authors Set Posters** Poster Area	08.30 to 10.30 **Session 4** Development of immune response	08.30 to 10.30 **Session 6** Clinical Allergy	08.30 to 10.30 **Session 8** Immunotherapy	07.30 to 18.30 **Speaker Ready Room Open** Room L	
	08.30 to 10.30 **Session 1** Development of Immune Response	09.00 to 18.00 **Posters on Display** Poster Area	09.00 to 13.00 **Posters on Display** Poster Area	09.00 to 18.00 **Posters on Display** Poster Area	08.30 to 10.10 **Session 10** Innate Immunity	
	09.30 to 18.00 **Posters on Display** Poster Area	11.00 to 13.00 **Poster Session 1** Poster Area	11.00 to 12.40 **Session 7** Eosinophils/ Basophils/ Macrophages	11.00 to 13.00 **Poster Session 2** Allergy and the Skin	09.00 to 18.00 **Posters on Display** Poster Area	
	11.00 to 12.40 **Session 2** Environmental Aspects of Allergy	13.00 to 14.30 **Lunch** Restaurant	13.00 to 18.00 **Boat Ride and Tour to the Christian's Island**	13.00 to 14.30 **Lunch** Restaurant	10.40 to 12.20 **Session 11** Asthma	
	13.00 to 14.30 **CIA Council Meeting** Hotel Fredensborg	14.30 to 16.10 **Session 5** Psychoneuro-allergology	Evening free – see optional program for proposals	14.30 to 15.05 **Paul Kallos Lecture**	12.20 to 13.00 **Carl Prausnitz Lecture**	
	12.40 to 14.45 **Lunch** Restaurant	16.15 to 17.00 **Business Meeting**		15.05 to 16.45 **Session 9** Effector Cells II	13.00 to 14.30 **Lunch** Restaurant	
	14.45 to 17.05 **Session 3** Effector cells (Mast cells)	17.00-17.45 **Relaxing Lecture**		Evening free – see optional program for proposals	14.30 to 15.50 **Session 12** General Session	
	18.30 to 21.00 **Tour to Baltic Sea Glass with Barbeque**	18.30 to 22.30 **Hans Christian Andersen evening**			18.00 to 22.00 **Banquet** Hotel Fredensborg	

*** If not mentioned otherwise, lectures take place in the "General Session Room" at the Hotel Griffen**

Schedule of Events

Tuesday, 24 August

14.00 to 19.30 Registration Desk Open
Hotel Griffen Lobby

19.30 to 22.00 Welcome Reception Ronne Theater

Wednesday, 25 August

07.00 to 09.00 Breakfast
Breakfast Rooms at Hotel
Fredensborg, Griffen and Hoffman

07.30 to 17.30 Registration Desk Open
Hotel Griffen Lobby

07.30 to 18.30 Speaker Ready Room Open
Room L, Hotel Griffen

07.30 to 09.30 Authors Set Posters
Poster Area, Hotel Griffen

08.30 to 10.30 **Session 1**

Development of Immune Response

Chairs: H. Löwenstein and J. Ring

1 **Denburg JA**, Hatfield H, Cyr M, Dunstan J, Holt P, Prescott S
Allergy is in the blood at birth

2 Hebenstreit D, Luft P, Schmiedlechner A, Horejs-Hoeck J and **Duschl A**
Interleukin-4/13 induced activation of signal transducer and enhancer of transcription (STAT) 6 is regulated by suppressors of cytokine signaling (SOCS)

3 **Izuhara K**
Identification and characterization of the IL-13-inducing genes in bronchial epithelial cells

4 **Holt P**
Th1-mediated inflammation as a pathogenic factor in allergic disease

5 **HayGlass K**, Stinson M, Simons FER
Ongoing Th1 vs Th2 chemokine production is differentially regulated by IL-4 vs IL-13

6 **Akdis M**, Blaser K, Akdis CA
Healthy or allergic immune response characterized by fine balance between specific T regulatory 1 and T helper 2 cells

09.30 to 18.00 Posters on Display
Poster Area, Hotel Griffen

11.00 to 12.40 **Session 2**

Environmental Aspects of Allergy

Chairs: H. Behrendt and A. de Weck

7 **Platts-Mills T**, Erwin E, Wickens K, Custis N, Woodfolk J, Crane J
The IgE ab response to cat allergen is decreased in titer as well as prevalence: use of Streptavidin CAP to measure IgE ab to Der p 1, Der p 2, and Fel d 1

8 **Custovic A**, Simpson A, Woodcock A, Lowe L
Lung function at age 3 years: effect of pet ownership and exposure to indoor allergens

9 **Traidl-Hoffmann C**, Jakob T, Mariani V, Hochrein H, Müller MJ, Wagner H, Ring J, Behrendt H
Tuning the immune response by allergen carriers: Th2-polarization induced by pollen associated lipid mediators (PALMs)

10 Schoell I, Untersmayr E, Hantusch B, Kalkura N, Betzel C, Keller W, Spitzauer S, Scheiner O, Boltz G, **Jensen- Jarolim E**
The molecular characteristics of allergens and food allergens, or what makes an antigen an allergen?

11 **Grönlund H**, Hedlin G, Kronqvist M, Vargas E, Kaiser L, Achour A, Grönneberg R, Valenta R, vanHage-Hamsten M
Fel d 1, the major allergen in cat; from structure to clinical application

13.00 to 14.30 CIA Council Meeting
Hotel Fredensborg

12.40 to 14.45 Lunch
Restaurant, Hotel Griffen

14.45 to 17.05 **Session 3**

Effector cells (Mast cells)

Chairs: S. MacDonald and S.E. Dahlén

12 Yu M, Tsai M, Tam S, **Galli, SJ**
Mast cells, and mast cell-dependant effects on tissue gene expression, contribute to multiple features of a chronic asthma model in mice

13 Gurish MF, Pablo Abonia J, Friend DS, Moore Jr FD , Carroll MC, Chan R, Oakes S, Austen Jr WG, Humbles A, Gerard C, Knight P, Kanaoka Y, Yasuda S, Austen KF, **Stevens RL**
Role of hTryptase epsilon in urokinase-type plasminogen activator/plasminogen pathways in the lung

14 **Baumruker T**, Olivera A, Bofill-Cardona E, Billich A, Rivera J, Urtz N
Activation of SPHK1 in mast cells is mediated by tyrosine kinase Lyn

Schedule of Events

Wednesday, 25 (continued)

15 **Saito H,** Nakajima T, Matsumoto K, Nagai H, Yamamoto T, Aoshima K
Comparative database for the orthologous genes expressed by various human and mouse inflammatory cell types

16 **Krilis S**, Cheng Qi J
The tetraspanin CD9 is essential for the IL-16-mediated chemotaxis and activation of human mast cell

17 Kitaura J, Kawakami Y, **Kawakami T**
Mast cell adhesion and migration induced by monomeric IgE

18 Gebhardt T, Bedoui S, Klempnauer J, Manns MP, **Bischoff SC**
Mast cell inhibition by beta-2-adrenoceptor agonists

18.30 to 21.00 Tour to Baltic Sea Glass with Barbeque

Thursday, 26 August

07.00 to 09.00 Breakfast
Breakfast Rooms at Hotel Fredensborg, Griffen and Hoffman

07.30 to 17.30 Registration Desk Open
Hotel Griffen Lobby

07.30 to 18.30 Speaker Ready Room Open
Room L, Hotel Griffen

08.30 to 10.30 **Session 4**

Development of immune response (isotype differentiation)

Chair: K. Blaser and Y. Mekori

19 **Achatz-Straussberger G**, Lamers M, Achatz G
Studies on the regulation of IgE expression by the use of "knock in"-mice

20 **Aalberse R**, Stapel S
Lessons on the mechanism of atopic sensitization from the ratios of IgG1/IgG4 antibodies to allergen

21 **Akdis CA**, Akkoc T, Jensen-Jarolim E, Blaser K, Akdis M
Increased apoptosis of circulating memory/effector T helper (Th) 1 cells in atopic diseases as a mechanism for Th2 predominance

22 **Redegeld F**, van der Heijden MW, Kool M, Blokhuis B, Kraneveld AD, Nijkamp FP
Immunoglobulin free light chains in immediate and delayed hypersensitivity reactions

23 **Kere J,** Laitinen LA, Laitinen A, Haahtela T, Hudson TJ, Alenius H, Polvi A, Laitinen T
A high IgE and asthma susceptibility gene on chromosome 7p

24 Flaswinkel H, Koellisch G , Soewarto D, Hrabe de Angelis M, Pfeffer K, Ring J, Wolf E, Behrendt H, **Jakob T**
From phenotype to genotype: novel mutations in hyper IgE mice generated by genome wide mutagenesis

09.00 to 18.00 Posters on Display
Poster Area, Hotel Griffen

11.00 to 13.00 **Poster Session 1**
Poster Area, Hotel Griffen

Pathophysiology

Moderator: S. Galli and A. Mori

25 **Zwadlo-Klarwasser G**, Bostanci Ö, Gräber S , Kiehl K, Heise R, Merk HF, Baron JM
Detection of the human organic anion transporter 3 (OAT3) in antigen presenting cells

26 Mempel M, Antonio Aguilar J, Ring J, **Ollert M**
Induction and recruitment of allergen-specific CD8+ T cells in the course of IgE mediated allergy

Schedule of Events

Thursday, 26 August (continued)

27 **Luger E**, Achatz G, Radbruch A, Berek C
Allergen specific IgG and IgA memory B cells in atopic and non-atopic individuals

28 **Hummelshoj H**, Ryder LP, Poulsen LK
Expression of plasma cell markers CD38, CD138, intracellular IgE and X-box binding protein in U266

29 Oberndorfer I, Geisberger R, Crameri R, **Achatz G**
Indications for the existence of an isotype specific signal transduction

30 **James A**, Kumlin M, Dahlen B, Sampson T, Dahlen SE
Flow cytometric analyses of leukotriene pathway enzymes in blood leukocytes

31 Hackett TL, **Warner JA**
Mediators of acute and chronic inflammatory response in human lung tissue

32 **Masini E**, Nistri S, Vannacci A, Marzocca C, Novelli A, Mannaioni PF, Bani D
Relaxin inhibits activation and chemotaxis of human neutrophils in vitro by a nitric oxide-dependent mechanism

33 **Lötvall J**, Johansson A, Sergejeva S, Sjöstrand M.
Eosinophilopoiesis on bone marrow and airways after airway allergen exposure

34 **Kumlin M**
Further studies on hyperosmolar challenge of mast cells

35 Feng C, Beller EM, **Boyce JA**
Prostaglandin E2 inhibits eicosanoid generation and cytokine production by human mast cells through a non-EP2 receptor-dependent mechanism

36 **Gurish MF**, Pablo Abonia J, Friend DS, Moore, Jr. FD, Carroll MC, Chan R, Oakes S, Austen, Jr. WG, Humbles A, Gerard C, Knight P, Kanaoka Y, Yasuda S, Austen KF, Stevens RL
Ischemia-reperfusion injury of skeletal muscle is mediated by mouse mast cell protease 5

37 **Andersen HB**, Holm M, Hetland TE, Dahl C, Junker S, Hoffmann HJ, Schioetz PO
Optimization of culture methods; cord blood derived mast cells

38 **Nilsson G**, Xiang Z
Active cutaneous anaphylaxis is attenuated in mice deficient in the pro-survival gene A1

Dendritic Cells

Moderators: P. Holt and B. Stadler

39 Iris Bellinghausen I, Bettina Klostermann B, Ingo Böttcher I, **Saloga J**
Dendritic cells and regulatory T cells in the allergic immune response and its modulation

40 **Wollenberg A**, Poeck H, Häusler E, Giese T, Wagner M, Schaller M, Rothenfusser S, Wetzel S, Hornung V, Kochan J, Endres S, Hartmann G
Human plasmacytoid dendritic cells, though rare in atopic dermatitis lesions, express functional high-affinity IgE-receptors

41 **Schäkel K**, von Kietzell M, Schulze L, Rieber EP
Differential T cell programming by pro-inflammatory blood dendritic cells

42 **Jahnsen FL**, Farkas L, Kvale EO, Lund-Johansen F
Plasmacytoid dendritic cells activate allergen-specific Th2 memory cells: modulation by CpG oligodeoxynucleotides

Allergens

Moderators: W. Thomas and W. M. Becker

43 **Nouri-Aria KT**, Pilette C, Durham SR
Nasal mucosa TGF-B mRNA expression is associated with elevated Phl p 5 specific serum IgA2 response in grass pollen immunotherapy treated patients

45 **Breitenbach M**, Simon B, Denk U, Schneider P, Ebner C, Crameri R
Cloning, expression, characterization and clinical testing of NADP dependent mannitol dehydrogenase

46 **Raulf-Heimsoth M**, Yeang HY, Lundberg M, Arif SAM, Brüning T, Rihs HP
Improvement of the in-vitro diagnostic of natural rubber latex allergy and estimation of cross-reactivity by application of recombinant and natural single allergens

47 **Spangfort MD**, Støvhase RB., Wurtzen PA, Ipsen H, Hansen L, Lund K, Lenhard T
Immunological characterisation of recombinant Phl p 6 and comparison with natural Phl p 6

48 **Henmar H**, Hansen L, Mærkedahl LL, Ipsen H, Würtzen PA
Mite allergic patients exhibit herterogeneous immunological responses to allergen extracts from related mites

Schedule of Events

Thursday, 26 August (continued)

Epidemiology and Risk Factors

Moderators: T. A.E. Platts-Mills and J. Warner

49 Lødrup Carlsen, KC, Løvik M, Granum B, **Carlsen KH**
Soluble CD14-levels are decreased in boys, with maternal smoking and with recurrent otitis in two-year old children

50 **Bauchau V**, Durham S
A large scale study of the epidemiology of allergic rhinitis in Europe

51 **Arruda LK**, Silva JM, Camara AA, Cardoso MR, Arruda E, Chapman MD, Platts-Mills TAE, Ferriani VPL
Risk factors for persistent wheezing in young children in a subtropical environment

52 Satinover S, Reefer A, Custis N, Platts-Mills TAE, Arruda K, Pomes A, Chapman M, **Woodfolk J**
Co-Sensitization to cockroach and mite is not simply explained by tropomyosin cross-reactivity

53 **Buters JT**, Schober W, Lubitz S, Belloni B, Eberlein-König B, Grimm V, Behrendt H
Xenobiotic metabolism and IgE mediated hypersensitivity reactions

54 **Glovsky M**
Can Chinese elm pollen cause asthma?

106 **Israel E**
Genotype stratified prospective crossover trial of regularly scheduled albuterol treatment in asthma

Diagnostics

Moderators: R. Aalberse and E. Jensen-Jarolim

55 **Baron JM**, Ott H, Schröder C, Heise R, Krischer S, Heimann G, Merk HF
A novel allergen chip technique for the quantitative analysis of serum IgE antibodies

56 Schröder CM, **Ott H**, Mahler V, Erdmann S, Merk HF, Baron JM
Microarray-based IgE profiling in patients with latex allergy

57 Lilja G, Nordlund M, Andersson K, Ostling J, Lundgren T, Marknell DeWitt A, Vieths S, **Lidholm J**
Analysis of specific IgE to vegetable foods in a cohort of birch-pollen allergic children and adolescents using recombinant allergens

58 Shek LPC, Soderstrom L, **Ahlstedt S**, Beyer K, Sampson HA
In vitro methods for monitoring the development of clinical tolerance to foods

Infection and Allergy

Moderators: H. Renz and G. Gleich

59 **Grüber C**, Kakat S, Stapel S, Wahn U, Aalberse R, Nilsson L
Down-regulation of IgE to tetanus toxoid and diphtheria toxoid by covaccination with cellular (but not acellular) Bordetella pertussis vaccine in children

60 Arnold R, **König W**
Peroxisome proliferator-activated receptor gamma ligands possess antiviral and anti-inflammatory activity in the course of respiratory syncytial virus infection

61 **Herz U**, Bluemer N, Uthoff H, Spenner A, Renz H
Influence of nonpathological exposure to bacteria during pre- and early postnatal period protects allergy in murine neonates

62 **de Paulis A**, Prevete N, Fiorentino I, Walls AF, Curto M, Petraroli A, Castaldo V, Ceppa P, Fiocca R, Marone G
Basophils Infiltrate the Gastric Mucosa in Helicobacter pylori-Infected patients and respond chemotactically to H. pylori-derived peptide Hp(2-20)

63 **Rohde G**, Borg I, Arinir U, Wiethege A, Loeseke S, Bufe A, Schultze-Werninghaus G
Respiratory Viral Infection in Chronic Obstructive Pulmonary Disease

64 **Suzuki S**, Shimojo N, Kohno Y
Differences in Bifidobacterium species in early infancy and development of allergy

13.00 to 14.30 Lunch
Restaurant, Hotel Griffen

14.30 to 16.10 **Session 5**

Psychoneuroallergology

Moderators: J. Bienenstock and G. Marone

65 **Renz H**, Hahn C, Virchow C, Nockher WA
Neurotrophins in Allergic Bronchial Asthma: Modulators of Immunological and Neuronal Plasticity

66 **Busse W**, Jackson MJ, Crisafi G, Swenson C, Rosenkranz M, Johnstone I, Davidson R
Brain Responses in Asthma Analyzed by Imaging of the Nervous System (BRAIN Study)

67 **Fischer A**, Groneberg D, Braun A, Braun-Dullaeus R, Undem B
NGF and NT-4/5 cooperatively mediate allergen-induced sensory neuroplasticity in a guinea pig model of asthma

Schedule of Events

Thursday, 26 August (continued)

68 **Luger T**
Alpha melanocyte stimulating hormone and related peptides meditate immediate as well a delayed type hypersensitivity reactions

69 **Bonini Se**, Bonini St, Bracci Laudiero ML, Lambiase A, Levi-Schaffer F, Micera A, Procoli A, Rumi C, Aloe L
The modulatory role of nerve growth factor in allergic inflammation and tissue remodelling

16.15 to 17.00 Business Meeting

17.00 to 17.45 Relaxing Lecture

18.30 to 22.30 Hans Christian Andersen evening

Friday, 27 August

07.00 to 09.00 Breakfast
Breakfast Rooms at Hotel Fredensborg, Griffen and Hoffman

07.30 to 13.00 Registration Desk Open
Hotel Griffen Lobby

07.30 to 13.00 Speaker Ready Room Open
Room L, Hotel Griffen

08.30 to 10.30 **Session 6**

Clinical Allergy

Chairs: E. Simons and S. Durham

70 **Pichler WJ**, Depta J, Altznauer F
Direct drug interactions with T cell receptors

71 **de Weck AL**, Sanz ML, Gamboa PM
Hypersensitivity to NSAID drugs: a new integrated approach to its pathophysiological understanding and diagnosis

72 **Higashi N**, Higashi A, Mita H, Sakakibara H, Dahlen SE, Dahlen B, Akiyama K, Taniguchi M
Urinary LTE4 concentration after intravenous aspirin challenge – A new diagnostic approach for Aspirin-intolerant asthma (AIA)

73 **Novak N**, Bieber T
Plasmacytoid dendritic cells bearing the high affinity receptor for IgE in Atopic dermatitis

74 Shreffler WG, Beyer K, Chu THT, Burks AW, **Sampson HA**
In vitro methods for assessing the potential severity of food allergic reactions

75 **Ring J**, Plötz SG, Darsow U, Braun-Falco M, Simon HU, Behrendt H
Anti-Interleukin-5 in the treatment of hypereosinophilic skin diseases

09.00 to 13.00 Posters on Display
Poster Area, Hotel Griffen

11.00 to 12.40 **Session 7**

Eosinophils/Basophils/Macrophages

Chairs: J. Denburg and T. Tomioka

76 **Moqbel R**, Odemuyiwa SA, Ghahary A
Human eosinophils regulate T-cell functions through induction of indoleamine 2,3-dioxygenase

77 Woerly G, Dombrowicz D, **Capron M**
Human eosinophils express non-TLR receptors involved in innate immune responses

Schedule of Events

Friday, 27 August (contiued)

78 **Nagase H**, Okugawa S, Ota Y, Yamaguchi M, Matsushima K, Yamamoto K. Ohta K, Hirai K
Comparison of expression and function of toll-like receptors in eosinophils and neutrophils

79 MacGlashan DW, Sora R, Vonakis BM, Langdon JM, **MacDonald SM**
Signaling effects induced by human recombinant histamine releasing factor in basophils with the hyperreleasable phenotype

80 **Ono SJ**, Nakamura T, Ohbayashi M, Toda M, Hall D, Horgan C
A specific CCR3 chemokine receptor antagonist inhibits both early and late phase allergic inflammation

13.00 to 18.00 Boat Ride and Tour to the Christian's Island

Evening free – see optional program for proposals

Saturday, 28 August

07.00 to 09.00 Breakfast
Breakfast Rooms at Hotel
Fredensborg, Griffen and Hoffman

07.30 to 17.30 Registration Desk Open
Hotel Griffen Lobby

07.30 to 18.30 Speaker Ready Room Open
Room L, Hotel Griffen

08.30 to 10.30 **Session 8**

Immunotherapy

Chairs: P. Norman and D. Proud

81 **O'Hehir R**
A candidate vaccine for specific immunotherapy in latex allergy: hypoallergenic mutants of the major allergen Hev b 6.01

82 Jutel M, Jaeger L, Fiebig H, Suck, R Narkus A, **Cromwell O**
Specific immunotherapy with recombinant grass pollen allergens is clinically effective in the management of allergic rhinoconjunctivitis

83 Lenzo J, Jarnicki A, Holt P, **Thomas W**
Inhalation allergy and desensitisation to a cysteine protease allergen

84 **Gafvelin G**, S Thunberg S, Hans G, Kronkvist M, Akdis C, Cromwell O, Pauli G, Valenta R, van Hage-Hamsten M
Therapy-induced alterations of the allergic response through a novel allergy vaccine based on genetically modified allergens

85 **Larché M**, Alexander C, Verhoef A, Kay AB
T cell peptide therapy improves objective and subjective outcome measures in asthma and rhinitis

86 **Durham SR**, Wachholz P, Larsen JN, Aalberse, R, Till SJ
Inhibition of allergen-IgE binding to B cells following a successful grass pollen immunotherapy: influence of withdrawal following two years' treatment

87 **Liu A**, Domdey A, Millner A, Søndergaard LB, Würtzen PA
Identification of third markers on human CD4+CD25+ T regulatory cells from peripheral blood

09.00 to 18.00 Posters on Display
Poster Area, Hotel Griffen

11.00 to 13.00 **Poster Session 2**
Poster Area, Hotel Griffen

Schedule of Events

Saturday, 28 August (continued)

Allergy and the Skin

Moderator: H. Merk and A. Kapp

88 **Simon D**, Vassina E, Yousefi S, Braathen LR, Simon HU
Reduced dermal infiltration of cytokine-producing inflammatory cells in atopic dermatitis following short-term topical tacrolius treatment

89 **Schmid-Grendelmeier P**, Disch R, Blaser K, Crameri R
Human manganese superoxide dismutase - a stress-inducible enzyme - can elicit IgE- and T cell-mediated reactions in atopic dermatitis

90 **Withdrawn**

91 **Thomas P**, Summer B, Ghoreschi K, Barnstorf S, Roider G
Peripheral blood mononuclear cells of nickel-allergic and non-allergic individuals display different apoptotic threshold upon exposure to nickel and metal ions released by euro coins

92 **Zuberbier T**, Grunow K, Babina M, Guhl S, Giesen C, Welker P, Kalkbrenner F, Henz BM
Effects of cannabinoid agonists on histamine release from activated human skin mast cells

93 **Raap U**, Wedi B, Kapp A
Neuroimmunological Crosstalk in Atopic Dermatitis

94 **Darsow U**, Ripphoff E, Ring J
New methods for quantitative and qualitative assessment of itch

95 **Torres MJ**, Antúnez C, Mayorga C, Santamaría-Babi LF, Cornejo-García JA, Blanca M
Expression of two neuropeptide receptors (calcitonin gene related peptide and somatostatin) in peripheral blood mononuclear cells from patients with atopic dermatitis and non-atopic controls

96 **Santamaría-Babi LF**, Antúnez C, Torres MJ, Mayorga C, Cornejo-García JA, Blanca M
Neuropeptide influence on cytokine production in peripheral T cells in atopic dermatitis patients with acute and chronic lesions and in controls

Food Allergy

Moderators: B. Björkstén and T. Werfel

97 **Straumann A**, Kristl J, Conus S, Spichtin HP, Hans-Uwe Simon HU
Eosinophilic esophagitis: new clinical and pathophysiological insights

98 **Dirks CG**, Platzer MH, Pedersen MH, Jensen LB, Bindslev-Jensen C, Poulsen LK, Skov PS
Histamine release for determination of systemically absorbed allergenic proteins in humans

99 **Schöll I**, Untersmayr E, Bakos N, Boltz-Nitulescu G, Scheiner, O, Jensen-Jarolim E
Anti-acid medication promotes oral sensitization and hyperreactivity to hazelnut: experiments and preliminary epidemiology

100 **Swoboda I**, Bugajska-Schretter A, Verdino P, Keller W, Sperr WR, Valent P, Valenta R, Spitzauer S
From diagnosis to therapy of fish allergy

101 Fiedler EM, Lee H, Kuhn M, **Worm M**
Oral threshold levels and in vivo basophil activation in hazelnut allergic patients during oral provocation tests

102 **Untersmayr E**, Bakos N, Schöll I, Kundi M, Walter F, Szalai K, Riemer A, Boltz-Nitulescu G, Scheiner O, Jensen-Jarolim E
Anti-ulcer drugs promote IgE formation to dietary proteins: an epidemiological study

103 **Hoffmann-Sommergruber K**, Asero R, Bohle B, Bolhaar S, Breiteneder H, Fernandez-Rivas M, E Gonzalez-Mancebo E, Knulst A, Ma Y, van Ree R, Zuidmeer L
Apple allergy: different patient allergen recognition patterns across Europe - studied by the use of recombinant allergens

104 **Erdmann S**, Hoffmann-Sommergruber K, Schmidt A, Sauer I, Moll-Slodowy S, Scheiner O, Merk HF
Pollen associated food allergy: diagnosis by recombinant allergens and CD63 expression of basophils

Asthma

Moderators: B. Kay and G. Schultze-Werninghaus

44 **Mori A**, Taniguchi M, MaedaY, Hasegawa M, Mita H, Akiyama K
T cell IL-5 production to Candida albicans secretory aspartic proteinase 2 is related to IgE-independent late asthmatic response

105 Pizzichini MMM, Jayaram L, Cook R, Pizzichini E, Boulet LP, Lemiere C, Cartier A, **Hargreave FE**
Does sputum cell counts alter asthma exacerbations? The LOMA Study

107 Custovic A, Simpson A, **Soderstrom L**, Ahlstedt S
Probability of persistent wheeze and early childhood asthma increases with increasing specific IgE antibody levels

108 Gleeson M, **Clancy R**, Lemmert K
21 year longitudinal study of healthy infants - correlates with atopy, atopic disease and asthma (The RIFYL Study)

109 **Nakagawa T**, Hoshino M
Angiogenesis and remodeling of airway vasculature in asthma

Anaphylaxis and Drug Allergy

Moderators: W. Pichler and R. Schellenberg

110 **Blanca M**, Mayorga C, Torres MJ, Rodriguez-Bada JL, Antúnez C, Cornejo-García JA, Fernandez T, Rodriguez R, Moya MC, Romano A.
Immediate adverse reactions to cephalosporins. Study of in vitro crossreactivity by determining specific IgE to different betalactams

Schedule of Events

Saturday, 28 August (continued)

111 **Withdrawn**

112 **Simons FER**
Lack of availability of epinephrine for first-aid treatment of anaphylaxis worldwide

113 Ruëff F, Armin S, Gabriele W, **Bernhard P**
Indications of a systemic inflammatory response to Hymenoptera venom immunotherapy

Immunotherapy

Moderators: H. Löwenstein and R. O'Hehir

114 **Crameri R**
Modular antigen translocation molecules (MAT): a new concept for the development of efficient vaccines

115 **Domdey A,** Liu A, Millner A, Søndergaard I, Würtzen PA
Human regulatory T-cell-lines cultures maintained with CD3/CD28 expander beads

116 **Werfel T**, KBreuer K, Rueff F, Przybilla B, Worm M, Grewe M, Ruzicka T, Brehler R, Wolf H, Schnitker J, Kapp,
Allergen-specific immunotherapy is effective in atopic dermatitis

117 Darcan Y, Seitzer U, Galle J, Becker WM, Ahmed A, **Petersen A**
Allergy prophylaxis by DNA vaccination inhibits specific IgE response and lung pathologic parameters in a mouse model

118 **Larsen JN**, Aalberse RC, Ipsen H, Wachholz P, Durham SR
'Blocking IgG' induced by specific immunotherapy is reduced following treatment termination

119 Grönlund H, Kaiser L, Saarne T, Gafvelin G, **van Hage-Hamsten M**
Fel d 1 mutants - new candidates for treatment of cat allergy

120 **Ejrnaes AM**, Svenson M, Larsen JN, Jacobi HH
Inhibition of rBet v 1-induced histamine release from human basophils by specific immunotherapy-patient IgG is generally not mediated by Fc RIIB

121 **Bergman KC,** Wolf H, Schnitker J, Petermann F
Quality of life and compliance in patients allergic to grass and rye pollen during a three-years treatment with specific immunotherapy (the LQC study)

122 **Walter F**, Schöll I, Untersmayr E, Boltz-Nitulescu G, Scheiner, O, Gabor F, Jensen-Jarolim E
Development of an oral allergen immunotherapy targeting M-cells

13.00 to 14.30 Lunch
Restaurant, Hotel Griffen

14.30 to 15.05 **Paul Kallos Lecture**

Introduction: H. Löwenstein
Waldmann, H
Reprogramming the immune system

15.05 to 16.45 **Session 9**

Effector Cells II

Chairs: M. Capron and H. Saito

123 **Howarth P**, Susan W, Salib R
Epithelial immunoexpression of transforming growth factor-beta, eotaxin and stem cell factor in allergic rhinitis, and their relationship to epithelial mast cell accumulation in naturally occurring disease

124 **Marone G**, Petraroli A, Giannattasio G, Granata F, Forte V, Morabito P, Triggiani M
Phenotypical and functional characterization of distinct populations of human lung macrophages

125 **Simon HU**, Altznauer F, Martinelli S, Fey MF, Schoeni MH, Yousefi S
Inflammation-associated block of apoptosis by survivin in neutrophils

126 **Befus D**, Gibbings D
Two forms of human CD8: possible implication of a novel 52 kDa form of CD8 on myeloid cells in allergic disease

Evening free – see optional program for proposals

Schedule of Events

Sunday, 29 August

07.00 to 09.00 Breakfast
Breakfast Rooms at Hotel Fredensborg, Griffen and Hoffman

07.30 to 17.30 Registration Desk Open
Hotel Griffen Lobby

07.00 to 08.15 CIA Council Meeting
Hotel Fredensborg

07.30 to 18.30 Speaker Ready Room Open
Room L, Hotel Griffen

08.30 to 10.10 **Session 10**

Innate Immunity

Chairs: P. Holt and L. Poulsen

127 **Björkstén B**, Julge K, Vasar M, Voor T, Böttcher M
Development of immune responses to allergens and clinical allergy on relation to microbial stimulati

128 Akkoc T, Sancak R, Yesil O, Yazi D, Bahceciler, **Barlan I**
Pretreatment with M. vaccae as an adjuvant along with intranasal allergen induced IL-10 but not IFN-gamma

129 **Breuer K**, Wittmann M, Kohlrautz V, Kempe K, Kapp A, Mai U, Dittrich-Breiholtz O, Kracht M, Werfel T
Alpha-toxin induces proliferation and secretion of IFN-y in T cells of atopic donors

130 **Foster P**
Intrinsic defect in T cell production of interleukin (IL) 13 in the absence of both IL-5 and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma

131 **Withdrawn**

09.00 to 18.00 Posters on Display
Poster Area, Hotel Griffen

10.40 to 12.20 **Session 11**

Asthma

Chairs: F. Adkinson and T. Nakagawa

132 **Kay AB**
Asthma provoked by inhalation of T cell peptides

133 **Vliagoftis H**, Ebeling C, Hollenberg M
Protease-activated receptor-2 enhances allergen-induced airway inflammation and hyperresponsiveness

134 Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Rückert B, Mantel PY, Menz G, Akdis CA, Blaser K, **Schmidt-Weber CB**
Glucocorticoids promote T regulatory cells in asthma and in vitro by increasing FOXP3 expression

135 **Wenzel SE**, Balzar S, Chu HW, Cundall M
The phenotype of steroid dependent severe asthma is not associated with increased lung levels of TH-2 cytokines

136 **Dahlén SE**, Sundström E, Låstbom L, Ryrfeldt Å
The three mediator paradigm in bronchoconstriction

12.20 to 13.00 **Carl Prausnitz Lecture**

Introduction: J. Ring
S. Prusiner

13.00 to 14.30 Lunch
Restaurant, Hotel Griffen

14.30 to 15.50 **Session 12**
General Session, Hotel Griffen

Effector Cells III

Chairs: J. Rivera and W. Schmutzler

137 Solarewicz K, **Jutel M**, Verhagen J, Akdis M, Blaser K, Akdis CA
T-cells and eosinophils in bronchial smooth muscle cell apoptosis

138 **Stadler BM**, Müller L, Marti P, Vogel M, Stadler MB
Autoimmunity and allergy: a bioinformatic approach

139 **Levi-Schaffer** F, Munitz A, Bachelet I
Expression and function of inhibitory and activatory receptors on mast cells and eosinophils: new mechanisms in mast cell-eosinophil cross-talk

140 **Razin E**
Regulation of MITF transcriptional activity in mast cell by two endogenous suppressors

15.50 to 16.00 Closing Remarks
J. Ring, H. Löwenstein

18.00 to 22.00 Banquet
Hotel Fredensborg

Monday, 30 August

07.00 to 09.00 Breakfast
Breakfast Rooms at Hotel Fredensborg, Griffen and Hoffman

Optional Tours

Wednesday 25 August
10.30 to 14.00
Theme: Bornholm arts and crafts
Morning program – The Art Museum of Bornholm
The craftspeople of Bornholm exhibit at The Art Museum of Bornholm.
Guided by the museum Curator, Lars Kærulf, combined with multi media communications. The craftspeople will be present for further information and purchase of items will be possible. A light lunch at The Art Museum of Bornholm is included.
Price per person: $46.00 USD.
A minimum of 30 people is required.
The price includes return transport by coach from your hotel.
The price includes a refund from your hotel lunch.

Thursday 26 August
10.30 to 14.00 – Theme: Bornholm stories and tales
The Medieval Centre of Bornholm
Stories of the people of the Middle Ages, staged at the Medieval Centre of Bornholm. A Medieval lunch will be served with local Bornholm brew at the Nobleman's Farm
Price per person: $40.25 USD.
A minimum of 40 people is required.
The price includes return transport by coach from your hotel.
The price includes a refund from your hotel lunch.

Saturday 28 August
Theme: Discover Bornholm
15.00 to 18.00 Discover Bornholm
The power, the arts, the faith and the play make up the four essential human driving forces for the evolution of man. The culture of and nature on Bornholm make up unique possibilities to experience and explore the development in the island's humanistic Lifeworld – through time. Toolboxx.dk takes the participants by the hand, and lead them into the Bornholm experience-universe. We follow the tracks of culture in nature, and together explore the codes of understanding human invention and creativity.
The participants can choose one of four tracks that all end and meet by Hammershus:

A. The power
- Is explored starting from the woods of Almindingen – where you are taken by horse-drawn carriages from Lilleborg and Gammelborg (early castle ruins) in Almindingen to Hammershus on Hammeren.
Transport: Horse-drawn carriage.

B. The arts
- Is explored starting from Helligdomsklipperne (The Sacred Cliffs) – where you are sailed around the northern tip of Bornholm, passing Hammeren and Hammershus to Hammerhavn.
Transport: Speedboat

C. The faith
- Is explored starting from the Round churches – tales of the monks, the knights, and the church.
Transport: Vintage bus.

D. The play
- Is explored starting from the beach of Dueodde – kite flying and beach volley.
Transport: Coach
Price: Track A, B and C (each): $46.00 USD per person
Track D: $34.50 USD per person
A minimum of 25 people per track is required.
The price includes refreshments and return transport from your hotel.

Saturday 28 August
Theme: The art and music of Bornholm
18.30 to 21.30 – The Art Museum of Bornholm
The art and music of Bornholm at The Art Museum of Bornholm.
Guided by Lars Kærulf, the museum Curator, combined with multi-media communications. Meet the craftspeople and designers and purchase or order their work of art and design. There will be impressive Choral music in the unique architectural rooms of the museum – art and music in symbiosis.
A light dinner will be served at the Art Museum of Bornholm.
Price: $86.25 per person.
A minimum of 40 people is required.
The price includes return transport by coach from your hotel.

Saturday 28 August
18.30 to 21.30 – Optional tour to Hammershus
At Hammershus the group will have picnic with different delicacies and wine from Bornholm. After the picnic, the twilight tour at the ruins will begin. Guides will take teams around the ruins and tell the existing and fascinating story spiced with funny anecdotes.
Price: $69.00 USD per person

Individual tours in the Bornholm nature and culture
We recommend that the participants themselves experience the Bornholm nature and culture. Special 2-3-hour tours will be offered, where the participants in smaller groups will experience the woods, the beach, the water and the creative people of Bornholm. Special recommendations will be available.
The means of transport will be mini-coaches or bikes and the tours can be booked at your hotel reception no later than 12 Noon the day prior to the tour.
Friday and Saturday 27 and 28 August
Recommended restaurants
Gastronomen, Sandvig
Restaurant Le Port, Vang
Restaurant Bokulhus, Gudhjem
Restaurant Andi's, Gudhjem
Melsted Badehotel, Melsted by Gudhjem
Brasseri Truberg, Nexø
Christianshøjkroen, Almindingen
Restaurant Di 5 Stâuerna, Radisson SAS Fredensborg Hotel, Rønne

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Abstracts Listing

1

ALLERGY IS IN THE BLOOD AT BIRTH

Judah A Denburg[1], Holly Hatfield[1], Michael Cyr[1], Jan Dunstan[2], Patrick Holt[3], Susan Prescott[2]
[1]Medicine, McMaster University, Hamilton, ON, Canada; [2]Paediatrics, University of Western Australia, Perth, Australia; [3]Cell Biology, Telethon Institute for Child Health Research, Perth, Australia

Alterations in eosinophil-basophil (Eo-B) progenitor numbers and phenotype in blood, marrow and tissue parallel allergic disease expression, indicating the contribution of processes to allergic tissue inflammation. We have examined whether or not alterations in $CD34^+$ cord blood (CB) progenitors at birth might relate to the development of the allergic diathesis, based on a previously demonstrated association of a *reduction* in $CD34/45^+$ cell expression of GM-CSFRα with increased risk for atopy (Upham et al, J Allergy Clin Immunol 104:370, 1999).

We have now further investigated whether phenotypic switching of CB progenitors towards the Eo-B pathway predicts atopy in early infancy. First, we showed an *inverse* correlation between maternal skin prick test responses to common allergens, and IL-5R∀/GM-CSFR∀ expression on CB $CD34^+$ cells at birth. We next performed CB analyses of samples taken at University of Western Australia in a double-blind study of atopic, pregnant women randomized to supplemental fish oil (PUFA, n=40) or olive oil (n=43) from 20 weeks gestation until delivery.

At birth, CB mononuclear cells were isolated and analyzed by flow cytometry for $CD34^+$ cell expression of cytokine receptors IL-5Rα, IL-3Rα, GM-CSFRα, CXCR4 and CCR3. CB Eo/B colony-forming cells (CFU) in methylcellulose assays were also enumerated.Percentages of CB $CD34^+$ cells were found to be higher after PUFA than placebo ($p<0.003$). Co-expression of cytokine or chemokine receptors on CD34 cells was not altered by PUFA supplementation. However, there were significantly more IL-5 responsive, but not IL-3 or GM-CSF responsive, CB Eo/B-CFU in the PUFA group compared to the control group ($p<0.03$), correlating with clinical outcomes at one year:

- The number of IL-5 responsive Eo/B-CFU and the % $CD34^+$ cells positively predicted *atopic dermatitis* at age 12 months.
- The number of IL-5 responsive Eo/B-CFU positively predicted *wheeze* at 1 year.

Thus, dietary n-3 omega PUFA supplementation during pregnancy in atopic mothers alters infant cord blood hemopoietic progenitors and their cytokine responsiveness. This supports the concept that *dysmature hemopoietic processes at birth may play a key role in the subsequent development of atopy*. Targeting these processes may be helpful in the prevention and treatment of allergic disease.

2

INTERLEUKIN-4/13 INDUCED ACTIVATION OF SIGNAL TRANSDUCER AND ENHANCER OF TRANSCRIPTION (STAT) 6 IS REGULATED BY SUPPRESSORS OF CYTOKINE SIGNALING (SOCS)

Daniel Hebenstreit, Petra Luft, Angela Schmiedlechner, Jutta Horejs-Hoeck and Albert Duschl
Institute of Chemistry and Biochemistry, University of Salzburg, Austria

Cytokine receptors signal through Jak/STAT pathways, which appear to be quite straightforward compared with other signaling chains. Furthermore, the expression of receptor chains, Jak kinases or STAT factors is usually constitutive. Despite of that, STAT proteins show remarkable flexibility in inducing or suppressing different genes in various cell types. One layer of complexity could by added by SOCS proteins, which inhibit Jak/STAT signaling. We have studied expression of the chemokine Eotaxin-3/CCL26, which is involved in attracting eosinophils to sites of allergic inflammation. IL-4/IL-13-induced activation of the eotaxin-3/CCL26 gene in human dermal fibroblasts was shown to be a STAT6-dependent process mediated by a single STAT6 binding motif located upstream of the transcription initiation site. Transfection of SOCS-1 and SOCS-3 but not SOCS-2 expression vectors inhibited IL-4/IL-13-induced secretion of eotaxin-3/CCL26 in the human embryonic kidney cell line HEK293. Further, using eotaxin-3/CCL26 promoter reporter gene constructs, we observed that IL-4 and IL-13 induced luciferase activity was strongly reduced upon cotransfection of SOCS-1 and SOCS-3 but not SOCS-2 expression vectors. Nuclear extracts of IL-4/IL-13 induced cells transfected with SOCS-1 or SOCS-3 did not form complexes with oligonucleotide probes corresponding to the STAT6 binding site in the eotaxin-3/CCL26 promoter in EMSA studies. In contrast, complex formation upon SOCS-2-transfection was comparable to mock-transfected cells. The levels of phosphorylated STAT6 in IL-4 and IL-13 treated cells were markedly reduced when the cells had been transfected with SOCS-1 or SOCS-3, confirming the role of these negative regulators for the IL-4 and IL-13 induced activation of eotaxin-3/CCL26 gene expression. We have recently reported that the SOCS-1 gene is itself regulated via three adjacent STAT6 binding sites in its promoter, which indicates that STAT6 and SOCS-1 can suppress each other via a negative feedback loops. Strategies for signal modification like this one may explain some of the complexities observed in Jak/STAT dependent gene regulation by cytokines.

3

Identification and characterization of the IL-13–inducing genes in bronchial epithelial cells

Kenji Izuhara
Department of Biomolecular Sciences
Saga Medical School

Bronchial asthma is a complex disease characterized by airway inflammation involving a Th2-cytokine, IL-13. A substantial body of evidence has accumulated pointing to the pivotal role of IL-13 in the pathogenesis of bronchial asthma, based on the analyses of mouse models, expression profiling in the bronchial lesions, and genetic association of single nucleotide polymorphisms. However, it has not been elucidated how IL-13 induces the asthmatic phenotypes. To clarify this point, we have applied microarray analyses to human bronchial epithelial cultures to search genes regulated by IL-13. Consequently, expression of squamous cell carcinoma antigen-1 (SCCA1) and SCCA2, the cysteine and serine protease inhibitors, respectively, was the highest in the bronchial epithelial cells stimulated by IL-4 and IL-13, and was augmented in the asthmatic cDNA library. Furthermore, serum levels of SCCA were also elevated in asthmatic patients. It has been shown that these two molecules have distinct properties; SCCA1 inhibits cysteine proteinases such as cathepsin K, L, and S, whereas SCCA2 inhibits serine proteinases such as cathepsin G and mast cell chymase, in spite of the close homology between SCCA1 and SCCA2. Although several intrinsic target proteinases for SCCA1 and SCCA2 have been found, the biological roles of SCCA1 and SCCA2 still remain obscure. A mite allergen, Der p 1 is one of the most immunodominant allergens, and also acts as a cysteine proteinase probably correlated with the pathogenesis of allergic diseases. We explored the possibility that SCCA proteins target Der p 1, and it turned out that SCCA2, but not SCCA1, inhibited the catalytic activities of Der p 1. These results suggest that SCCA2 acts as a cross-class serpin targeting an extrinsic cysteine proteinase derived from house dust mites, and that it may have a protective role against mite-causing biological reactions. Furthermore, SCCA2 itself or a low-molecular compound mimicking the structure of SCCA2 may be applied to a therapeutic reagent for treatment allergic diseases caused by mite allergens.

4

Th1-mediated inflammation as a pathogenic factor in allergic disease

Patrick G. Holt
Division of Cell Biology, Telethon Institute for Child Health Research and Centre for Child Health Research, The University of Western Australia, Perth, Western Australia

Our earlier description of sluggish postnatal development of Th1 function in $CD4^+$ T-cells as a determinant of genetic risk for atopy has been confirmed in subsequent studies. More recent evidence from our group suggests that this "window" period of attenuated Th1 function in high risk (HR) children is restricted to infancy, during which diminished negative feedback via IFNγ may contribute to preferential development of Th2-polarised memory against allergens. We have also recently shown that beyond infancy, Th1 maturation frequently accelerates in atopics resulting in expression of high levels of Th1 immunity including within allergen-specific Th-memory. Additionally, recent studies have suggested a contribution from Th1 cytokines in allergy-associated tissue inflammation. However small sample size coupled with the variability of Th-cell responses amongst genetically heterogenous study

populations have limited the capacity to draw firm conclusions regarding the precise role of Th1 cytokines.

We are seeking to elucidate this issue via two prospective cohort studies spanning the period birth-high school, employing study populations of n~200, providing adequate statistical power for multivariate modelling of associations between immunological parameters (including Th1/Th2 cytokines) and clinical phenotypes. The first study comprises a cohort of HR children in whom atopic outcomes at 2 years have been related to allergen-specific and polyclonal cytokine response patterns in peripheral blood mononuclear cells (PBMC) collected between birth and outcome age. The second study focuses upon a cohort sampled at 11 years, and comprised detailed analysis of immune response parameters in relation to current asthma/allergy phenotypes.

In the infant cohort, regression modelling identified PHA-induced ***IFNγ production by CD8$^+$ T-cells*** as the strongest independent predictor of atopy ($p=0.007$). In the cohort of 11 year olds, regression analyses identified IL-5 and/or eosinophilia as the factors most strongly associated with SPT wheal size ($p=0.000$) and with BHR ($p<0.001$), in each case ***in independent association with IFNγ reactivity*** ($p=0.003$ and $p=0.023$). Taken together with recent smaller published studies, these findings suggest that beyond the period of allergen-specific Th-memory priming during which Th1 cytokines are atopy-antagonistic, Th1 cytokine production (including from CD8$^+$ T-cells) may synergise with Th2 cytokines to drive atopy pathogenesis.

5

Ongoing Th1 vs Th2 chemokine production is differentially regulated by IL-4 vs IL-13

Kent HayGlass, Monique Stinson, Estelle Simons
Depts of Immunology and Pediatrics/Child Health, University of Manitoba, Winnipeg, Canada

Chemokine production and responsiveness play multiple roles in ongoing human immediate hypersensitivity diseases and in maintenance of immune responses responsible for clinical tolerance. Here, we investigate the inter-relationships between differential chemokine and cytokine production in young adults with (i) seasonal allergic rhinitis, (ii) mild to moderate asthma associated with grass pollen sensitivity and in (iii) non-atopic controls. Given that atopic and non-atopic humans exhibit differential responsiveness to chemokine (ie CXCR3 ligand selective enhancement of IFNγ production, FASEB J. 2004), we hypothesized that these populations exhibit differential production and regulation of *allergen*-driven chemokine responses. Type1 (~Th1, CXCR3 family) vs Type 2 (CCR4 family) responses were quantified in vivo via cross-sectional and longitudinal analysis of plasma and in vitro upon re-stimulation with allergen. Both type 1 and type 2 immunity associated chemokine responses were readily identified and quantified, typically exhibiting ng/ml levels. CXCL10 levels were indistinguishable in control, atopic and asthmatic groups under all conditions tested. In marked contrast, allergen-dependent CCL17 (TARC) responses were elevated in atopic populations ($p<0.005$), with the strongest recall responses evident among asthmatics. All were allergen-driven, CD4 T cell dependent, and blocked by anti-CD80/86 or CTLA4-Ig. CCL17 responses were markedly enhanced by addition of rIL-13, but not rIL-4, at levels that are characteristic of those seen in Ag responses. Similarly, CCL17 was markedly inhibited by anti-IL-13, but not anti-IL-4.
We conclude that (i) differential chemokine production is readily quantified at ng/ml levels (cf. pg/ml range of classical Th2 cytokines), demonstrating their enhanced utility for sensitive longitudinal analysis of changes in immune capacity (ii) Ag-specific recall chemokine responses exhibit characteristics associated with T cell dependent activation, (iii) seasonal grass pollen exposure results in markedly elevated type 2 chemokine production in atopic adults, particularly those with asthma, (iv) type 2 chemokine production characteristic of well established responses is highly susceptible to regulation by IL-13 but only marginally by IL-4, and (v) these alterations in type 2 chemokine responses are independent of detectable changes in the CXCR3 chemokines studied. Support: CIHR, Canada Research Chairs.

6

Healthy or allergic immune response characterized by fine balance between specific T regulatory 1 and T helper 2 cells

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Immune response to nonpathogenic environmental antigens and molecular mechanisms, which lead to either allergy or normal immunity appears as a crucial question. The regulation of normal or allergic immune response to allergens is still poorly understood, and the mechanism of specific immunotherapy (SIT), normalizing the allergic response to such allergens is slowly being elucidated. We investigated the immunoregulatory mechanism of both normal and allergic responses to the major house dust mite (HDM) and birch pollen allergens – Der p 1 and Bet v 1 respectively, as well as the immunologic basis of SIT to HDM in rhinitis and asthma patients. Single allergen-specific T cells constitute less than 0.1 % of the whole CD4$^+$ T cell repertoire and can be isolated from peripheral blood of humans according to their cytokine profile. Freshly purified IFN-γ-, IL-4- and IL-10-producing allergen-specific CD4$^+$ T cells display characteristics of Th1-, Th2- and T regulatory 1 (Tr1)-like cells, respectively. Although in different proportions, healthy and allergic individuals exhibit all three allergen-specific subsets, suggesting that a change in dominant subset may lead to allergy development or recovery. In healthy individuals, Tr1 cells consistently represent the dominant subset against common environmental allergens, such as Der p 1, Bet v 1, Cor a 1, Pyr c 5, Phl p 2, Api m 1 and Api m 2, which *in vivo* expands after high dose allergen exposure. In contrast, allergen-specific IL-4-secreting T cell frequency is high in allergic individuals. Tr1 cells suppress Th2 cells by using multiple suppressive mechanisms, including IL-10 and TGF-β as secreted cytokines and CTLA-4 and PD-1 as surface molecules. In addition, SIT induced increased specific suppressive activity in the whole CD4$^+$ CD25$^+$ T cells in allergic individuals. Neutralization of cytokine activity showed that T cell suppression was mainly mediated by IL-10 and TGF-β during SIT and in normal immunity to mucosal allergens. Together these results demonstrate a deviation to a regulatory/suppressor T cell response during SIT and the decisive role of the fine balance between allergen-specific Tr1 cells and Th2 cells in the generation of a healthy or an allergic immune response.

7

The IgE ab response to cat allergen is decreased in titer as well as prevalence: use of Streptavidin CAP to measure IgE ab to Der p 1, Der p 2, and Fel d 1

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Although the presence of a cat in the home has been associated with decreased prevalence of sensitization to cat dander allergens, the explanation of this phenomenon is not clear. In particular the effect of cat exposure on the response to other allergens is not well defined. The present study was carried out in New Zealand where houses have high dust mite allergens [>20µg Der p 1/g dust] and 60% of the houses have a cat. Sera from 224 children enrolled in an ISAAC study were tested for IgE ab to mite and cat using Pharmacia CAP. The IgE ab response to cat was strikingly decreased compared to dust mite. This was apparent from: the lower overall prevalence of IgE ab to cat (41/224 vs. 99/224); decreased prevalence of IgE ab to cat among children who lived in a house with a cat [20/141 vs. 21/83; $p<0.05$]; and the lower mean titer of IgE ab to cat [1.8 IU/ml compared to 18 IU/ml for mite ($p<0.001$)]. To further investigate the results on titre of IgE ab, allergens were biotinylated and bound to Streptavidin CAP provided by Pharmacia. Assays on 210 sera demonstrated that in keeping with the results for allergen extract the titer of IgE ab to Fel d 1 was tenfold lower than that of Der p 1 or Der p 2. Thirty-eight sera had IgE ab ≥ 17.5 IU/ml for Der p 1 while only 4 had IgE ab ≥ 17.5 IU/ml to Fel d 1 ($p<0.001$). Comparable results for those shown here for Fel d 1 have recently been found for IgE ab for Can f 1 from dogs. Our results establish that the IgE ab response to cat allergens is not only lower in prevalence but that it is also tenfold lower in titer than IgE ab to mite. In addition our results show no effect of cat ownership on the response to dust mite and no relevant differences in endotoxin exposure. The results support a model where cat allergen induces “control” of the immune response in both “tolerant” and allergic children.

8

Lung Function at Age 3 Years: Effect of Pet Ownership and Exposure to Indoor Allergens

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Background: We investigated the effect of pet ownership and exposure to indoor allergens on lung function in 3 year-old children.

Methods: Children were followed from birth to age 3 years, when skin-prick testing and lung function (specific airway resistance-sR_{aw} by whole body plethysmography) were performed. We measured allergen levels in dust collected from homes, and defined high exposure as Der p 1>2μg/g in mattress, Can f 1>10μg/g and Fel d 1>8μg/g in the lounge floor.

Results: There was no effect of cat or dog ownership at birth or age 3 years on lung function, and no association between lung function and mite, cat or dog allergen exposure. Sensitized children exposed to high levels of sensitizing allergen had significantly poorer lung function (n=49, sR_{aw} kPa/s; GM [95% CI] 1.20 [1.13-1.28]) than children who were not sensitized and not exposed (n=114; 1.08 [1.04-1.12]), not sensitized, but exposed (n=282; 1.07 [1.05-1.10]), or sensitized and not exposed (n=53; 1.12 [1.06-1.18], p=0.005). In a multivariate model, independent significant associates of lung function were maternal and paternal asthma, and the combination of sensitization and exposure to sensitizing allergen, with significant interaction between them. Lung function was substantially worse in sensitized and highly exposed children with both asthmatic parents (2.23 [1.68-2.97]), compared to those with neither (1.09 [1.04-1.16]) or just one of these features.

Conclusions: Pet ownership, sensitization without exposure or exposure in non-sensitized individual have no effect on lung function. However, the combination of specific sensitization and exposure to sensitizing allergen is associated with significantly poorer lung function in early life.

9

Tuning the immune response by allergen carriers: Th2-polarization induced by pollen associated lipid mediators (PALMs)

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The immune response of atopic individuals against allergens is characterized by an increased release of Th2 cytokines by allergen-specific T-helper cells. However, the way in which the Th cell cytokine profile is matched to the type of invading allergen, and why these profiles sometimes derail and lead to disease, is not well understood. We recently demonstrated that pollen grains not only function as allergen carriers but are also a rich source of bioactive lipid mediators stimulating and attracting cells of the innate immune system. In this study we show that soluble factors from pollen (APE, aqueous pollen extracts) modulate dendritic cell function that results in an altered T cell polarization. APE dose-dependently inhibited the LPS induced IL-12 production of monocyte derived dendritic cells, while IL-6 release remained unchanged. The ensuing T-cell response of heterologous naïve T-cells stimulated by dendritic cells (DC) matured in the presence of APE resulted in a dramatic shift from a Th1 to a Th2 phenotype as compared to the response obtained with DC matured in the presence of LPS alone. Furthermore, APE induced a significant downregulation of LPS induced release of Th1 chemokines such as RANTES and IP10 suggesting a reduced attraction of Th1 T cells by APE-matured DCs. The chemical analysis of APE revealed the presence of the recently described dinor isoprostanes, the phytoprostanes, derived from linolenic acid. We identified phytoprostane E1, F1, B1 in nanomolar concentrations. From the wide array of phytoprostane identified in APE the cycopentenones PPJ1 type 1 and type 2 and PPE1 exhibited the most prominent effect on the LPS induced IL12 reduction and Th2 switch. Signaltransduction studies revealed that PPARg is involved in the mechanisms induced by APE. Studies are underway to dissect the signal transduction of these lipid mediators. In summary, our results demonstrate that pollen associated lipid mediators (PALMs) act as important regulatory signals that modulate DC function in a fashion that favors Th2 polarization.
CTH and TJ contributed equally.

10

The molecular characteristics of allergens and food allergens, or what makes an antigen an allergen?

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The question whether allergens have common features is longstanding and it touches their potency to sensitize as well as their capacity to elicit an allergic response. 1.) Sensitization phase: We suggest that conformational epitopes are the first critical prerequisites for IgE binding of allergens. Mimotope technology identified IgE epitopes of the discontinuous, conformational type on Bet v 1 from birch and Phl p 5 from grass pollen. Only after persistence of an ongoing allergy, but not in the first instance, also linear IgE epitopes become evident. The conformational epitopes are not only responsible in the effector phase, but they are obviously also prerequisites in sensitization. In a murine model of food allergy we demonstrate that dietary allergens as e.g. fish parvalbumin, harbour sensitization capacity only when their structure is preserved. This is of impact, as many food proteins (and food allergens) are digestion-labile and do not act as allergens when they are regularly digested. Anti-acidic conditions in the stomach, therfore, turn harmless proteins into potential allergens. 2.) Effector phase: To explain their potency for IgE crosslinking, allergens are routinely illustrated as multimeric molecules, offering a panel of epitopes for interaction with IgE. However, taking Bet v 1, the major birch pollen allergen, as a model, only one IgE-epitope could be revealed by mimotope technology. There are accumulating reports on dimeric allergens. Indeed, in dynamic light scattering analysis Bet v 1 formed dimers and even multimers at a wide pH range (pH 1.2 – 8.2), and behaved monomeric only in the presence of glycerol. *In vivo*, dimeric Bet v 1 was capable to trigger histamine release in the skin of allergic mice, whereas monomeric Bet v 1 was not. Taken together, we suggest that conformation is a requirement for sensitization and IgE induction, at least by the oral route. Moreover, small allergens as Bet v 1 may crosslink IgE, because they naturally form homodimers or homooligomers.

11

Fel d 1, the major allergen in cat; from structure to clinical application

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The domestic cat (*Felis domesticus*) is among the most common and potent sources of allergens, causing allergic disease on a worldwide basis. Crude cat dander extracts, composed of several allergenic and non-allergenic molecules, are used in clinical practise for diagnostics and specific immunotherapy. For biological reasons, the variation of individual allergens and antigens are difficult to assess in given extract batches. Therefore single allergens have been produced and isolated by recombinant techniques and tested as candidates to replace allergen extracts. Previously we have solved the monomer structure of Fel d 1 using a fusion molecule of chain 2 and 1 (2+1). In this study we present the overall structure of Fel d 1, including the site of dimerization and the analysis of IgE responses in serum from children and adults allergic to cat.
The novel rFel d 1 fusion constructs were produced by a set of overlapping oligonucleotides using PCR and expressed in *E coli*, chain 1 N-terminal of chain 2, (1+2). Crystals were grown in 22.5 % PEG3350, 0.5 M CaCl and diffraction data were collected to solve the 3-D structure of the Fel d 1 tetramer to 1.65 Å resolution.

Ninety-nine clinically well documented cat allergic patients were enrolled in the study. Serum was collected from 68 children (range; 2-17 years, mean age; 10.7 years) and 31 adults. Age-matched control groups, 50 atopic non-cat dander sensitized children and 25 allergic, not cat sensitized adults, were analysed for presence of specific IgE antibodies to rFel d 1 by ELISA and cat dander extract by CAP (Pharmacia Diagnostics).
IgE responses to Fel d 1(2+1) detected specific IgE in all cat allergic patients and in none of the control groups, displaying a sensitivity and a specificity of 100%. In comparison, the cat extract based CAP test demonstrated 93% sensitivity. Comparison with the extract CAP and rFel d 1 showed a correlation of r=0.86
Specific IgE measurements in serum from cat allergic children using rFel d 1 is a promising marker for accurate diagnostics and the data suggest rFel d 1 to be considered as a candidate for specific immunotherapy.

12

Mast cells, and mast cell-dependent effects on tissue gene expression, contribute to multiple features of a chronic asthma model in mice

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The importance of mast cells (MCs) in the development of many of the inflammatory, patho-physiological and chronic tissue changes in asthma is controversial. To elucidate the importance of mast cells in regulating patterns of local gene expression in chronic asthma, and the expression of several important phenotypic characteristics of the disorder, mast cell-deficient WBB6F1- Kit^{W}/Kit^{W-v} (W/W^{v}) mice, the wild type WBB6F1- +/+ (+/+) mice, and W/W^{v} mice that had been reconstituted with bone marrow-derived cultured MCs (BMCMCs) of +/+ origin were sensitized (i.p.) and then repeatedly challenged (i.n.) with ovalbumin (OVA) in the absence of artificial adjuvant. We found that OVA sensitization/challenge significantly increased the numbers of mast cells in the lungs of +/+ and +/+ BMCMC-reconstituted W/W^{v} mice, compared with PBS controls. We also found that mast cells can significantly enhance the expression of antigen-induced acute bronchoconstriction, lung inflammation, airway hyper-responsiveness to methacholine, mucus gland hyperplasia and mucin hypersecretion, subepithelial and total lung collagen deposition, and hyperplasia/hypertrophy of bronchial smooth muscle. In addition, cDNA microarray analysis revealed a pattern of antigen-induced and MC-dependent gene expression in the lung, which included effects on genes that are known to be associated with the development of some of the key features of asthma that are listed above. These results show that, in this model of chronic, antigen-induced asthma in mice, mast cells have a critical role in influencing patterns of antigen-induced gene expression in the lung, as well as in contributing significantly to several important acute, late phase and chronic manifestations of the disorder.

13

Ischemia-reperfusion injury of skeletal muscle is mediated by mouse mast cell protease 5

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Ischemia-reperfusion injury is a significant problem in physical and surgical trauma, myocardial infarction, and organ transplantation. The finding that the hypoxic changes in muscle viability and permeability barriers are attenuated in mast cell-deficient W/Wv mice relative to wild-type mice implicated a role for this immune cell in the pathological response. Analysis of injured BALB/c and C57BL/6 mice revealed the extent of injury was strongly correlated with level of mast cell degranulation in these mast cell-sufficient strains. Thus, transgenic mice unable to express varied mast cell mediators were evaluated to identify the relevant factor. Mouse mast cell protease 5 (mMCP-5) is the major serine protease stored in the secretory granules of skeletal muscle mast cells, and it is the protease that is most similar to human mast cell chymase. Here we report that mMCP-5 plays an essential role in hindlimb ischemia-reperfusion injury. We also show that the classical complement pathway, in concert with mMCP-5, plays an additional key role in muscle damage, but not via the MC-regulatory anaphylatoxins C3a or C5a.

14

Activation of SPHK1 in mast cells is mediated by tyrosine kinase Lyn

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Lately, Sphingosine kinase (SPHK) has been recognized as an essential signaling molecule that mediates the intracellular conversion of sphingosine to sphingosine-1-phosphate. In mast cells, induction of sphingosine kinase and generation of sphingosine-1-phosphate have been originally linked to the initial rise in Ca^{2+}, released from internal stores, and to the degranulation reaction. These events either precede or are concomitant with the activation of PLCγ and the generation of inositol trisphosphate. These findings positioned the activation of sphingosine kinase relatively proximal to the FcεRI and sphingosine-1-phosphate as an alternatively / additionally (to inositol trisphosphate) used lipid mediator in the Ca^{2+} activation.

Here we show that sphingosine kinase type 1, interacts directly with the tyrosine kinase Lyn and that this interaction leads to a recruitment of this lipid kinase to the high-affinity receptor for immunoglobulin E (FcεRI) after stimulation. Furthermore, we provide evidence that a complex of both molecules features enhanced lipid - and tyrosine kinase activity. The *in vivo* relevance of these findings is demonstrated by the fact that 5 min after FcεRI triggering, enhanced sphingosine kinase activity is associated with the γ-chain in sphingolipid-enriched rafts of mast cells and that bone marrow-derived mast cells from Lyn knock-out mice, as compared to syngeneic wild type cells, are defective in the initial induction of sphingosine kinase activity.

15

Comparative database for the orthologous genes expressed by various human and mouse inflammatory cell types

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Animal disease models have been used as surrogates for human and have been informative. The use of mouse models for diseases related to allergy and immunology has increased because of the rapidly developing technologies to selectively knockout genes contributing to the pathogenesis of the disease. Regarding the relevance of these models of allergic diseases such as asthma, however, controversy still exists. Clinical trials sometimes fail due to the fact that the results obtained in animal studies cannot be reproduced in human. Recently, many human and mouse orthologous genes have become available at genome-wide level in electronic format, which facilitates interspecies comparisons. However, it had not been proven whether these structure-based orthologs are similarly regulated. Thus, we started to construct the interspecies comparative database of whole genome expression. Using this database, it is also possible to identify mast cell-, basophil-, eosinophil-specific genes which are not expressed by other cell types and we can screen the genes that are suitable for pharmaceutical development such as G-protein coupled receptor (GPCR).

As has been previously reported in part using the previous version of oligonucleotide microarray (GeneChip®), we found the transcriptional levels of several CC chemokines markedly increased in both human and mouse mast cells after stimulation via high-affinity IgE receptor. As

such, we present comparative database for orthologous genes expressed by several types of inflammatory cells including mast cells using the new version of the GeneChip® covering over 50,000 types of transcripts. We also identified GPCR genes that were selectively expressed by mast cells, basophils and/or eosinophils as follows. They were histamine H4 receptor, prostaglandin E receptor (type 3a2), ADORA3 adenosine A3 receptor, P2Y2 purinergic receptor, chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), egf-like module containing, mucin-like, hormone receptor-like sequence 1 (EMR-1), CCR3 chemokine receptor 3, C3a receptor, orphan GPCR114 and orphan GPCR34 probably responsible for lysophosphatidyl serine.

Utilizing this database, it will be possible to select the information obtained from animal models just where the orthologous genes are similarly functioning. Also, it should minimize the efforts required for pharmaceutical development.

16

The Tetraspanin CD9 is Essential for the IL-16-mediated Chemotaxis and Activation of Human Mast Cell

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IL-16 plays a key role in numerous immune responses due to its ability to induce the chemotaxis and activation of T cells, monocytes, dendritic cells, eosinophils, and mast cells. The ability of IL-16 to inhibit HIV-1 infection also has implicated a protective role for this proinflammatory cytokine in AIDS patients. While CD4 appears to be the primary IL-16 receptor on the surface of the T cell, other IL-16-receptors must exist because monocytes and dendritic cells isolated from CD4-null mice are IL-16 responsive. We now show that the IL-16-reponsive mast cell line HMC-1 lacks CD4 protein on its surface, and that the
IL-16-mediated chemotactic and Ca^{2+} mobilization responses of this cell line can be blocked effectively by anti-CD9 mAbs but not by anti-CD4 mAbs or mAbs directed against other tetraspanins. Similar findings were obtained with non-transformed cord blood-derived human mast cells. The chemotactic response of HMC-1 cells to IL-16, as well as the binding of the cytokine to the cell's plasma membrane, were inhibited by CD9-specific antisense oligonucleotides. The tetraspanin therefore is essential for the IL-16-mediated chemotaxis and activation of human mast cells. In support of this conclusion, IL-16 bound to CD9-expressing CHO cell transfectants and activated a CD9-dependent, phosphatidylinositol 3-kinase/phospholipase C-γ/ inositol trisphosphate-dependent signalling pathway. This is the first report of a tetraspanin that plays an essential role in a cytokine-mediated chemotactic response of the mast cell.

17

Mast cell adhesion and migration induced by monomeric IgE

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We and others recently demonstrated that monomeric IgE binding to its receptors (FcεRI) on mast cells renders the cells resistant to growth factor depletion-induced apoptosis (Immunity 14, 791, 2001; ibid 14, 801,2001). More recently, we showed that, upon receptor binding, various IgE molecules display a wide spectrum in their ability to induce cytokine production and other activation events (PNAS 100, 12911, 2003): highly cytokinergic (HC) IgEs can induce mast cell survival more potently than poorly cytokinergic (PC) IgEs as well as degranulation, receptor internalization, and DNA synthesis. Although PC IgEs can induce survival, they cannot induce other activation events efficiently. Both types of IgE can induce FcεRI aggregation with more extensive aggregation being induced by HC IgEs. IgE-induced survival signal is mediated by FcεRI γ subunit (Sakurai et al, J. Immunol. In press). We have extended previous observations that IgE can induce mast cell adhesion to fibronectin (Blood, 102, 1405, 2003) by showing the dependence of the adhesion on Src, Syk, Tec, and PKC family kinases. Furthermore, our results demonstrate that HC, but not PC, IgEs can induce the migration of mast cells by a mixed mechanism of increased cell movement toward an IgE source (chemotaxis) and random movement (chemokinesis). Both adhesion and migration are dependent on β1 integrins. This migration requires Src, Syk, and PKC family kinases, PI3K and p38. A minor portion of IgE-mediated migration involves an autocrine or paracrine mechanism of mediators that are released from IgE-activated mast cells and attract naïve mast cells. Recent studies have established that IgE is synthesized in local mucosal tissues of nasal cavity and lung in allergic rhinitis and asthma, respectively, creating the inflammatory microenvironment high in IgE concentrations (Immunology 98, 646, 1999; Eur. J. Immunol. , 31, 3422, 2001). Therefore, we propose that IgE-mediated adhesion and migration are involved in mast cell accumulation in inflammatory sites where local IgE synthesis is ongoing.

18

Mast cell inhibition by β2-Adrenoceptor Agonists

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To characterize β2-adrenoceptor (β2AR)-mediated modulation of growth and function of human intestinal mast cells (MC). MC were isolated from intestinal surgery specimens, purified up to 95% using the MACS™ system, and subsequently cultured for 14-21 days in the presence of stem cell factor (50ng/ml) with our without addition of adrenaline, noradrenaline, or salbutamol. Mediator release was induced by IgE receptor crosslinking upon incubation with mAb22E7 (100ng/ml) in the presence or absence of β2AR agonists. Histamine and prostaglandin D2 (PGD2) in the supernatant were measured by RIA and EIA, respectively. MC adhesion assays were performed with fibronectin coated plates and human umbilical vein endothelial cells (HUVEC). Actin polymerization in activated MCs was assessed by flow cytometry using fluorescent phallacidin.. β2AR agonists dose-dependently reduced MC growth to 23.4% (salbutamol), 25.3% (adrenaline), and 40.5% (noradrenaline) of control conditions (all at 10^{-6}M, n=5). Activation-induced histamine release was inhibited to 4.1% (salbutamol), 21.6% (adrenaline), and 35.8% (noradrenaline, all at 1μM, n=5)) of control conditions in a dose-dependent fashion. PGD2 release was nearly abolished in the presence of all β2AR agonists at 1μM (n=3). Moreover, the presence of β2AR agonists strongly inhibited MC adhesion to HUVECs as well as activation-induced adhesion to fibronectin (n=3). In addition, all β2AR agonists clearly inhibited actin polymerization in MC induced by SCF (10ng/ml) or mAb22E7. Our data show that β2AR agonists profoundly regulate human intestinal MC survival and function. Such findings may be of importance for our understanding of intestinal neuroimmune functions and of therapeutic strategies used in allergic disorders.

19

Studies on the Regulation of IgE Expression by yhe Use of "Knock In"-Mice

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Immunoglobulin E (IgE) is the key effector molecule of allergic reactions. We have previously shown that expression of the membrane form of IgE is essential for the recruitment of cells that secrete IgE. In further studies we could show that the mRNA for th epsilon heavy chain gene is expressed at very low levels due to poor processing and polyadenylation of the transcript. This contrasts with the expression of mRNA for the membrane form of other Ig isotypes. It correlates well with the low level of expression of mIgE protein after class switch to IgE and the normally extremely low serum levels of IgE. As a result of an alternative splicing event, immunoglobulins are found as a secreted product and/or as a membrane bound receptor. For posttranscriptional modification, both variants use different polyadenylation-addition sites (internal or external polyadenylation sites). Therefore, the production of the two types of RNA are determined by alternative splicing or rather, alternative polyadenylation. The ratio between the amount of secreted vs. the amount of membrane-bound Ig that is produced by a single cell is determined by the efficiency with which the internal or external polyadenylation sites are used and by the stability of the ensuing mRNA's. Whether the regulation of splicing for the IgE heavy chain mRNA is similar to that described for IgG and IgA remains to be established. Preliminary data from our laboratories suggest a different regulation: expression of mRNA for the secreted form of IgE is favoured over that for the membrane form in resting B cells. Factors that influence the alternative polyadenylation are largely unknown. However, because expression of mIgE is essential for recruitment of IgE-producing cells in the immune response, clarification of this issue is of great importance. A step forward in the analysis of this tight regulation was done with the construction of sIgE-mIgE chimaeric "knock-in"-mice with composite membrane exons and/or chimaeric polyadenylation sites which are responsible for the transcriptional regulation.

20

Lessons on the mechanism of atopic sensitization from the ratios of IgG1/IgG4 antibodies to allergen

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The TH2 response in man is usually associated conceptually with antibodies of the IgE and IgG4 isotype. Both these isotypes depend on IL-4/IL-13 for the isotype switch. In contrast, IgG1 (human) is inducible by TH1 cytokines and IgG1 antibodies to atopic allergens have therefore been taken to reflect a distinct type of immune reactivity.
We investigated the ratio IgG1/IgG4 to atopic allergens in atopic and non-atopic subjects. Conventional RAST- ELISA- or blotting-based approaches for the measurement of IgG1 antibodies proved unreliable. The likely explanation is that the spectrum of antigens that induces IgG1 responses is very much wider than that of IgG4 (and IgE), particularly because it includes all kinds of anti-microbial specificities that are absent from the IgG4/IgE repertoire. Two precautions proved to be essential for the reliable detection of IgG1 antibodies to atopic allergens: 1) highly purified allergens have to be used and 2) the use of a test system that is selective for high-affinity antibodies is important. These requirements are well met by an assay system based on ^{125}I-labelled purified allergens.
The results indicate that the IgG1 response to allergens from mite and pollen is very similar to the IgG4 (and IgE) response, with very little if any antibody in subjects without IgE response. In contrast, IgG1 antibodies to Fel d 1 (cat) and Can f 1 (dog) are often found in subjects without IgE responses. In none of these responses (mite, pollen, cat or dog) the IgG4 response is markedly higher than the IgG1 response, unless there is prolonged antigen exposure. The latter situation is akin to the high IgG4/IgG1 ratio known to occur in beekeepers and in subjects after allergen-specific immunotherapy.
These results are compatible with the hypothesis that atopic sensitization reflects a TH2 response to weak immunological stimulation, whereas the IgG4 dominance seen upon prolonged, strong immunological sensitization reflects the selective proliferation/ differentiation of IgG4-switched B cells.

21

Increased apoptosis of circulating memory/effector T helper (Th) 1 cells in atopic diseases as a mechanism for Th2 predominance

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T cells constitute a large population of cellular infiltrate in allergic inflammation and a dysregulated, Th2-biased peripheral immune response appears as an important pathogenetic factor. Because apoptosis is a powerful mechanism for deleting T cells, it raises the interesting possibility that unequal apoptosis of Th1 and Th2 effector cells may lead to preferential deletion of one subset over another. Accordingly, mechanisms underlying cytokine switch and fate of T cells are being elucidated in several human and mouse models. In atopic dermatitis, circulating cutaneous lymphocyte-associated antigen-bearing (CLA^+) $CD45RO^+$ T cells with skin-specific homing property represent an activated memory/effector T cell subset. They express high levels of Fas and Fas-ligand and undergo activation-induced cell death (AICD). The freshly purified CLA^+ $CD45RO^+$ T cells of atopic individuals display distinct features of *in vivo* triggered apoptosis such as pro-caspase degradation and active caspase-8 formation. Particularly, the Th1 compartment of activated memory/effector T cells selectively undergoes AICD, skewing the immune response towards surviving Th2 cells in atopic dermatitis patients. The AICD of circulating memory/effector T cells was confined to atopic individuals, whereas non-atopic patients such as psoriasis, intrinsic-type asthma, contact dermatitis, intrinsic type of atopic dermatitis, bee venom allergic patients and healthy controls did not show any evidence for enhanced T cell apoptosis *in vivo*. In the second model, we analyzed apoptotic features of *in vitro* differentiated Th1 and Th2 cells. Th1 cells of atopic individuals expressed increased Fas, Fas-ligand and activation and degradation of caspases. They were significantly more susceptible to apoptosis compared to Th1 cells of healthy individuals and Th2 cells of the same donor. In the third model, we looked at apoptotic features of T cells in Balb/c and C57B6 mice. Balb/c mice as high IgE responders and susceptible strain in allergic inflammation models showed significantly increased T cell AICD together with increased caspase activation and Th2 cytokine predominance. These data demonstrate that the unequal susceptibility to AICD between Th1 and Th2 cells causes an imbalance in T cell subsets leading to Th2 response in atopic diseases. In conclusion, we propose a novel mechanism for understanding how Th2 cells and cytokines are dominant in atopic individuals.

22

Immunoglobulin Free Light Chains in Immediate and Delayed Hypersensitivity Reactions

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Ig free light chains elicit immediate hypersensitivity-like responses

Recent work from our group showed that Ig free light chains (FLC) may not be an useless spillover of Ig production by B cells. We have shown that Ig FLC (both hapten and Der p 2-specific) can dose-dependently transfer antigen sensitivity upon intravenous/local injection into naïve mice. A second encounter with the cognate antigen induced mast cell activation and edema formation (ear swelling) in the sensitized animals. Mast cells were shown to be crucial in this hypersensitivity-like response elicited by Ig FLC: no ear swelling could be induced in mast cell-deficient animals and mast cell reconstitution restored the response. Moreover, mast cells in ear tissue showed morphological signs of degranulation after challenge of FLC-sensitized mice. Using in vitro cultured primary mast cells provided direct evidence that crosslinking of surface proteins with Ig FLC stimulated release of granule mediators and production of lipid mediators. The nature of the Ig light chain-binding proteins (receptor) on mast cells is currently under investigation. Previous experiments in gamma-chain null animals showed that gamma-chain associated receptors were not involved.

Ig free light chains are crucial in contact sensitivity responses

Cutaneous sensitization of mice with low molecular weight compounds followed by a second contact with the appropriate antigen on the ear induces contact sensitivity (CS) reactions marked by a biphasic ear swelling response. Mast cells seem to be crucial for a full development of CS responses, although it is unclear what mechanism of mast cell activation is involved. Also, B cells were shown to be essential in the development of this typical T-cell-mediated immune response. We have demonstrated that contact sensitization with dinitrofluorobenzene, picryl chloride/DNFB and oxazolone results in production of Ig FLC specific for the hapten used. Moreover, we were able to inhibit development of clinical signs of contact sensitivity by treating picryl chloride/DNFB-sensitized animals with F991, an Ig free light chain antagonist, a peptide compound which prevents binding of light chains to their receptor. In conclusion, we propose that Ig FLC might play a role in both immediate and delayed hypersensitivity responses. These insights in the immunological role of Ig FLC reveal challenging concepts in the treatment of allergic disorders.

23

A high IgE and asthma susceptibility gene on chromosome 7p

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Our group uses strategies of genetic mapping and positional cloning to identify new molecular players in asthma and allergy. Starting in 1994, we recruited to genetic studies families residing in the Kainuu province, eastern central Finland. Candidate locus studies and a genome scan suggested that in this data set, a significant determinant of high IgE levels maps to chromosome 7p. The same locus was found important also in other population samples: a significant linkage result was obtained for asthma in a Quebec family collection (Laitinen et al., Nature Genet 28:87, 2001). To proceed with positional cloning, we constructed a detailed sequence, marker and gene map across the implicated 20 cM region and excluded the TCRG gene as the genetic effector in the locus (Polvi et al. Eur J Hum Genet 10:658, 2002).

Recently, we identified a strong candidate gene for high IgE and asthma susceptibility in the region by a strategy of high-resolution association mapping (submitted for publication). The newly identified gene has interesting properties directly relevant to the allergic phenotypes, such as alternative splicing with one protein isoform upregulated in bronchial biopsies from asthmatic airways, and upregulation of mRNA in a mouse model of airway inflammation. Genetic markers within a conserved haplotype associate with high IgE or asthma in 3 population samples studied, and an analysis of haplotypes in populations as distinct as Finnish and French Canadians suggest a common old origin for the risk effect. This new candidate gene can now be considered together with several other, recently identified asthma susceptibility genes in large studies comparing their individual and joint effects.

24

From Phenotype to Genotype: Novel Mutations in Hyper IgE Mice Generated by Genome Wide Mutagenesis

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Laboratory animals carrying mutations are of great value to study the biological function of genes. Using a genome wide mutagenesis approach (ENU-Mouse-Mutagenesis) followed by a broad phenotype screen of basal immunological blood parameters we have generated and identified several independent mouse mutants with increased plasma IgE levels. One of these mutants, that showed a selective hyper IgE phenotype (while other immunoglobulin isotypes where normal), also displayed dramatically reduced T cell numbers in pB, LN and spleen lymphocyte subpopulations as determined by flowcytometry. In contrast B and NK cell counts were normal. Thymocyte analysis of mutants indicate a block in late T cell differentiation where numbers of CD4, CD8 double positive cells appeared to be normal, while single positive cells where almost absent. Functional challenge by OVA sensitization showed an absence of humoral immune response (OVA specific IgE, IgG2a, IgG1) in homozygote while the response in heterozygote was normal. Chromosomal mapping revealed a link to a 2.1 cM region localized on chromosome 1. Sequencing of selected candidate genes revealed a novel point mutation at the kinase domain of zeta chain associated protein kinase 70 kD (ZAP70). ZAP70 is involved in early TCR signalling and in thymocyte development. In humans mutations in the ZAP70 kinase domain have been reported in patients with combined immuno-deficiency (SCID) characterized by the absence of peripheral CD8+ T cells and the presence of non-functional CD4 T cells. A second mouse mutants with increased IgE levels was identified that displays reduced numbers of NK cells and moderately reduced numbers of T cells in pB. Further functional phenotype analysis and chromosomal mapping are underway. In conclusion, using a genome wide mutagensis approach we have identified a number of novel mouse mutants with hyper IgE phenotype. One of them showed a combined phenotype of hyper IgE, a late block in T cell differentiation and an almost complete absence of peripheral T cells. Chromosomal mapping and sequencing allowed us to identifiy a novel ZAP70 mutation . Detailed characterization of ZAP70 signalling pathway may reveal novel genotype/phenotype relationships and may lead to a novel animal model for human SCID.

25

DETECTION OF THE HUMAN ORGANIC ANION TRANSPORTER 3 (OAT3) IN ANTIGEN PRESENTING CELLS

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We recently have shown that human blood monocytes, macrophages and dendritic cells express a cell type specific pattern of cytochrom P450 isoenzymes and transport polypeptides responsible for the uptake and export of xenobiotics (1,2). This indicates the ability of antigen presenting cells to metabolize a variety of drugs including allergens. Here we studied the gene and protein expression of OAT3 a transporter so far only described in kidney, liver and choroid plexus. Here we measured OAT3 expression in blood monocytes, monocyte derived macrophages and dendritic cells by using RT-PCR- and FACS-analysis. We found that monocytes, macrophages as well as dendritic cells contain mRNA for OAT3. Using FACS analysis with living cells OAT3 protein was found to be expressed on the cell membrane of the cells. Our data show for the first time that blood derived antigen presenting cells carry the human organic anion transporter 3. Even though the substrate specificity of the OAT3 transporter is only partially known the identification of a further transport protein associated with the import of xenobiotics underlines the important role of antigen presenting cells in the metabolism of drugs most probably also of other allergens.

1. Baron, J., Jugert, F.K., Zwadlo-Klarwasser, G., Mukhtar, H., Merk, H.F. Biochem Pharmacol 56, 1105-1110 (1998)
2. Baron, J.M., Schiffer, R., Bostanci, Ö., Merk, H.F., Zwadlo-Klarwasser, G. Allergo J 12, 41 (2003)

26

Induction and Recruitment of Allergen-Specific CD8+ T Cells in the Course of IgE-Mediated Allergy

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Previous studies performed both in humans and in rodents have shown that $CD8^+$ T lymphocytes may be important in IgE-mediated allergic inflammation. However, the relative impact of allergen-specific $CD8^+$ T cells during the various natural and therapeutic phases of IgE-mediated allergy such as sensitization, allergen challenge and allergen immunotherapy is not well understood. In this study, we used major histocompatibility complex (MHC) class I multimer technology (tetramers) to analyze the induction and the phenotype of allergen-specific $CD8^+$ T cells both in a murine (C57BL/6) model of alum-ovalbumin (OVA)-induced IgE-mediated allergy and in HLA-A*0201-positive allergic patients undergoing allergen-specific immunotherapy (SIT) with venom or grass pollen extracts. Using the murine MHC class I H2-Kb-OVA peptide SIINFEKL ($OVA_{257-264}$) tetrameric complex, we observed increased frequencies of $OVA_{257-264}$-specific $CD8^+$ T cells in peripheral blood and spleen at the peak of the primary response of allergic sensitization which was characterized by a marked increase of OVA-specific IgG1 and IgE following i.p. injection of OVA. $OVA_{257-264}$-specific $CD8^+$ T cells were still present and inducible three months after the last OVA injection, and expressed amongst others the $CD62L^{lo}$ phenotype, thus indicating the induction of effector memory cells in the course of allergic sensitization. After inhalative allergen challenge with OVA aerosol to induce airway inflammation, a decrease of $OVA_{257-264}$-specific $CD8^+$ T cells in blood was paralelled by a marked migration of $OVA_{257-264}$-specific $CD8^+$ T cells to nonlymphoid organs such as the effector organ lung and liver. The induction of allergen-specific $CD8^+$ T cells during SIT was investigated in patients after definition of HLA-A*0201-restricted honey bee phospholipase A_2 (PLA_2) and grass pollen allergen (Phl p5a) nonapeptides. Using tetrameric complexes involving these peptides, a significant increase in the frequency of PLA_2- and Phl p5a-specific $CD8^+$ T cells with an activated phenotype was observed in the peripheral blood during SIT. Based on these results, further studies can be initiated with a focus on the pathogenetic and/or regulatory role of $CD8^+$ T cells during allergic sensitization and allergen specific immunotherapy.
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27

Allergen Specific IgG and IgA Memory B Cells in Atopic and Non-Atopic Individuals

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Clinical manifestations of type 1 hyperreactivity are triggered when allergens cross-link effector cell-bound IgE and aggregate the underlying high affinity FcεRI. While the IgE antibody-mediated release of mediators constitutes the immunopathological basis for immediate symptoms, much less is known concerning the role of allergen-specific responses of isotypes other than IgE. Our analysis of the major birch pollen (Betv1) specific immune response showed for the first time that Betv1 specific memory B cells are present in tonsils and peripheral blood of both atopic and non-atopic individuals. RT-PCR of single Betv1 specific B cells revealed highly mutated sequences mainly of the IgG and IgA isotype. Striking was the high percentage of VH3-30 or VL3l gene rearrangements. This result was confirmed by an *in vitro* antibody affinity selection via phage panning of a human VH/VL phage library (Griffin.1) using recombinant Betv1. Recombinant antibodies with VH3-30 or VL3l rearrangement had the highest relative affinities as measured by plasmon resonance technology. As Betv1 plasma cells were detectable in tonsils only in atopic individuals, the question rises, how the differentiation of Betv1 specific memory B cells in non-atopic individuals is controlled and why Betv1 specific IgE antibodies as in case of atopic disease are not synthesized. Summarizing, molecular and functional mechanisms of Betv1 specific immune reactions were studied to understand the dramatic reactions in case of major birch pollen induced allergies and to develop an idea for possible further therapeutic strategies.

28

Expression of plasma cell markers CD38, CD138, intracellular IgE and X-box Binding Protein in U266

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Introduction: Terminally differentiated antibody-secreting plasma cells are the end-stage effector cells of the humoral immune response but their lifetime is debated.
The presence of long-lived IgE producing plasma cells could counteract the effect of immunotherapy treated allergic individuals and could explain the longevity of allergic sensitization in drug-, insect- and food allergic patients who have not been exposed for many years.
Aim: To do design a method for detecting plasma cells in vivo. A new plasma cell marker, the transcription factor XBP-1, is highly expressed during plasmacytic differentiation. The IgE-producing plasma cell line U266 was used to study the expression of intracellular IgE and XBP-1 and the distribution of the plasma cell markers CD38 and CD138.
The expression of XBP-1 was verified by a PCR based method.
Methods: U266 was analysed for intracellular IgE and XBP-1 and surface CD38, CD138 and CD19 by flow cytometry. mRNA were analysed for XBP-1 by RT-PCR.
Furthermore IL-4 and aCD40 stimulated B-cells were analysed for the expression of mRNA XBP-1 in RT-PCR.
Results: The methods for detecting intracellular protein and mRNA XBP-1 were optimized and validated. U266 was positive for intracellular IgE (91%), intracellular
XBP-1 (82%) and CD138 (89%). However, only 3% were positive for the plasma cell marker CD38. The nonplasma cell marker CD19 was expressed in less than 3% and only few were positive for surface IgE (9%). Unstimulated naive B-cells were low for both intracellular IgE (4%), surface IgE (2%), intracellular XBP-1 (5%), CD138 (7%) and CD38 (6%). The B-cell marker CD19 was expressed in 95% in these cells.
Furthermore, mRNA XBP-1 were highly expressed in U266 and IL-4 and aCD40 stimulated B-cells compared to unstimulated naive B-cells.
Conclusion: We have developed at method for detecting the plasma cell markers XBP-1, CD138 and intracellular IgE in the cell line U266 that was not found in unstimulated naive B-cells. It was not possible to detect CD38 on U266 which are normally found on human plasma cells. However this method seems to differentiate between naive B-cells and U266/plasma.

29

Indications for the Existence of an Isotype Specific Signal Transduction

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From the numerous surface markers of a B lymphocyte, the B cell antigen receptor complex (BCR) is probably the most powerful one in influencing developmental processes of the cell. The BCR consists of the membrane bound immunoglobulin (mIg) but, depending on the state of differentiation, may be associated with a couple of other transmembrane proteins, most notably Igα and Igβ. In principle, the BCR has two main functions: signal transduction and internalization of antigen for subsequent presentation to T cells. Considering the huge diversity of possible responses, it is becoming apparent that the cytoplasmic signalling cannot be interpreted as a simple on-off mode, but an integration of strength, duration and complexity of the signals initiated upon receptor stimulation. The BCR plays a central role in almost all processes along the developmental pathway of B cells, i.e. the generation, maturation, survival and activation of B cells. Obviously, between Ig subclasses, there should be no differences in the transduction of signals regulating the central features of the immune system such as the control of allelic exclusion, early cellular transitions and in the function of the membrane-bound immunoglobulin as a receptor for antigen capture and presentation. However, our recent experiments indicate that, apart from the signal transduction pathways via Igα/Igβ cytoplasmic tails of the mIgs itself have the capacity to engage in the signal transduction pathway, influencing the quantity and quality of the immune response. We were able to identify proteins, which directly interact with the cytoplasmic tails of mIgs, thus representing an additional signalling machinery. However, it is not yet clear whether the cytoplasmic tails of the Ig-molecules or the cytoplasmic tails of the coat proteins or both tails together are necessary for transducing the whole panel of these signals. It has become clear that BCR-mediated signalling at different maturational stages has distinct biologic consequences. Therefore, a pressing challenge for future research lies in unravel differences in these pathways and identifying operative effectors.

30

Flow cytometric analyses of leukotriene pathway enzymes in blood leukocytes

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Introduction

Leukotrienes (LTs) are eicosanoid mediators of central importance to asthma and airway inflammation, generated predominantly by myeloid leukocytes. LTs are generated by the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism. Upon cell stimulation, free arachidonate is presented to 5-LO by its activating protein FLAP and further metabolized by either LTA_4 hydrolase to the potent chemoattractant LTB_4, or by LTC_4 synthase to the bronchoconstrictive cysteinyl-LTs (CysLTs).

Aim

Synthesis of LTs depends on expression and activity of 5-LO pathway enzymes. Our aim was to investigate the suitability of flow cytometry as a novel technique for measuring the expression and regulation of LT synthetic proteins under in vivo conditions.

Methods

Mixed blood leukocytes were obtained from normal volunteers (n=5) fixed with 4% paraformaldehyde, saponin permeabilised and incubated with antibodies against 5-LO, FLAP, LTA_4 hydrolase or LTC_4 synthase. Cell markers for CD16, CD14, CD5 and Major Basic Protein were used to identify populations of neutrophils, monocytes, lymphocytes and eosinophils respectively. We examined the repeatability of experiments by performing measurements in the same subjects on 4 separate occasions.

Results

Results confirmed previous knowledge of LT generation by human blood leukocytes. Neutrophils are known to synthesize more LTB_4 than CysLTs and

accordingly showed high immunofluorescence for LTA_4 hydrolase with 90% of neutrophils staining positively versus only 6% of eosinophils. Eosinophils are the main producers of LTC_4 and consequently expressed high levels of LTC_4 synthase (88% of eosinophils showed positive LTC_4S staining compared to 6% of neutrophils). The variability between measurements made in one subject on four occasions was generally low. In eosinophils the average standard deviation of LTC_4 synthase measurements was 20% (range 10-30%). Similarly, in neutrophils the average standard deviation of LTA_4 hydrolase measurements was 23% (range 12-29%).

Conclusion

Flow cytometry is a relatively simple and rapid technique showing good repeatability for the measurement of LT pathway enzymes. This methodology may be useful for examining effects of pharmacological interventions on the LT synthetic pathway or for comparing enzyme expression in different patient groups to provide vital information regarding the regulation of LT production in asthma and other disorders.

31

Mediators of acute and chronic inflammatory response in human lung tissue

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We have examined the effect of 100ng/ml LPS on the production of 7 different mediators from human lung tissue over 48hrs. Tissue came from 24 patients (12F/12M, average age 60.8 ±2.0 yrs, average FEV1/FVC=0.69 ±0.02) undergoing resection for cancer. Tissue was maintained in culture and supernatant collected at 1,2,4,6,24, and 48 hr time points. Supernatant was analysed for TNFα, IL-6, IL-8, IL-1β, IL-10, IL-5, and TIMP-1 using commercial ELISAs, and results confirmed using Western blot. Release of the pro-inflammatory cytokine TNFα, was statistically elevated in the LPS stimulated lung at 1, 2, and 4hrs, peaking at 6hrs (median=11.4 ng/mg of tissue versus 0.01 ng/mg of tissue in the control, P=0.0001) and remained elevated until 48hrs. A second pro-inflammatory cytokine, IL-6, was statistically increased in the LPS stimulated supernatant at 6hrs, with a maximum response at 24hrs (median=196.5 ng/mg of tissue compared to 37.5 ng/mg of tissue in the control, P=0.005) and remained elevated at 48hrs. The release of the chemokine, IL-8, followed a similar pattern with a maximum response at 24hrs (median=2.3 ng/mg of tissue versus median=1.1 ng/mg of tissue in the control, P=0.005) and remained elevated. However, not all pro-inflammatory cytokines were increased following LPS stimulation, as there was no difference in IL-1(beta) at any of the time points. Intriguingly, the anti-inflammatory cytokine, IL-10 was elevated at 24 hrs (median=10.88ng/mg of tissue compared to undetectable levels in the control, P=0.03) and remained elevated at 48hrs. As expected we found no differences in IL-5 after LPS stimulation. We noted increased production of the anti-proteinase, TIMP-1, over the 48hrs but this did not reach statistical significance. However, we noticed a strong correlation between the level of TNFα at 6hrs and the level of TIMP-1 at 24hrs (rho=0.638, P=0.01). In summary, LPS stimulated production of TNFα early on in the response of human lung tissue. This was followed by IL-6, IL-8 and IL-10 production at 24hrs. However in the case of IL-1β and IL-5 there was no change in production up to 48hrs.

32

Relaxin Inhibits Activation and Chemotaxis of Human Neutrophils In Vitro by a Nitric Oxide-Dependent Mechanism

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In animal models of inflammation, the pregnancy hormone relaxin was shown to reduce the recruitment of leukocytes, especially neutrophils, in inflamed tissues. The current study was designed to clarify whether relaxin could inhibit activation and chemotaxis of isolated human neutrophils challenged with N-formyl-Met-Leu-Phe (fMLP) and N-phorbol-12-myristitate-13 acetate (PMA), if so, whether the nitric oxide (NO) biosynthetic pathway was involved, as occurs in other relaxin targets. Human neutrophils were preincubated with 1, 10 and 100 nmol/l porcine relaxin for 1 h before activation with MLP (10 mmol/l) or PMA (0,1 nmol/l). In selected experiments, the NO synthase inhibitor L-NMMA (100 umol/l) was added to the samples 30 min. before relaxin. In other experiments, chemically inactivated relaxin (10 nmol/l) was substituted for authentic relaxin. Untreated, non-activated neutrophils were the controls. Relaxin reduced significantly and in a concentration-dependent fashion the expression of the surface activation marker CD11b, as well as the generation of superoxide anion, the rise of intracellular Ca2+, the release of cytoplasmic granules and the *in vitro* chemotactic migration. These effects of relaxin were blunted by L-NMMA and could not be reproduced by inactivated relaxin. Relaxin also increased neutrophil iNOS expression and NO generation. This study provides evidence that relaxin inhibits the activation of human neutrophils stimulated by proinflammatory agents. This novel property of relaxin could be of relevance in toning down maternal neutrophil activation during pregnancy, thereby counteracting the occurrence of pregnancy-related disorders such as pre-eclampsia, which is regarded as an excess maternal inflammatory response to pregnancy.

33

Eosinophilopoiesis in bone marrow and airways after airway allergen exposure

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Allergen-induced increase of eosinophils in airways may results from the recruitment of these cells from the bone marrow (BM) via the blood, but also hypothetically through local airway eosinophilopoiesis. Also, the phenotype of airway eosinophils may depend on whether these cells have differentiated within the BM or in the airways. Clearly, a major stimulator of eosinophilopoiesis is IL-5, but the cellular source of the IL-5 in the microenvironment in this process is not known.
C57BL/6 or Balb/c mice were sensitized (OVA) and subsequently exposed to allergen repeatedly. Newly produced cells were labelled with a thymidine analog (BrdU). Adoptive transfer of of BrdU-labelled bone marrow cells (i.v.) from allergen-exposed mice to another sensitized and allergen exposed mouse, resulted in approximately 50% of BAL eosinophils after allergen being BrdU-positive, proving that these cells came from the donor bone marrow, and thus that bone marrow eosinophils have the capacity to traffic to the airways. In separate experiments, repeated airway allergen exposure increase the number of BAL and BM eosinophils, but also BAL CD34-cells as well as BAL CD135(+)within the CD34(+) cell population, providing evidence of the presence of primitive myeloid progenitors in airways. Culture in vitro of BAL CD34-cells in the presence of IL-5 provided evidence of eosinophil colony forming cells in the airways after airway allergen exposure, although the colony formation is numerically greater during culture of BM CD34-cells. Morphological analysis of BAL suggest that at least 10% of BAL eosinophils may have undergone their final differentiation within the airways. CD34-cells from both BM and blood have the capacity to release substantial amounts of IL-5 upon stimulation in vitro, and the extent of this release may be similar to that of CD3-cells in this model.
We conclude that the extent of airway eosinophilia in sensitized and allergen exposed mice depend to a great extent on traffic of newly produced eosinophils from the bone marrow, but also that local airway maturation of eosinophils from primitive cells may contribute.

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34

Further studies on hyperosmolar challenge of mast cells

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Introduction

Eicosanoids are lipid mediators formed from arachidonic acid via enzymatic catalysis by cyclooxygenase to prostaglandins (PG) or by 5-lipoxygenase to leukotrienes (LTs). The cysteinyl-LTs (LTC_4, D_4 and E_4) are known mediators of allergic and asthmatic responses and PGD_2 is a proposed mediator of allergic reactions and bronchoconstriction. Being the primary cell activated by antigen through the high-affinity Fc receptor for IgE, the mast cell is of outmost importance in allergic reactions. Both the cys-LTs and PGD_2 are predominant mediators formed in the mast cell.

Aim

In order to further explore the role of the mast cell and its mediators in different types of asthmatic responses, mast cell activation and mediator release upon hyperosmolar challenge with mannitol was studied *in vivo* and *in vitro*.

Methods

Inhalation provocation with mannitol was used to cause a hyperosmolar challenge of the airways and cause bronchoconstriction in similarity to the events during exercise-induced bronchoconstriction. Urinary excretion of the PGD_2 metabolite, $9\alpha 11\beta$-PGF_2 was used as a marker of mast cell activation and urinary LTE_4 as an index of whole body production of the cysteinyl-LTs. Mast cell activation and the release of LTE_4 and PGD_2 upon hyperosmolar challenge with mannitol was studied *in vitro* in cultures of human cord blood derived mast cells (CBMC). All eicosanoids were measured with enzyme immunoassays.

Results

Mannitol-induced bronchoconstriction in asthmatics was associated with increased excretion of urinary $9\alpha 11\beta$-PGF_2, significantly different from non-asthmatics not experiencing bronchoconstriction. Urinary LTE_4 was significantly elevated in both groups with no difference between asthmatics and non-asthmatics.
Mannitol elicited release of PGD_2 and to a lesser extent LTC_4 in cultures of CBMC. A synergy between IgE-dependent stimulation and hyperosmolar challenge was observed solely for the release of LTC_4.

Conclusion

Taken together, hyperosmolar challenge *in vivo* and *in vitro* with the release of PGD_2 supports the hypothesis of a role for the mast cell in EIB. The predominant formation of PGD_2 as opposed to LTC_4 in response to mannitol, both *in vivo* and *in vitro*, may indicate a major role for PGD_2 in such asthmatic responses.

35

Prostaglandin E_2 Inhibits Eicosanoid Generation and Cytokine Production by Human Mast Cells Through a non-EP2 Receptor-Dependent Mechanism

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Prostaglandin (PG)E_2 mediates diverse functions in vivo through 4 known receptors (EP1-4), and is unique among eicosanoids for its anti-asthmatic properties. When inhaled exogenously by susceptible asthmatic human subjects, PGE_2 blocks early and late asthmatic responses, decreases eosinophil and basophil influx during the late phase, and completely blocks incremental production of cysteinyl leukotrienes (cys-LTs) and asthmatic responses to aspirin ingestion in subjects with aspirin-intolerant asthma. Since mast cell (MC) activation is a key event in both the biphasic asthmatic response and aspirin-induced asthma attacks, we sought to define the effect of PGE_2 on activation responses of cultured human MCs (hMCs) derived in vitro from cord blood. hMCs expressed mRNA encoding EP1, EP2, and EP4, as well as 3 of the 8 known splice variants of the human EP3 receptor (EP3-I, EP3-II, and EP3-VI, respectively). Exogenous PGE_2 stimulated a dose-dependent calcium flux in cultured hMCs, and potently induced the accumulation of cyclic AMP (cAMP) at a range of 0.01-10 μM. Butaprost, a selective agonist of the Gs-coupled EP2 receptor, did not mimic the effect of PGE_2, even at doses as high as 100 μM. PGE_2 interfered with both cys-LT and PGD_2 generation by IL-4-primed hMCs activated by FcεRI-stimulated hMCs, and strongly inhibited the production of tumor necrosis factor (TNF)-α by these hMCs in response to either FcεRI cross-linkage or staphylococcal peptidoglycan (PGN); exocytosis was unaffected. PGE_2 potently induced the expression of inducible cAMP early repressor (ICER), a product of the cyclic AMP response element modulator gene and a known inhibitor of cytokine transcription. Thus PGE_2 acts dominantly through a non-EP2 receptor-dependent mechanism to stimulate cAMP-dependent inhibitory signaling pathways, interfering with proinflammatory cytokine gene induction and eicosanoid generation. These findings may bear on the ability of exogenous PGE_2 to attenuate MC activation in vivo and prevent challenge-induced asthmatic responses.

36

Ischemia-reperfusion injury of skeletal muscle is mediated by mouse mast cell protease 5

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Ischemia-reperfusion injury is a significant problem in physical and surgical trauma, myocardial infarction, and organ transplantation. The finding that the hypoxic changes in muscle viability and permeability barriers are attenuated in mast cell-deficient W/Wv mice relative to wild-type mice implicated a role for this immune cell in the pathological response. Analysis of injured BALB/c and C57BL/6 mice revealed the extent of injury was strongly correlated with level of mast cell degranulation in these mast cell-sufficient strains. Thus, transgenic mice unable to express varied mast cell mediators were evaluated to identify the relevant factor. Mouse mast cell protease 5 (mMCP-5) is the major serine protease stored in the secretory granules of skeletal muscle mast cells, and it is the protease that is most similar to human mast cell chymase. Here we report that mMCP-5 plays an essential role in hindlimb ischemia-reperfusion injury. We also show that the classical complement pathway, in concert with mMCP-5, plays an additional key role in muscle damage, but not via the MC-regulatory anaphylatoxins C3a or C5a.

37

Optimization of Culture Methods; Cord Blood Derived Mast Cells

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Introduction
Mast cells (MCs) are effectors of both innate and adaptive immunity. Culturing human MCs from stem cells have therefore been the objective of many laboratories.
In vitro differentiation of human MCs is influenced by numerous factors including stem cell factor (SCF), interleukins (IL-3, IL-4, IL-6), IgE and fetal calf serum (FCS). Moreover, cell-density and biological variation may have an impact on phenotypic in vitro differentiation of mast cells.
We have compared the resulting MC phenotype from three different culture protocols.

Materials and Methods
CD133+ cord blood derived human MCs were cultured under serum-free conditions for 7 or 12 weeks in the presence of IL-6, SCF, and initially IL-3 for 1 or 3 weeks. Final differentiation was induced by fetal calf serum for respectively 1 or 2 weeks. The influence of IL-4 for 3 days on mature MCs was investigated measuring FcεRIα and CD117 expression by FACS analysis. IgE/anti-IgE mediated histamine release was quantitated. Alcian Blue staining showed metachromasi.

Results
We observed high biological variation in proliferation potential among batches of cord blood stem cells (n=4). Overall, IL-3 had a positive impact on multiplication ratios, and enhanced the effect of IL-4 on seven week cultures. More than 50% of mature cells stained positive for CD117 (positive difference) at both 7 and 12 weeks cultures and more than 60% for FcεRIα. However, IL-4 resulted in modest decrease in CD117 expression, but had no effect on FcεRIα.
Histamine content in 7 weeks cultures was 24.4±14.0 pg/cell. In 12 weeks cultures 24.9±11.0 pg/cell**.**
IgE/anti-IgE mediated release of histamine (HR) in 7 weeks cultured cells showed batch variation but always increased by 15% in the presence of IL-4. No significant HR was found in 12 weeks cultured cells.

Conclusions
Significant variations of phenotypes were observed between culture batches. At this stage it is unclear whether the in vitro phenotype heterogeneity reflects the in vivo heterogeneity of MCs or whether it is the result of culture conditions.
The phenotypes of MCs cultured 7 or 12 weeks were comparable. However, short term cultured cells were functionally superior.
Comprehensive characterisation of the particular phenotypes of MCs generated from different sources and cultivation protocols must be carried out.

38

Active Cutaneous Anaphylaxis is Attenuated in Mice deficient in the pro-Survival Gene A1

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The contribution of mast cells to the pathology of allergic diseases is facilitated by their long life span in tissues. To understand the mechanisms responsible for mast cell survival in allergy, the prosurvival Bcl-2 family gene expression in mast cells upon aggregation of the high affinity IgE-receptor, FcεRI, has been investigated. We have previously described that the expression of the prosurvival gene A1/bfl-1 is a prerequisite for mast cell survival after aggregation of FcεRI (Xiang Z et al J Exp Med 2001). To further investigate the role of A1-expression in mast cells we have examined passive cutaneouse anaphylaxis (PCA) and active cutaneouse anaphylaxis (ACA) in mice deficient in the A1 gene. PCA was assessed in wild-type and $A1^{-/-}$ mice by measuring Evans blue extravasation in the ear. The magnitude of the PCA reaction was found to be similar in $A1^{+/+}$ and $A1^{-/-}$ mice. We next used active cutaneous anaphylaxis to investigate mast cell numbers of the ear and ear swelling. Mice were actively sensitized by intraperitoneal injection of OVA in alum and then provocated by an intradermal injection of the antigen. The number of mast cells was the same in $A1^{+/+}$ and $A1^{-/-}$ mice after the sensitization period. However, after provocation $A1^{-/-}$ mice had significantly lower number of mast cells compared to wild-type. Furthermore, the ear thickness was significantly diminished in $A1^{-/-}$ mice. Our findings suggest that A1/Bfl-1may account for the survival and regranulation of mast cells in allergic and inflammatory responses mediated by FcεRI aggregation, thus providing a potential target for allergy therapy.

39

Dendritic Cells and Regulatory T Cells in the Allergic Immune Response and its Modulation.

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Dendritic cells (DC) and T cells play key roles in the initiation and regulation of adaptive immune responses. We investigated their functions in the human allergic immune response and its modulation.
Exposure of immature blood/monocyte-derived DC (MoDC) to allergens leads to their activation initiating distinct signals like phosphorylation of STAT 6 and subsequent (low) production of IL-13. IL-13 enhances IL-4 production of cocultured autologous T cells with no effect on IFN-gamma production in an allergen-specific setting employing allergen-pulsed MoDC matured in the presence of TNF-alpha and IL-1-beta (and IL-4, which had been washed out before coculture). Arresting MoDC in an immature state by pre-treatment with IL-10 at the time when they are fed with allergen results in the induction of "anergy" in cocultured allergen-specific T cells which can be broken by addition of external high doses of IL-2. T cells induced by IL-10-MoDC do not proliferate or produce cytokines normally (except IL-10, which is produced at higher levels) even if stimulated with allergen-pulsed mature MoDC. When such "anergic" T cells are cocultured with fresh autologous T cells they suppress their proliferation and cytokine production. An even more profound suppression of proliferation and Th1-cytokine production of allergen-specific T cells can be achieved by coculture of such cells with autologous CD25+CD4+ regulatory T cells even if derived from atopic donors. In most cases these cells can also suppress the production of Th2 cytokines (in about 80% of atopic donors), however this suppression is dependent on allergen concentration and the type of allergen. An additional way to modify the allergic immune response is the use of allergen-DNA which can be transfected into MoDC very efficiently using adenoviral vectors. The induced response of autologous T cells by such cells is much more dominated by CD8+ T cells and type 1 cytokine production.
These findings underline the important roles of DC and regulatory T cells in the orchestration of allergic immune responses and provide promising therapeutic perspectives.

40

Human plasmacytoid dendritic cells, though rare in atopic dermatitis lesions, express functional high-affinity IgE-receptors

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Epidermal dendritic cells (DC) of inflamed skin include Langerhans cells (LC) and inflammatory dendritic epidermal cells (IDEC). Another DC subset is the plasmacytoid dendritic cell (PDC) characterized by the production of large amounts of interferon (IFN-α and IFN-β) upon viral infection.

Using flow cytometry for simultaneous enumeration of LC, IDEC and PDC, we recently identified PDC in lesions of lupus erythematosus, psoriasis, contact dermatitis and atopic dermatitis. In normal looking skin, PDC and IDEC were low or absent. Lesions of psoriasis and contact dermatitis contained moderate numbers of IDEC and PDC. Lupus erythematosus contained many PDC but only few IDEC. In contrast, many IDEC but only very few PDC were detected in atopic dermatitis lesions. We hypothesized a role for FcεRI on PDC inflamed skin lesions.

Flow cytometric immunophenotyping revealed surface expression of the IgE-binding FcεRIα chain on PDC isolated from peripheral blood and inflamed skin. FcεRIα expression was confirmed by immunogold labeling and electronmicroscopic PDC examination.

Real-time RT-PCR was performed for FcεRIα, FcεRIβ and FcεRIγ chain in highly purified blood PDC. FcεRIα and FcεRIγ chain were present in all samples, whereas FcεRIβ could not be detected in our samples, thus resembling published data from LC and monocyets.

Calcium influx was investigated with human-myeloma-IgE loaded PDC. A strong calcium influx was seen about 180 seconds after FcεRIα ligation with a murine anti-human IgE antibody in all PDC samples. An ommission of IgE-loading and the ionophore ionomycin served as negative and positive control. The time kinetics of this influx induced by the physiological stimulus were different from those induced by ionomycin: Ligation of FcεRIα was followed by delayed calcium influx after 3 minutes, whereas ionomycin induced an instant calcium influx. Calcium levels returned to normal about 12 minutes after FcεRIα ligation, whereas ionomycin led to a prolonged increase of intracellular calcium.

We conclude that PDC and IDEC are selectively recruited to the skin lesions depending on the type of skin disease, and that the lack of PDC in atopic dermatitis may predispose these patients to viral infections such as eczema herpeticum. Furthermore, PDC express functional FcεRI of the non-mediator-releasing FcεRIαγγ type.

41

Differential T cell programming by pro-inflammatory blood dendritic cells

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The immunoregulatory potential of DCs is known to critically depend on their state of maturation. Immature DCs are believed to induce tolerance or Th2 cells, while mature DCs can program Th1 cells. Little is known about the functional state of native human DCs circulating in blood and their response to external stimuli. Focussing on a recently described population of pro-inflammatory blood DCs, identified by the mAb M-DC8, we studied their requirements and dynamics of maturation that determines their responsiveness to external signals and their immunoregulatory function. We show, that freshly isolated DCs purified by magnetic sorting as well as M-DC8+ DCs enriched by negative depletion, undergo profound phenotypical and functional changes during the first 3 hours of culture. The majority of DCs expressed the maturation marker CD83 and upregulated expression of HLA-DR molecules on their cell surface. When immediately after isolation from blood the DCs were stimulated by the TLR4-ligand lipopolysaccharide (LPS) they showed an impeded maturation and failed to produce IL-12, yet they markedly produced TNF-a. In contrast, the same stimuli applied to DCs after an initial culture period of 6 hours fastened DC maturation, induced IL-12p70-production and boosted the secretion of TNF-a. To test the biological relevance of the spontaneous maturation of M-DC8+DCs we cocultured them with allogeneic cord blood T cells. LPS-stimulation at the initiation of the cocultures induced a preferential programming of Th2 cells. In contrast, LPS-stimulation of DCs after 6 hours of maturation in culture very efficiently induced Th1 cells.

These data indicate that within a short period of time, M-DC8+ blood DCs can change their responsiveness to TLR-4 stimulation and switch their T cell programming capacity.

42

Plasmacytoid dendritic cells activate allergen-specific Th2 memory cells: modulation by CpG oligodeoxynucleotides

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Background: Plasmacytoid dendritic cells (PDC) accumulate in the nasal mucosa of allergic rhinitis patients, but their function in upper airway allergy has not been determined. CpG oligodeoxynucleotides (ODN), potent adjuvants in immunotherapeutic strategies in animal models, are especially effective at activating PDC. These cells are therefore potential targets for immunomodulation in humans.
Objective: In this study, PDC were compared with CD11c+DC, a very potent antigen presenting cell, for their capacity to induce allergen-dependent activation of Th2 memory cells. Then, we investigated whether CpG ODN-activated PDC were able to modulate the allergen-specific Th2 memory response.
Methods: DC were isolated from patients with upper airway allergy and co-cultured with autologous CD4+ T cells with or without grass pollen extract and CpG ODN. In some experiments cells were restimulated with allergen-pulsed monocyte-derived DC. T-cell activation was measured by their proliferative response and cytokine production.
Results: PDC stimulated allergen-dependent T-cell proliferation and Th2 cytokine production as efficiently as CD11c+DC. In parallel experiments both DC subsets stimulated virus-specific T cells to produce high levels of IFN-γ and low IL-5, demonstrated that the T-cell responses were antigen-specific. CpG ODN-activated PDC inhibited allergen-dependent proliferation of Th2 memory cells and markedly increased IFN-γ production in PDC/T cell co-cultures; the former effect depended on the CpG ODN-induced IFN-α/β production by the PDC.
Conclusion: Our results demonstrated that PDC efficiently drive allergen-dependent Th2 memory responses, suggesting that they play an active role in the allergic reaction. However, in the presence of CpG ODN, PDC were responsible for production of cytokines with strong Th2 counteracting properties (IFN-γ and IFN-α), indicating that mucosal PDC may be targets for CpG ODN-based immunotherapeutic strategies against airway allergy.

43

Nasal mucosa *TGF-β* mRNA expression is associated with elevated *Phl p 5* specific serum IgA2 response in grass pollen immunotherapy treated patients.

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Successful grass pollen injection immunotherapy (IT) is associated with a inhibition of seasonal allergic responses mediated possibly through IL-10 and TGF-β, inducing IgG4 and IgA production respectively. We have assessed the IgA response in patients with seasonal allergic rhinitis during a double-blind, placebo-controlled trial of grass pollen IT, and related the findings to TGF-β expression in the nasal mucosa. Serum concentrations of *Phl p 5* specific total IgA as well as IgA1, IgA2 and J chain were measured by ELISA using monoclonal antibodies before treatment and after 2 years of IT. The results were expressed as arbitrary units. TGF-β mRNA was assessed by *in situ* hybridization in nasal biopsies taken before and after 2 year IT in placebo- and IT- treated patients. *Phl p 5* specific serum IgA (total) was significantly increased only in the IT-treated group [median 0.3 (I.Q. range 0.1, 0.6) before and [2.8 (0.1, 0.6)] after treatment, but not in the placebo-treated patients [0.3 (0.1, 0.7)] before and [0.4 (1.5, 3.7)] after treatment (p=0.0001). Serum *Phl p 5* IgA2 showed similar pattern to total IgA and was significantly increased after 2 years of IT as compared with placebo [35.6, (29.4-49.9) versus 8.15 (1-17.8), p<0.0001]. In contrast, *Phl p 5* IgA1 was increased to a much lower extent and only at the peak pollen season. Mucosal-derived, joining chain-containing IgA to *Phl p 5* was significantly increased after 2 years of IT as compared with placebo (108, 94.11-151.33 versus 80.22, 54.39-116.47, p=0.02). Moreover, levels of IgA2 antibodies to *Phl p 5* correlated with TGF-β expression in the nasal mucosa (p=0.01, r=0.60). These data revealed that IgA responses to grass pollen immunotherapy is mostly of the IgA2 subclass, and suggest that *Phl p 5* serum IgA2 antibodies are possibly produced within the nasal mucosa under control of local TGF-β expression.

44

T cell IL-5 production to *Candida albicans* secretory aspartic proteinase 2 is related to IgE-independent late asthmatic response

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To identify possible allergens responsible for nonatopic asthma, peripheral blood mononuclear cells (PBMC) obtained from atopic asthmatics, nonatopic asthmatics, and healthy controls were incubated with various allergen molecules. IL-2, IL-4, IL-5, IL-13, and IFN-γ productions were measured by specific ELISAs. T cell proliferation was assessed by ^{3}H-thymidine uptake. Proliferative response to crude *Candida albicans* (CA) extract was not statistically different among the three groups, indicating a common sensitization to CA antigen. Significant amount of IL-5 was produced by PBMC obtained from several nonatopic asthmatics upon incubation with crude CA extract and a purified protein, secretory aspartic proteinase 2 (SAP2). IL-5 production was not detectable for the PBMC obtained from healthy control subjects in response to SAP2. Upon intradermal and bronchial challenge of SAP2, late but not immediate skin and bronchial responses were induced for the IL-5-producing asthmatics, respectively. Neither IAR nor LAR was detectable for the IL-5-nonproducing asthmatics, indicating the specificity of the responses. IgE-dependent mechanism was ruled out by negative RAST, histamine releasing test, or immediate skin reaction. Significant amount of IL-13 was also produced upon incubation with SAP2 by the PBMC obtained from the SAP2 responders, whereas those producing IL-13 but no significant IL-5 upon SAP2 stimulation did not exhibit LAR. Nonatopic asthma may result from an IgE-independent, T cell-dependent immune-recognition, and *in vitro* cytokine synthesis represent a reliable diagnostic test for "T cell allergens".

45

Cloning, expression, characterization and clinical testing of NADP dependent mannitol dehydrogenase

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Cladosporium herbarum is one of the most important and world-wide occurring allergenic fungal species. Sensitization to *Cladosporium* (and fungi in general) has often been associated with asthma, chronic idiopathic urticaria, and allergic dermatitis. It is important to identify the major allergens of this mould species and to define its allergen repertoire. In IgE-immunoblots approximately 60% of all *C. herbarum*-allergic patients show reactivity with a 29kD protein band. The protein was purified to homogeneity from *C. herbarum* cell extracts and the N-terminal sequence was determined. Enzymatic analysis of the purified native 29 kD protein revealed that this allergen represents a NADP-dependent mannitol dehydrogenase (MtDH) which interconverts mannitol and D-fructose. It is a soluble, non-glycosylated cytoplasmic protein. Two-dimensional protein analysis showed that MtDH is represented probably by a single isoform. MtDH is an enzyme induced by osmotic stress as well as by oxidative stress. The reaction product, mannitol, is an osmoprotectant for fungal and plant cells. The cDNA encoding this protein was cloned from a library in lambda-ZAP constructed from hyphae and spores. The recombinant non-fusion (rnf) protein was expressed in E. coli using the pMW172 vector system and purified to homogeneity and its immunological and biochemical identity with the natural protein was shown by enzyme activity tests and by IgE immune blots with patients' sera. In parallel, also the 6XHIS-tagged form of the protein was expressed in E. coli and purified to homogeneity. Again, about 60 % of the patients recognized the rnfMtDH. This protein therefore is a major allergen of *C. herbarum*. The rnf protein is also being used in a clinical study for skin prick testing (SPT) of a panel of *C. herbarum*-allergic patients. The results of this study will be presented.
The MtDH of the allergenic mould, *Alternaria alternata*, was cloned and expressed as rnf and as 6XHIS-tagged protein using methods very similar to the ones employed for MtDH of *C. herbarum*. The MtDHs of the two moulds are 74% identical in sequence. The two allergens partially cross-react, however, only about 30% of the patients allergic to *A. alternata* show IgE reactivity towards *A. alternata* MtDH.

46

Improvement of the *in-vitro* diagnostic of natural rubber latex allergy and estimation of cross-reactivity by application of recombinant and natural single allergens

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Natural rubber latex (NRL) allergy had become a widespread problem with extensive health and economic implications which result in intensive scientific efforts to identify the allergen sources. The impact of the single allergens for an advanced *in-vitro* NRL diagnostic and for the evaluation of cross-reactivity was studied in 68 health care workers (HCW) with NRL-related symptoms and a positive NRL skin prick test. 90% of the sera were positive to latex ImmunoCAP spiked with rHev b 5 ("k82 new"; Pharmacia*Diagnostics*). In contrast only 76% of these sera displayed positive specific IgE to latex without additional rHev b 5 (correlation 0.93). Eight out of nine sera with an exclusive IgE-response to the "k82 new" were monosensitized to rHev b 5. Using a panel of single latex allergens the following profile was detected: 13% were positive to rHev b 1, 56 % to nHev b 2, 6% to rHev b 3, 68% to rHev b 5, 53% to rHev b 6.01, 10% to rHev b 8, 1% to rHev b 9 and rHev b 10, 18% to rHev b 11 and 66% to nHev b 13. None of the sera revealed a positive IgE response to rHev b 12 (lipid transfer protein). Positive IgE response to rHev b 8 was measured in rBet v 2-positive sera, whereas rHev b 10 and/or rHev b 9 positive sera were also positive to mould allergens. The diagnostic efficiency for testing a mixture of four recombinant latex allergens (rHev b 1, 5, 6.01, 8) was 95%: only three out of 52 sera showed no IgE binding to these mixture-ImmunoCAPs. These sera were positive to nHev b 2 and/or nHev b 13. Based on the analysis of the sensitisation profiles Hev b 2, 5, 6.01 and 13 are major allergens for HCWs. Together with Hev b 1, the major allergen for spina bifida patients and patients with multiple surgery, this panel should be included in sufficient amounts in a standardized latex diagnostic extract. Further allergens like Hev b 8, 9, 10, 11 and 12 have to be considered for testing specific cross-reactivities.

47

Immunological characterisation of recombinant Phl p 6 and comparison with natural Phl p 6

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Rationale: Phl p 6 is one of the major allergens of *Phleum pratense* grass pollen. Recombinant expression of Phl p 6 allows a thorough characterisation of the protein. Here, we explore the authenticity of the recombinant molecule (rPhl p 6) with its natural counterpart.
Methods: rPhl p 6 was expressed in *Pichia pastoris* and both the recombinant and the natural molecules were purified to homogeneity by identical methods including a Cu-chelate column and size exclusion chromatography. The immunological properties of the molecules were compared by their ability to inhibit the binding of nPhl p 6 to grass-pollen allergic patients´ IgE, by Histamine-release assay with grass allergic patients` blood, T-cell reactivity, and by using monoclonal antibody based immuno-assays.
Results: rPhl p 6 and nPhl p 6 inhibit the binding of biotinylated nPhl p 6 to serum IgE to a similar extend. Blood cells from grass allergic patients were incubated with either of the molecules and degranulation was measured as release of histamine in a similar allergen-dose dependent manner. Phl p 6-specific T-cell reactivity was investigated using Phl p 6-specific T-cell lines and clones. The readouts used were proliferation and cytokine production. All cultures tested responded equally to both nPhl p 6 and rPhl p 6. The kinetic parameters of the binding of three monoclonal antibodies raised against Phl p 6 were identical for the two molecules.
Conclusion: Large amounts of recombinant Phl p 6 can be produced by heterologous expression in yeast. The recombinant and the natural molecule show similar activity in the immunological assays.

48

Mite allergic patients exhibit heterogeneous Immunological responses to allergen extracts from related mites

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Introduction: Different combinations of purified or recombinant allergens have recently been suggested as a new generation of vaccines for specific immunotherapy.
The reactivity of mite allergic patients to mite allergens from related mites differently distributed in separate geographical areas may help to define the correct treatment for specific patient populations and is important to illustrate the diversity of patient populations in general.
Methods: We have characterized 9 extracts from dust and storage mites with respect to protein and allergen content, IgE and mAb reactivity and affinity, IgE inhibition, histamine release, and T-cell reactivity using cells and sera from mite allergic patients and from Der p 1 and Der p 2-sensitized mice as well as mite allergen specific mAb.
Results: Comparable protein composition was found in the individual extracts but storage mites lack the characteristic group 1 band. The binding of mAb and patient IgE confirms that group one is only found in dust mites and that group 1 and 2 are the most abundant allergens in all extracts investigated. Moreover, there is a high degree of cross-reactivity on the antibody level exhibiting distinct affinities towards the allergens from each mite species. IgE inhibition demonstrates that the cross-reactivity is only partial and highly heterogeneous. Basophil histamine release suggests that the allergens from the individual species may be clinically relevant and that the reactivity differ from patient to patient at the effector-cell level. The reactivity pattern towards dust and storage mites for T-cell lines responding to both Der p 1 and 2 raised from individual patients is directly related to the degree of reactivity to each of these major allergens. Blocking antibodies in mice exhibit a graded pattern of cross-reactivity comparable to the partial cross-reactivity observed at the IgE level.
Conclusion: The present data clearly demonstrates that the sensitization pattern of the individual patient populations should be taken into account when new vaccines are considered for immunotherapy. In addition, the heterogeneous reactivity observed within a population of Danish mite allergic patients calls for a thorough examination of the final products to ensure that it is safe and efficient in the majority of the patients. Finally, the blocking activity of Der p 1 or Der p 2-specific mouse sera suggests that the degree of homology of the allergens from each species is not high enough to induce highly cross-reactive blocking antibodies able to fully inhibit the binding of human IgE to all these clinically relevant mite allergens from different mite species.

49

Soluble CD14-levels are decreased in boys, with maternal smoking and with recurrent otitis in two-year old children.

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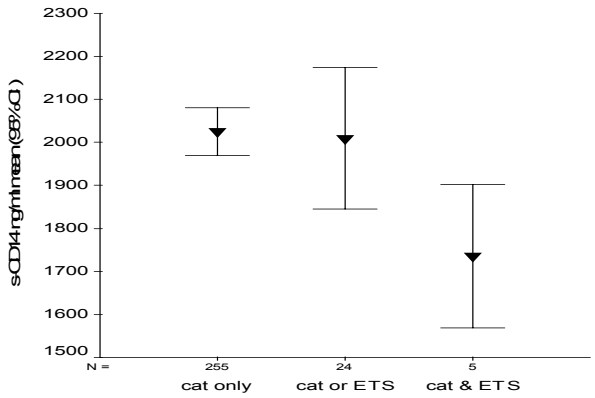
CD14 receptor (membrane bound) and soluble form (sCD14)) is involved in innate immune responses through the binding of bacterial components. Studies suggest that increased levels of sCD14 may be associated with lower levels of IgE or allergic sensitisation. We therefore aimed to explore whether sCD14 was reduced in children with allergic diseases at the age of two years.

Methods: s-CD14 was analysed by ELISA kits in frozen stored serum of 411 two-year old children recruited from the birth cohort study "Environment and Childhood Asthma" in Oslo. Six-monthly questionnaire recordings of clinical disease from 0-24 months of age were collected from the parents. Clinical investigation, structured parental interview and blood samples were obtained at age two in 241 children with recurrent bronchial obstruction (rBO)(91 with atopic dermatitis ever (AD)) and 170 controls without BO (44 with AD).

Results: sCD14(ng/ml) (mean, 95 % c.i.) was significantly lower among boys (1947, 1890-2004), n=228) than girls (2035, 1973-2096)(p=0.04) and reduced with any (n=138)(1916, 1848-1983) compared to no (2022, 1968-2075) maternal smoking (p=0.018).



Combined cat and maternal smoking were associated with further reduced sCD14 (figure). In a linear regression model a significant interaction between cat and maternal smoking was found, when controlling for personal and parental allergic diseases, pet exposure and respiratory infections. sCD14 was significantly negatively associated with rBO and AD only after controlling for variables listed above, whereas sCD14 increased significantly with increasing number of otitis media (OM) episodes (1964,1906-2023 in the lower compared to 2082, 1989-2174 the upper quartile of OM).

Conclusion: sCD14 was reduced among boys, children with maternal smoking and particularly so if they also had a cat at home, as well as with both AD and rBO. OM on the other hand was associated with increased sCD14 in two year old children.

50

A large scale study of the epidemiology of allergic rhinitis in Europe

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Background: Allergic rhinitis (AR) is a frequent condition with an increasing prevalence which may have a large impact on quality of life and productivity. More accurate estimates of the extent of this condition in the general population are needed.

Objective: To estimate the prevalence of AR, the proportion undiagnosed, and the proportion and characteristics of persistent AR (new ARIA definition) in the general, adult population.

Method: Two-step cross-sectional population-based survey in Belgium, France, Germany, Italy, Spain and UK. Step 1: Highly sensitive screening for AR was conducted by a computer-assisted telephone interview; the screening was based on presence or history of symptoms or self-awareness of the condition. Step 2: Confirmation of AR by a clinical diagnosis in a subset of the subjects screened positive was conducted in three to five clinical centres per country. Specific IgE tests (grass pollen, tree pollen, weed pollen, dust, mould and animal danders) and a disease-specific questionnaire were also administered.

Main Results:
A total of 9645 telephone interviews were conducted between February and April 2001 and 725 subjects received clinical diagnoses between May and August 2001. Prevalence of AR in the general population was estimated at 23%, ranging from 17% in Italy to 29% in Belgium. Sensitization to grass was the most frequent, followed by dust and trees; sensitization was not homogeneous among countries. Among the patients with investigator-confirmed AR, 45% had not reported a previous diagnosis of AR by a physician. The following factors were associated with the lack of a previous physician-based diagnosis: no asthma, shorter disease duration, shorter symptom duration, less severe symptoms, and not smoking. Overall 29% of the subjects were classified as having persistent AR. There was no difference in the proportion of SAR (49%) among subjects with intermittent or persistent AR. Compared to subjects with intermittent AR, subjects with persistent AR were more often sensitized to pollen and less often sensitized to dust. Symptoms severity scores were higher in subjects with persistent AR than in subjects with Intermittent AR.

Conclusions: This study confirms that allergic rhinitis has a high prevalence in Europe and is frequently undiagnosed. Persistent allergic rhinitis has some unique characteristics, confirming the usefulness of the new ARIA definition.

51

Risk Factors for Persistent Wheezing in Young Children in a Subtropical Environment

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Previous studies in Brazil have shown that infection with respiratory viruses, particularly respiratory syncytial virus (RSV) and family history of allergy were strongly associated with acute wheezing among infants, and that sensitization to inhalant allergens was the major risk factor for wheezing among children 2 to 12 years old. We have evaluated risk factors associated with persistence of wheezing beyond the first two years of life among children living in a subtropical area. Eighty children who had been seen at the Emergency Room for an episode of wheezing in the first 2 years of life were followed for 2 years in this prospective study. Children underwent clinical evaluation and skin prick testing to inhalant and food allergens two years following the index episode of wheezing. Detection of respiratory viruses and analysis of house dust samples for exposure to major allergens from mites, cockroach, cat and dog (by ELISA) were carried out at enrollment. Total IgE and specific IgE antibodies (Pharmacia CAP system) were measured at the beginning of the study and at the end of follow-up. Multivariate analysis was performed to identify factors associated with persistent wheezing. Seventy-three children (44 boys) completed the study. After 2 years, 38 (52%) presented 3 or more wheezing episodes treated with beta-2 agonists in the past 12 months (persistent wheezers). Overall, 33 children (45%) were sensitized to at least one allergen at the end of follow-up. Sensitization to mites and cockroach was observed in 88% and 57.5% of the children, respectively. The number of sensitized children was higher among persistent wheezers as compared to transient wheezers (71% and 17%, respectively, $p<0.001$). Risk factors for persistence of wheezing were: family history of allergy (OR=22.1; p=0.03), exposure to levels of cockroach allergen Bla g 1 > 2U/g in the home (OR=7.1; p=0.047) and allergic sensitization at age 2-4 (OR=11.3; p=0.002). Breast-feeding for at least one month was a protective factor (OR=0.09; p=0.012). In conclusion, environmental exposure to high levels of cockroach allergen in infancy, sensitization to indoor allergens at age 2-4 and family

history of allergy were strongly and independently associated with persistent wheezing in children.

52

Co-Sensitization to Cockroach and Mite is Not Simply Explained by Tropomyosin Cross-Reactivity.

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Tropomyosins derived from mite species (Der p 10 and Der f 10) and German or American cockroach species (Bla g 7 and Per a 7 respectively) have the potential to elicit cross-reactivity. This could explain the high prevalence of immediate hypersensitivity skin tests to mite among subjects with positive skin tests to cockroach (CR). Cross-reactivity to tropomyosins was tested using 130 sera with measurable IgE ab to CR extract (CAP assay, Pharmacia). Serum samples were obtained from 5 geographic regions: 1) Atlanta, Georgia (n=56); 2) Wilmington, Delaware (n=36); 3) Charlottesville, Virginia (n=23); 4) New Zealand (n=9); 5) Brazil (n=6). IgE ab to mite extract was measured by CAP assay while specific IgE ab to recombinant Der p 10 (rDer p 10) and rPer a 7 were measured using a novel Streptavidin CAP assay. The geometric mean titer of IgE ab to CR extract was 4.98 IU/ml (range=0.37 to 97.1 IU/ml, n=130). The frequency of sensitization to mite extract (either *D. pteronyssinus* or *D. farinae* or both species) was 83% (108/130). Among mite-sensitized subjects, there was no quantitative correlation between IgE ab to *D. pteronyssinus* and CR ($r= -0.051$, $p=0.6$, n=106) or *D. farinae* and CR ($r=0.084$, $p=0.4$, n=103). Furthermore, only 19% (21/108) of subjects with IgE ab to mite had IgE ab to rPer a 7. Nineteen of the 21 sera with IgE ab to rPer a 7 exhibited IgE ab binding to rDer p 10 and anti-tropomyosin IgE ab titers were strongly correlated ($r=0.899$, $p<0.001$). No anti-Per a 7 or anti-Der p 10 IgE abs were measurable in mite-negative sera. Quantitation of specific IgE ab to multiple CR allergens (rBla g 2, rBla g 4, rBla g 5) in a single patient without IgE ab to rPer a 7 demonstrated mono-sensitization to Bla g 5 (70.6 IU/ml). Thus, the IgE ab profile to CR allergens may be unique to each patient. We conclude that co-sensitization to CR and mite only partially reflects tropomyosin cross-reactivity. Instead, consistent with climatic and social issues, concomitant exposure to mite and CR allergens with potent IgE ab-inducing properties is a more likely explanation.

53

Xenobiotic Metabolism and IgE mediated Hypersensitivity Reactions.

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PAH (polycyclic aromatic hydrocarbons) coated on diesel particles are suggested to be adjuvant factors in IgE mediated allergic reactions (Diaz-Sanchez et al. 2000). However, it is unknown whether the parent PAH or its metabolites, generated by xenobiotica metabolizing enzymes in exposed individuals are responsible for this effect and whether atopic patients display a different xenobiotica metabolizing enzyme profile compared to controls.
Methods: Organic extracts from particulate matter of ambient air collected from a densily motorized road (PAH) as well as an individual PAH (phenanthrene) and its metabolites were tested with the murine mast cell line L138.8A and primary human skin mast cells for IL-4 expression, enriched human basophils for CD63 upregulation, and murine and human mast cells for ß-hexosaminidase release. The main metabolites of phenanthrene were identified by a newly constructed humanized cDNA-expression system for xenobiotica metabolizing enzymes.
RNA from atopic donors was isolated from whole blood using an RNA stabilization solution (Pax Gene®, Preanalytics, USA). Biotinylated cDNA was generated and hybridized against a micro array containing 524 xenobiotic metabolism or toxicologic relevant genes (ToxChip®, Genescan, Germany). Detection was performed using a gold-particle labeled antibody against biotin (Qiagen Hilight® method).
Results: PAH increased the activation of human basophils measured as CD63 expression by 40-80% ($p<0.05$). Phenanthrene, the major component of PAH, but not its metabolites 9,10-diol- and 1,2-diol-phenanthrene increased the ß-hexosaminidase release from mast cells. In addition, IL-4 expression in L138.8A cells was strongly increased.
Mal d 1 sensitive volunteers expressed more SULT1A1 and MKK4 but less HSP105b. MCS patients expressed two-fold more microsomal epoxidehydrolase or cytochrome P450 2C19, both groups compared to age and sex matched controls.
Conclusions: The parent compound phenanthrene but not its metabolites showed an adjuvant effect in IgE mediated reactions. The xenobiotic metabolizing enzyme profile of an individual appears to be an additional factor in IgE mediated allergic reactions.

54

Can Chinese elm pollen cause asthma?

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Chinese elm trees produce copious amounts of pollen in the fall and are a major cause of allergic rhinitis in Pasadena. Many studies over the past 20 years have shown that episodes of pollen-induced asthma occur especially after periods of rainfall or thunderstorm. In addition, combustion particles from automobile exhaust are known to act as catalysts in the allergic response. We investigated whether Chinese elm pollen allergens contribute to the respirable aerosol as well as their mechanism of release from flowers. Outdoor air sampling was performed with a liquid impinger with a 2.5 µm size cut. Immunological analysis showed levels of up to 260ng of Chinese elm allergen per cubic meter of air. The mechanism of release of respirable allergens from fragmented elm pollen was found to be similar to the process recently described for flowering grasses (Taylor et al., J. Allergy Clin. Immunol. 2002;109:51-56) and birch trees (Taylor et al., Clin Exp Allergy, 2004 in press). In all cases, pollen remains on the open anthers in the absence of wind or other disturbances. If wetted, pollen can rupture within minutes. Fragmented cytoplasm is emitted through the pore region of the pollen grain. Drying winds release this cytoplasmic debris of allergen-loaded particles in the size range of 30 nm to 4.5 µm. The small size of these particles, combined with their abundance in the air, suggests that they can readily deposit in the lower airways and may trigger asthmatic reactions in susceptible people in cities with high levels of combustion pollutants.

55

A novel allergen chip technique for the quantitative analysis of allergen-specific IgE antibodies

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Background
Currently, in vitro determination of specific IgE antibodies relies on extracts derived from natural allergen sources although these contain various allergen components and are therefore difficult to standardize. Recently, chip technology has been introduced into allergology facilitating the detection of specific IgE antibodies against a range of highly standardized allergen components. It was the aim of our study to correlate the results of a component-based diagnostic tool, the allergen chip technique (ACT), with an established extract-based method of IgE detection.
Method
We recruited forty atopic adults in whom the diagnosis of type I hypersensitivity had been established by a detailed patient history, a standardized clinical examination and a commercially available fluorescence enzyme immunoassay (FEIA). Computer-assisted quantitative analysis of interacting human IgE antibodies with an array of 11 recombinant and 5 highly-purified natural allergen components was performed incubating only 20 µl of patient serum on a solid-phase allergen chip.
Results
Analysing serum probes of 40 patients, we detected positive reactions against a panel of recombinant and highly purified natural allergen components. We found good to excellent correlations of the ACT with the established FEIA system for the majority of tested allergens. However, allergen chip results for two recombinant allergen components did not correlate significantly due to a markedly lower rate of positive results in FEIA testing.
Conclusion The allergen chip technique is a novel and promising tool for clinical and research purposes in allergology. This test shows good to excellent correlation with an established in vitro diagnostic tool for a panel of recombinant and highly purified natural allergen molecules. However, as ACT reactivity patterns of two allergen components were

found to differ significantly from FEIA technique results, we strongly recommend that each individual allergen component has to be evaluated before implementation on the allergen chip.

56

Microarray-based IgE profiling in patients with latex allergy

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Background

Immediate reactions to natural rubber latex (NRL) still have to be considered a clinically relevant phenomenon with up to 17% of exposed health care workers sensitized to liquid latex. Currently, in vitro- and in vivo-testing relies on poorly standardized extracts of varying allergen content hampering their diagnostic performance. However, several latex proteins have recently been characterized and synthesized as recombinant allergens and are now available for a component-resolved diagnosis in patients with latex allergy.

Methods

We studied seven adult patients in whom type 1 sensitization to NRL had priorily been confirmed by an established fluorescence enzyme immunoassay and/or skin prick testing. 20 µl of each patient's serum was incubated with a solid-phase allergen chip containing a panel of microarrayed recombinant latex allergens (rHev b 3, rHev b 5-10). Latex-specific IgE antibodies were captured onto the allergen chip and visualized by laser scanning of fluorescence-labeled anti-human IgE antibodies.

Results

We evaluated the individual reactivity pattern of seven patients with latex sensitization and detected specific IgE antibodies to rHev b5 (n=2), rHev b6 (n=5) and rHev b8 (n=4) while no patient reacted to rHev b3, rHev b7, rHev b9 or rHev b10. Interestingly, one of the analysed samples showed a strong reactivity to the panallergen profilin (rHev b 8) but lacked specific IgE antibodies to the other latex allergen components. Semiquantitative test results of the allergen chip technique correlated excellently with the established enzyme immunoassay classification.

Conclusion

Microarrayed recombinant latex allergens are a useful and highly standardized tool in establishing the individual IgE profile of patients with latex sensitization. Thus, this component resolved approach allows the distinction between patients reacting to panallergens (i.e. profilin) and those individuals who are genuinely sensitized to latex allergen molecules. Further studies are needed to correlate specific IgE reactivity patterns and the clinical course of latex allergy in larger populations.

57

ANALYSIS OF SPECIFIC IGE TO VEGETABLE FOODS IN A COHORT OF BIRCH-POLLEN ALLERGIC CHILDREN AND ADOLESCENTS USING RECOMBINANT ALLERGENS

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Background: Allergic reactions to certain vegetable foods is common among birch pollen sensitised individuals. The gold standard for diagnosis of such pollen-related food allergies consists of laborious challenge procedures. More widely available and cost-effective diagnostic methods, with favourable performance characteristics, are highly desired.

Purpose: To assess the use of natural food extracts and recombinant food allergens for detection of specific IgE in a cohort of birch pollen allergic patients with self-reported food allergy symptoms.
Methods: Case history regarding symptoms of food allergy to hazelnut, apple, carrot, pear, cherry and celery was obtained from 97 Swedish consecutive birch pollen allergic patients, 3-18 years of age. A serum sample from each subject was analysed for specific IgE to birch pollen extract and recombinant components, food extracts, recombinant food allergens and bromelain, all using UniCAP.
Results: The proportion of subjects who had been knowingly exposed and could provide a definite answer regarding tolerance or reaction ranged from 63% to 98% between the different foods. Among those patients, reported allergic symptoms were most common to hazelnut (73%), followed by apple (68%), pear (49%), carrot (45%), cherry (29%) and celery (10%). Eighty-five percent of all subjects reported symptoms to at least one of the six foods while 10% reported tolerance to all foods. Using natural food extracts, positive IgE test results were obtained from 83-91% of the subjects reporting symptoms, except for cherry, to which only one of 23 subjects (4%) tested positive. Specific IgE reactivity to at least one recombinant allergen derived from each respective food was detected in 91-100% of the same subjects. IgE reactivity to bromelain was detected in 10% of the entire cohort. Among the recombinant allergens tested, IgE reactivity was most common to members of the PR-10 family (Bet v 1 homologues). Frequently, the levels of IgE measured to allergens of this family significantly exceeded those to natural food extract. The higher sensitivity of recombinant food allergens as assay reagents was accompanied by a more frequent detection of specific IgE also among subjects reporting no symptoms of food allergy.

58

In Vitro Methods for Monitoring the Development of Clinical Tolerance to Foods.

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About 3% of young American children are allergic to milk and/or egg. While the vast majority of children outgrow these allergies, there are no good laboratory indices to predict when and in whom this occurs. We sought to determine if monitoring food-specific IgE levels over time could be used as a predictor for determining when patients develop clinical tolerance. Eighty-eight patients with egg and 49 patients with milk allergy who underwent repeated double-blind placebo-controlled food challenges were included in the study. Using the Pharamcia CAP-System FEIA®, specific IgE (sIgE) levels to milk and egg were retrospectively determined from stored serum samples obtained at the time of the food challenges. Logistic regression was used to evaluate the relationship between tolerance development and the decrease in sIgE levels over a specific time period between the two challenges. Twenty-eight of the 66 egg- and 16 of the 33 milk-allergic patients lost their allergy over time. For egg, the decrease in sIgE levels ($p = 0.0014$) was significantly related to the probability of outgrowing the allergy, with the duration between challenges having an influence ($p = 0.06$). For milk there was also a significant relation of decrease in sIgE levels ($p = 0.0175$) to the probability of outgrowing milk allergy, but no significant contribution with regard to time. Stratification into 2 age groups [those less than 4 years of age and those above at time of first challenge] had an effect, with the younger age group being more likely to outgrow the allergy in relation to the rate of decrease in sIgE. The median food-specific IgE level at diagnosis was significantly less for the "outgrown" group with regards egg allergy ($p<0.001$) with a similar trend seen for milk allergy ($p=0.06$). Using these results, we developed a model for predicting the likelihood of outgrowing milk and egg allergy based on the decrease in food-specific IgE over time. Using the likelihood estimates from this study could aid doctors in prognosis and in timing subsequent food challenges, thereby decreasing the number of premature and unnecessary DBPCFCs.

59

Down-regulation of IgE to tetanus toxoid and diphtheria toxoid by covaccination with cellular (but not acellular) *Bordetella pertussis* vaccine in children

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Background. Previous work has demonstrated that the IgE response to vaccine antigens is down-regulated by covaccination with cellular *Bordetella pertussis* vaccine. It is unknown whether this effect is also achieved by covaccination with acellular *Bordetella pertussis* vaccine.

Methods. In sera of infants vaccinated with diphtheria toxoid (D) and tetanus toxoid (T) (DT, N=68), cellular (DTPw, N=68), 2 or 5 component acellular *Bordetella pertussis* vaccine (DTPa2, N=64; DTPa5, N=65) at 2, 4, and 6 months of age, IgE to D and T was analyzed at 2, 7, and 12 months of age (RAST).

Results. Prior to vaccination no D-IgE was found. At 7 months of age, D-IgE was detected in 50.0% (DT), 55.7% (DTPa2) and 60.3% (DTPa5) of the sera (n.s.), but significantly less frequent in sera of DTPw vaccinated (9.7%, $P<0.001$). Prior to vaccination, only in one case borderline T-IgE was detectable. At 7 months, T-IgE was present in 59.1% (DT), 50.0% (DTPa2) and 74.5% (DTPa5) of the sera (n.s.), but only in 13.0% of DTPw vaccinated infants ($P<0.001$). Correspondingly, the median reum level of D-IgE was significantly lower in DTPw vaccinated (<0.01kU/L) than in DT vaccinated (0.35kU/L, $P<0.001$), DTPa2 vaccinated (0.39kU/L) or DTPa5 vaccinated infants (0.57kU/L). In sera of DTPw vaccinated, the median level of T-IgE (<0.01kU/L) was significantly lower than in DT vaccinated (0.52kU/L, $P<0.001$), DTPa2 vaccinated (0.34kU/L) or DTPa5 vaccinated infants (0.66kU/L). Results at 12 months of age reflect these findings.

Conclusion. Cellular (but not acellular) *Bordetella pertussis* vaccine down-regulates IgE to covaccinated antigens in infants, presumably by cell wall components. The possibility to down-regulate IgE formation in children by safe vaccination should be further investigated.

60

Peroxisome proliferator-activated receptor gamma ligands possess antiviral and anti-inflammatory activity in the course of Respiratory syncytial virus infection.

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Respiratory syncytial virus (RSV) is the major causative agent of severe lower respiratory tract disease and death in infants. The infection of the lung epithelial cell, the primary target for RSV, induces an overwhelming inflammatory response resulting in a prominent recruitment of inflammatory effector cells into the infected lung. Currently there exist no promising antiviral therapy. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors which form a subfamily of the nuclear receptor gene family consisting of three isotypes: PPARα, PPARβ and PPARγ. The activation of PPARα and PPARγ correlated with the inhibition of inflammatory responses. We hypothesized whether the inflammatory response induced by RSV-infection and the replication of the virus might be influenced by activation of PPARs. We determined constitutive expression of PPARß and PPARγ by RT-PCR in human lung A549 cells; expression of PPARα was not observed. Therefore, we analyzed the specific PPARγ agonists 15-deoxy-PGI_2 (15-d-PGI_2), f-L-Leu, and the thiazolidinediones ciglitazone and troglitazone with respect to RSV-infection. When A549 cells were exposed to these PPARγ agonists prior to RSV infection the RSV-induced upregulation of cell surface ICAM-1 and the release of the CXC chemokine IL-8 was significantly reduced. Moreover, when ciglitazone and troglitazone, respectively were added post RSV-infection strong anti-inflammatory responses were still observed. In case A549 cells were preexposed to PPARγ agonists, RSV-infection induced cytopathic effects to a lesser extent in cultured cell monolayers. A reduced cell surface expression of virus G glycoprotein, analyzed by FACS analysis, was concomitantly demonstrated; the release of infectious virus particles from RSV-infected A549 cells was significantly suppressed by all PPARγ agonists. Our data show that PPARγ agonists are able to inhibit the replication of RSV and to reduce the detrimental inflammatory response induced by RSV-infection. Thus PPARγ agonists may play a role in controlling RSV-infection in lung epithelial cells.

61

Influence of nonpathological exposure to bacteria during pre- and early postnatal period protects from allergy in murine neonates

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Recent epidemiological studies indicate that non-pathological exposure to components of microbes protects against allergy and asthma in children. These studies also indicated, that the exposure within the prenatal and early postnatal period plays an important and decisive role for this protective environmental effect. Among other bacterial components the exposure to endotoxin lipopolysaccharide (LPS) is currently of major interest. Several studies indicate an inverse relationship between the exposure to LPS and the development of allergic diseases later in life. To further investigate the role of the pre- and postnatal environment on the development of allergies we have established a murine model over the past years. An allergic TH-2 response was induced in pregnant mice by sensitization and aerosol allergen exposure. At birth the F1 mice demonstrated a suppressed TH-1 response as reflected by lowered frequencies and reduced levels of IFN-γ production. After allergen exposure these F1 mice developed a dichotomy response pattern. Development of an allergic response was completely prevented early in life against the same allergen to which the mother was already exposed. This effect was mediated by diaplacental transfer of allergen-specific IgG1 antibodies. In contrast, allergic sensitization against a different allergen early in life was accelerated in these F1 mice. This effect was mediated by maternal CD4 TH-2 cells induced by allergic sensitization during pregnancy. Based on these data we assessed the impact non-pathological exposure to LPS before conception and during pregnancy. Several weeks after birth offspring were sensitized to Ovalbumin followed by aerosol allergen challenges. Prenatal LPS-exposure enhanced TH-1 response at birth as indicated by increased IFN-γ, but not IL-4 and IL-5 production. Preconceptional and prenatal exposure to LPS protects from allergic sensitization in neonates after allergen re-exposure. In addition assessment of broncho-alveolar lavage fluids revealed a marked reduction in eosinophils and lymphocytes in these F1 mice. However, development of airway hyperresponsiveness in these F1 mice was not altered by LPS pre-exposure. This study provides further evidence that environmental factors may operate already in prenatal life in order to modulate the development of postnatal allergy.

Key words: *Mice, allergy, materno-fetal interaction, cytokines, Th1/Th2-immune response, lipopolysaccharide, airway inflammation, airway hyperresponsiveness*

62

Basophils Infiltrate the Gastric Mucosa in *Helicobacter pylori*-Infected patients and respond chemotactically to *H. pylori*-derived peptide Hp(2-20)

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Although basophils are usually find in the circulation, they can migrate to sites of allergic inflammation. Using the specific monoclonal antibody BB1, we identified basophils in the gastric mucosa of patients with moderate and severe gastritis and *Helicobacter pylori* infection. Basophils were not found in *H. pylori*-free individuals or in subjects with gastritis. Unlike the control peptide Hp1, the *H. pylori*-derived peptide Hp(2-20) was a potent basophil chemoattractant *in vitro*. Basophils from blood samples of healthy donors expressed mRNA for the formyl peptide receptors FPR, FPRL1 and FPRL2. When basophils were exposed to FMLP or Hp(2-20) they did not respond to a subsequent challenge with homologous stimulus. Exposure of basophils to a low concentration of FMLP, which binds with high affinity to FPR, but not to FPRL1 or FPRL2, did not affect the chemotactic response to Hp(2-20). In contrast, the chemotactic response to Hp(2-20) was reduced by a high

concentration of FMLP, which binds to FPRL1 and FPRL2. The FPR antagonist cyclosporin H prevented chemotaxis induced by FMLP, but not by Hp(2-20). In conclusion, Hp(2-20), through interaction with FPRL1 and FPRL2, could be responsible for basophil infiltration of the gastric mucosa of *H. pylori*-infected patients.

63

Respiratory Viral Infection in Chronic Obstructive Pulmonary Disease

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To investigate the role of respiratory viruses in chronic obstructive pulmonary disease (COPD) we established quantitative real-time PCR assays for the detection of respiratory syncytial virus (RSV), influenza A (Inf. A), rhinovirus (HRV) and parainfluenza 3 virus (PIV 3). The frequency of detection and the viral loads in respiratory tract specimens of patients with exacerbation of COPD (AE-COPD) or stable disease were analysed.
A total number of 184 hospitalized patients were included, 116 patients with AE-COPD (age 69 [41-84] years; 24F:92M) and a control group of 68 patients with stable COPD (age 68 [45-81] years; 10F:57M), who were hospitalized for other medical reasons.
In the AE-COPD study group RSV was detected in 22/116 (19%), Inf. A in 21/116 (18.1%), HRV in 13/116 (11.2%) and PIV 3 in 8/116 (6.9%). In the control group 17/68 (25%) were positive for RSV, 10/68 (14.7%) for HRV, 6/68 (8.8%) for Inf. A and 4/68 (5.9%) for PIV 3.
The median RSV viral load was 6.5 x 103 viral copies/ml in AE-COPD patients and 4.8 x 103 viral copies/ml in stable COPD (p=0.121). Similar low viral load values were found for PIV 3. Significant higher values were detected for Inf. A in AE-COPD (2.6 x 106 viral copies/ml) compared to stable COPD (5.8 x 104 viral copies/ml, p=0.009). 2.0 x 105 HRV copies/ml were found in AE-COPD compared to 1.2 x 104 copies/ml in the control group (p=0.413).
The rates of detection and the viral loads of the four respiratory viruses investigated seem to reflect different roles of these viruses. RSV and PIV 3 are found in AE-COPD and in stable COPD with very low viral loads, suggesting low-grade or persistent infection. For Inf. A on the other hand comparable rates of detection, but significant higher viral load values in AE-COPD were detected, indicating acute virulent infection with a significant impact on the exacerbation of COPD.

64

Differences in *Bifidobacterium* species in early infancy and development of allergy

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Background: *Bifidobacterium*, which is the most common intestinal bacteria in early infancy, may be related to development or prevention of allergic diseases.
Objective: To analyze the occupation and species differences of *Bifidobacterium* between atopic and nonatopic children.
Method: We prospectively follow up children born at a local hospital in Chiba prefecture until 2 years of age. Determination of atopy was performed on the basis of examination and skin prick test at 6 month and 2 year of age. Feces from atopics (n = 9) and nonatopics (n=9) were served for count of *Bifidobacterium* genus by Mitsuoka's culture method at 6 month and 2 year of age. Feces from two groups at 1, 3, 6 month and 2 year of age were analyzed for detection of *Bifidobacterium* species by PCR using 10 species-specific DNA primers.
Results: *Bifidobacteria* were detected in all children of the two groups at both 6 months of age and 2 years of age, and bacteria counts was not significantly different between the two groups. Atopics had significantly lower frequency of *Bifidobacterium adolescentis* in 3 month of age compared with nonatopics (14.3% vs 66.7%). Atopics had significantly higher frequencies of *Bifidobacterium bifidum* in 3 month of age and 6 month of age (71.8% vs 11.1%, 77.8% vs 22.2%, respectively). Any differences in frequencies of the species were found at 2 year of age.
Conclusion: Some intestinal *Bifidobacterium* species may be related to development or prevention of allergic diseases in infancy.

65

Neurotrophins in Allergic Bronchial Asthma: Modulators of Immunological and Neuronal Plasticity

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There is growing evidence that the immune and nervous system are closely related not only in physiological but also in pathological reactions in the lung. Recent evidence from our group indicates that neurotrophin production including nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) are elevated in the lung. Both, residential cells including airway epithelium and migratory cells including macrophages, T-cells and eosinophils serve as important sources. The effects of increased neurotrophin production are bi-directional. On the one hand, they control sensory nerve fibres in terms of function, neuropeptide synthesis and growth. On the other side, neurotrophins serve as important survival factors particularly for inflammatory cells such as eosinophils, T-cells and macrophages. Utilizing the model of segmental allergen provocation of mild to moderate asthmatic patients, it has been shown that neurotrophins prevent sufficient apoptotic cell death of lung, but not blood eosinophils. In addition, they augment the ongoing inflammatory reaction. The functional interaction between neurotrophins, immune and nerve cells has been extensively studied in both human and mouse models of experimental allergic asthma. In the latter system, the crucial role of the pan-neurotrophin receptor p75 has been investigated in p75 NTR -/- mice. Furthermore, NGF transgenic animals have been utilized to assess the contribution of NGF. The role of BDNF on differentiation and function of B-lymphocytes has been identified in BDNF -/- mice. In conclusion, our data support the concept that neurotrophins mediate immunological and neuronal plasticity within the neuro-immune network of bronchial asthma.

66

Brain Responses in Asthma Analyzed by Imaging of the Nervous System (BRAIN Study)

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Rationale: There is much interest that the central nervous system (CNS) affects asthma, but little information on the brain/lung processes involved. Consequently, we hypothesized that areas of the brain are activated in response to inhaled allergen (Ag) in asthma, and CNS locations are important in regulating the intensity of allergic inflammation in asthma. **Methods**: Identified asthma subjects underwent 3 blinded provocative challenges: saline, methacholine [immediate pulmonary response (IMM)] and Ag [IMM and late pulmonary response (LPR)]. Functional (f) MRI scans were performed 1 and 4 hrs after each challenge and timed to occur after the IMM and before the LPR. To monitor activity in the brain, subjects were required to discriminate asthma-relevant (e.g., wheeze) and asthma neutral (e.g., walk) words. Sputum samples were obtained pre- and 24 hrs post-challenge. **Results**: There was no change in FEV1 to saline challenge, an IMM only to methacholine (23.6±0.6% fall in FEV1), and an IMM (31.8±1.4%) and LPR (23.9±2.0) fall in FEV1 to Ag. Only Ag caused a significant increase in sputum eosinophils (eos) (pre- 5.7±3.6% and 24 hrs post-challenge 20.8±12.1%). We searched for areas in the brain that were correlated with the increase in sputum eos and changes in FEV1 during LPR to Ag. Both the anterior cingulate and insula cortices showed increased activation that was highly associated with the increase in eos. Activation increases in the insula also strongly predicted the fall in FEV1 during the LPR to Ag. **Conclusion:** These findings are the first to show specific changes in functional brain activation that are associated with the inflammatory process initiated by Ag challenge in asthma and have important implications for understanding the circuitry through which peripheral signals in the lung may feedback upon the brain and vice versa.
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67

NGF AND NT-4/5 COOPERATIVELY MEDIATE ALLERGEN-INDUCED SENSORY NEUROPLASTICITY IN A GUINEA PIG MODEL OF ASTHMA

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Allergen induced inflammation is associated with characteristic changes of the airway sensory innervation. In a distinct group of sensory neurons in the lower vagal (nodose) ganglion, allergen causes an induction of substance P (SP) expression. The mechanisms of inflammation-induced changes are not clear. Since neurotrophins are released during allergic inflammation and are known to modulate afferent nerve function, we have addressed the question which neurotrophins are involved in a guinea pig model of asthma and how blocking neurotrophins affects sensory neurons. Guinea-pigs were sensitized to ovalbumin and challenged by inhalation. The neutrophins nerve-growth factor (NGF), brain derived-neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT4/5) were measured by ELISA. Blockade of neurotrophins was performed *in vivo* by treatment with neutralizing antibodies (AB) and by antisense oligodesoxynucleotides (as-ODN) to each of the neurotrophins. We analyzed the effects on the transcriptional activation of the PPT-A gene by real-time PCR and tachykinin-protein expression in sensory ganglia by immunohistochemistry. Neurotrophin levels were elevated in sensitized and challenged animals as compared to sensitised and vehicle challenged controls. The percentage of SP-immunoreactive neurons in the nodose ganglion were 23.8% (±4.2% S.E.M.) in control animals, in OVA challenged 44.3% (±3.8%), after treatment with NGF s-ODN 44.1% (±5.3), NGF as-ODN 30.8% (±2.1%), BDNF s-ODN 43.6% (±3.8%), BDNF as-ODN 45.4% (± 4.4%), NT3 s-ODN 42.5% (±3.8%), NT3 as-ODN 46.2% (±5.0%), NT4/5 as-ODN 38.1% (±4.1%) NT 4/5 s-ODN 48.1% (±2.7%) and combined NGF as-ODN+NT4/5 as-ODN 20.9% (±3.5%), respectively. These data indicate that both NGF and NT4/5 mediate the allergen induced phenotypic switch of nodose sensory neurons. The corresponding receptors for NGF, tyrosinkinase A (Trk A), and for NT4/5 B tyrosinkinase B (Trk B) may be interesting targets to control allergen induced nerve activity in asthma.

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68

Alpha melanocyte stimulating hormone and related peptides mediate immediate as well as delayed type hypersensitivity reactions.

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There is a substantial body of evidence that the tridecapeptide α-melanocyte stimulating hormone (αMSH) exerts a variety of antiinflammatory and immunomodulating activities. The immunomodulating capacity of αMSH is primarily due to its effects on melanocortin receptor expressing endothelial cells, monocytes, macrophages and dendritic cells (DC). Accordingly, αMSH was found to downregulate the production of proinflammatory cytokines (IL-1, TNFα, IFNγ) and the expression of costimulatory molecules (CD86, CD40, ICAM-1) on antigen presenting cells (APC). In contrast, the production of suppressor factors such as IL-10 is upregulated by αMSH. At the molecular level these effects of αMSH are mediated via the inhibition of the activation of transcription factors such as NFκB. Not only αMSH but also its C-terminal tripeptide (αMSH 11-13, KPV) was able to bind to MC-1R and to modulate the function of APC. *In vivo*, using a mouse model of contact hypersensitivity (CHS) systemic as well as topical application of αMSH or KPV inhibited the sensitization as well as the elicitation phase of CHS and was able to induce hapten specific tolerance. To investigate the underlying mechanisms of tolerance induction *in vivo* transfer experiments using bone marrow derived immature DC have been performed. Using the same murine model of CHS, αMSH treated haptenized DC inhibited CHS and induced hapten-specific tolerance. Tolerance induction was found to be mediated by the generation of $CTLA4^+$ and IL-10 producing T-lymphocytes. The potent capacity of αMSH to modulate APC functions has been further supported in another experimental approach. *In vitro*, via activating monocytes αMSH has been shown to modulate IgE production by IL-4 and anti-CD40 stimulated B-lymphocytes. Moreover, in a murine model of allergic airway inflammation, systemic treatment with αMSH resulted in a significant reduction of allergen-specific IgE production, eosinophil influx and IL-5 production. These effects were mediated via IL-10 production, since IL-10 knock-out mice were resistant to αMSH treatment. Therefore, in the future therapeutical application of αMSH or related peptides (KPV) as well as αMSH/KPV pulsed DC may be a useful approach for the treatment of inflammatory, autoimmune and allergic diseases.

69

The modulatory role of Nerve Growth Factor in allergic inflammation and tissue remodelling

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Background. Nerve Growth Factor (NGF) is the major neurotrophin regulatoring differentiation, growth, survival and function of neurons. Published studies from our (and other) laboratories indicates that NGF and NGF receptors are also produced and expressed by several immune cells including B and T lymphocytes, mast cells, eosinophils and fibroblasts.

Aim. To present recent data extending the role of NGF to CD34+ haematopoietic stem cells and epithelial cells.

Results

1. CD34+ human mononuclear cells from both cord and peripheral blood express NGF and NGF receptors by flow cytometry, confocal microscopy and mRNA determination following RT-PCR in sorted purified populations. The expression of the NGF receptor TrkA is more marked in CD34+ cells expressing markers of an early differentiation (CD133, CD117+), while the appearance of markers of specific cell lineage differentiation is associated with a decrease in TrkA expression, more evident in the lymphoid lineage (CD19, CD3+) less marked in the myeloid one (CD33+).
2. In vivo, epithelial cells of mucosal tissues of allergic subjects express NGF and NGF receptors. In vitro, NGF stimulates epithelial proliferation, differentiation and mucin production.

Conclusions. Recent and previous data from our group suggest that NGF has a pleiotropic activity on immune cells and exerts a complex modulatory role at various steps of inflammatory and remodelling processes of allergic diseases.

70

Direct drug interactions with T cell receptors

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Immune reactions to drugs are normally explained by the hapten hypothesis, which implies that drugs require covalent binding to a larger molecule to become immunogenic. We recently extended this concept by proposing the p-i concept, namely that certain drug hypersensitivity reactions are due to a type of "pharmacological" interaction of the drug with immune receptors (p-i-concept). We found that chemically inert drugs, unable of covalently binding to peptides or proteins, can nevertheless activate certain T-cells if they happen to bear T-cell receptors (TCR) that can interact with the drug itself. This concept has been elaborated by *in vitro* studies using drug specific T-cell clones obtained from patients with various drug hypersensitivity reactions elicited by sulfamethoxazole, lidocaine, celecoxib, lamotrigine, carbamazepine and p-phenylendiamine. This p-i-model of drug hypersensitivity does not require biotransformation to a chemically reactive compound, as the T cell reacts within seconds and as the drug is only labile bound – in contrast to covalently bound haptens, which are presented as modified peptides by MHC molecules. To better analyze the conditions how drugs can stimulate T-cells we transfected into a TCR-negative mouse T cell hybridoma human TCRs isolated from two sulfamethoxazole (SMX) - reactive T cell clones (TCC). The transfectants reacted to SMX in the presence of antigen presenting cells (APC) and anti HLA-class II antibodies abrogated the response. Glutaraldehyde-fixed APCs, unable to process, were sufficient to elicit T cell stimulation, indicating a processing-independent direct interaction of the drug with the TCR. Most interestingly, APC and MHC-classII molecules were required for IL-2 synthesis, but some phospohorylation (ERK and Zap70) of the TCR-transfected hybridoma cells was seen after drug contact even in the absence of APC (and human MHC-molecules). Our data suggest that the drug first interacts with the TCR and gives an initial signal, but that for full T cell stimulation an additional MHC

interaction with the TCR is required. This finding opens new possibilities to modify T cell functions by drugs.

71

Hypersensitivity to NSAID drugs : a new integrated approach to its pathophysiological understanding and diagnosis

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Hypersensitivity to non steroidal anti-inflammatory drugs (NSAIDs) is a well known syndrome affecting the airways (rhinosinusitis, nasal polyps, asthma) and/or the skin (urticaria, angioedema) and represents 20 – 25 % of all hypersensitivity reactions to drugs. Although the favorite theory on its pathogenesis is nowadays a pharmacogenetic abnormality in the response to cyclooxygenase (COX)-1 inhibitors, much uncertainty remains. Furthermore, it is persistently claimed that only provocation challenge with NSAIDs permits to establish the diagnostic and that no in vitro tests are helpful to ascertain that condition.

However, since a number of years, various studies have indicated that blood basophils from patients hypersensitive to NSAIDs may produce, upon stimulation with such drugs in vitro, more sulfidoleukotrienes (CAST assay) than tolerant patients. This, however, has not been universally confirmed. Our recent study on 60 pattients and 30 controls , using in addition a flowcytometric basophil activation test has definitely shown that clinical hypersensitivity to NSAIDs is accompanied by basophil stimulation in vitro in about 75 % of the cases , with a specificity of 95-100 %. In addition, basophils from hypersensitive patients appear to be hypereactive non specifically to various stimulants, such as C5a.

A review of all known clinical and laboratory findings leads to an integrated view of the syndrome, which requires the joint effects of several factors to become clinically realized. First and foremost, a chronic inflammatory process, which may be of various origins (possibly viral) yields hyperreactive effector cells (mast cells, basophils, eosinophils) either in the skin or in the airways, explaining the final localization of symptoms. On these hyperreactive cells, NSAIDs act on the arachidonic acid metabolism in an altered fashion, most probably based on a pharmacogenetic abnormality leading to increased production of sulfidoleukotrienes. In addition, decreased synthesis of PGE2 removes an essential brake for mediator release by inflammatory cells. The simultaneous intervention of these three factors explains most of the clinical and pathophysiological findings in the NSAID hypersensitivity syndrome and opens logical approaches for diagnostic and treatment.

72

Urinary LTE4 concentration after intravenous aspirin challenge - A new diagnostic approach for Aspirin-intolerant asthma (AIA)

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Background: Intolerance to aspirin and other NSAIDs can not be confirmed without provocation test. Bronchial inhalation challenge occasionally shows false-negative reactions. On the other hand, oral aspirin challenge sometimes produces severe and long-lasting attacks.
Purpose: 1) To asses the safety and usefulness of a new diagnostic method for aspirin-intolerance: lysine-aspirin intravenous (i.v) challenge. 2) To measure urinary leukotriene E_4 (LTE_4) concentration before and after lysine-aspirin i.v challenge.
Methods: Single-blind, lysine-aspirin i.v challenge was conducted. Patients with history of AIA received every 40-60 minutes a doubling dose of aspirin (12.5-200mg as ASA). The results of i.v provocations were compared with oral and inhalation tests performed in the same patients at other occasions. Urine sample was collected during the periods of 0-3, 3-6, 6-9, and 9-24 h after the provocation.
Results: Median lysine-aspirin i.v threshold, defined as 20% drop in FEV_1, was 32.4mg as ASA (n=150, 12.5mg: n=31, 25mg: n= 70, 50mg: n=45, 100mg; n=4). There was no difference in the time course of fall in FEV_1 after the provocation between i.v challenge and inhalation challenge. On the other hand, the time–course of FEV_1 changes after i.v challenge was significantly shorter than the oral challenge. There were relatively a few extrapulmonary reactions and no severe reactions. In contrast, a group of aspirin-tolerant subjects (n=100) showed no systemic reaction after aspirin i.v challenge. The urinary LTE_4 concentrations after lysine-aspirin iv challenge in AIA patients significantly increased above basal concentrations (median urinary LTE_4: 134.4 pg/mg-cr for basal vs 678.1 pg/mg-cr for 0-3h, $p<0.001$, 1076.9 pg/mg-cr for 3-6h, $p<0.001$). Release of the mast cell mediator PGD_2 and other biomarkers were also followed.
Conclusion: Lysine-aspirin i.v challenge is safe and quick diagnostic method for aspirin-intolerance, comparable with inhalation challenge but not with the more difficult to control oral test. (words. 344)

73

Plasmacytoid dendritic cells bearing the high affinity receptor for IgE in Atopic dermatitis

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Background: The high affinity receptor for IgE (FcεRI) on myeloid dendritic cells has been shown to play a major role in atopic dermatitis (AD). Plasmacytoid dendritic cells (pDC) which are instrumental in the defense of viral infections are present in reduced amounts in the skin of AD patients, which are characterized by a high susceptibility to viral infections.
Objective: We explored phenotypical and functional characteristics of pDC in the peripheral blood of patients with AD and healthy individuals.
Methods: $BDCA2^+CD123^+$ pDC have been enriched from the peripheral blood of patients with AD and studied in functional assays.
Results: Skin homing molecules such as cutaneous lymphocyte antigen (CLA) and L-selectin CD62L were expressed in lower levels on pDC of patients with AD. PDC expressed high amounts of IgE-occupied FcεRI. Further on FcεRI-aggregation on pDC impaired the surface expression of MHC I and II, induced the production of interleukin-10 and enhanced the apoptosis of pDC. Importantly, FcεRI pre-activated pDC produced less IFN-α and IFN-β after stimulation with CpG motifs and enhanced the outcome of immune responses of the Th2 type .
Conclusion: From these data we conclude that (i) FcεRI-bearing pDC from patients with AD are different from pDC of healthy individuals, (ii) might be important in the pathophysiology of AD and (iii) contribute to the enhanced susceptibility of AD patients to viral infections.

74

In vitro Methods for Assessing the Potential Severity of Food Allergic Reactions.

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Overall, 1.5 million Americans are allergic to peanuts, or 0.6% of the population. The prevalence of peanut allergy in children < 5 years of age has doubled in the last 5 years to 0.8% of this age group. When children are diagnosed with peanut allergy, the most frequent question parents ask is whether their child could experience a “life-threatening” reaction. To date, no laboratory parameter has correlated with reaction severity. In previous studies we have shown that IgE epitope specificity correlates with the likelihood of “outgrowing” egg, milk and peanut allergy. However, screening large numbers of patients for epitope specificity has been impractical using current techniques. Therefore we sought to develop a peptide microarray-based immunoassay to map peanut epitope-specific IgE using microliter quantities of serum. A set of 213 overlapping twenty-residue peptides was synthesized corresponding to the primary sequences of Ara h1, 2 and 3. These were arrayed in triplicate along with the corresponding recombinant proteins onto glass slides and used for immunolabeling. Seventy-seven patient and 15 control sera were analyzed. The majority of patients (97%) had specific IgE to at least one of the recombinant allergens and 87% had detectable IgE to sequential epitopes. Microarray mapping correlated well with previous studies. However, the analysis of individual patients revealed remarkable heterogeneity in the number and patterns of epitope recognition. High epitope diversity, i.e. recognition of a large number of allergenic peanut epitopes, was found in patients with more severe allergic reactions. Also, sensitization of effector cells, i.e. human basophils and an FcεII-transfected rat basophil leukemia cell line, with more “diverse” IgE antibodies conferred greater reactivity to peanut allergen. This qualitative difference in IgE-specific epitope diversity may provide information about the potential severity of clinical reactivity in food allergic patients.

75

Anti-Interleukin-5 in the treatment of hypereosinophilic skin diseases

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Eosinophils and granulocytes represent a major component of the inflammatory infiltrate in various allergic and non-allergic skin diseases. Interleukin-5 (IL-5) has been found to be the relevant cytokine in recruitment and activation of eosinophils. Therefore, anti-IL-5 treatment was tried in several skin diseases with eosinophil participation.

Methods: Three patients with hypereosinophilic syndrome (HES), one patient with angiolymphoid hyperplasia with eosinophilia (ALHE) and 18 patients with atopic eczema (AE) were treated with anti-IL-5 (mepolizumab, 750 mg i.v.). In AE, a placebo group was included and the effect on clinical symptoms as well as atopy patch test (APT) was evaluated.

Results: All three patients with HES responded dramatically within the first days after anti-IL-5 injection both in clinical symptoms (skin lesions and itch) as well as in cellular and serum parameters: There was a significant drop in eosinophils, eosinophil cationic protein (ECP), IL-5, eotaxin, thymus- and activation-regulated chemokine (TARC); in lymphocyte cultures, secretion of IL-4, IL-5, IL-10 and IL-13 was decreased, while IL-2, IFNγ and TNF α remained unaffected. Furthermore, eosinophils and eosinophil products showed a strong decrease in skin biopsies. Similarly, there was a reduction in thickness and inflammatory infiltrate in the patient with AHLE. Patients with AE showed improvement in skin symptoms significantly more pronounced after mepolizumab than after placebo (P<0,04); also APT reaction sites decreased markedly after treatment which was particularly significant for cat and house dust mite reactions. Mepolizumab was tolerated without systemic or local side effects. There was no general immunosuppresion in lymphocyte parameters, but rather a shift from Th2 towards Th1 reactivity.

Conclusion: Mepolizumab effectively controlled eosinophilic dermatitis in patients with HES and improved inflammatory APT reactions as well as general skin symptoms in AE. Future studies with anti-IL-5 antibody in eosinophilic skin diseases are warranted.

76

Human eosinophils regulate T-cell functions through induction of indoleamine 2,3-dioxygenase

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Asthma represents a complex heterogeneous set of syndromes that are immunologically regulated. It is now generally accepted that asthma and allergic disease are characterized by a T-helper 2 (Th2) type response in the airways and systemically, where IgE, mast cells, eosinophils and Th2 type cytokines and chemokines predominate. Eosinophils are thought to be key effector cells in the airway tissue of asthmatic subjects putatively contributing to tissue damage and inflammation. However, the precise mechanisms by which the immune response is skewed towards the Th2 pattern remain elusive.

Indoleamine 2,3-dioxygenase (IDO), a rate-limiting enzyme in tryptophan catabolism, is inducible by IFNγ, resulting in kynurenine (KYN) production, which inhibits proliferation and induce apoptosis of Th1 cells; an important mechanism of inhibition of T-cell function by regulatory dendritic cells. Eosinophils increase during allergen challenge and may interact with T-cells. Our work has led us to the hypothesis that eosinophil-derived IDO play an immunomodulatory role in the maintenance of Th1-Th2 polarization in allergy.

To address this important question, human eosinophils purified from atopic and non-atopic donors and were probed for IDO expression by RT-PCR and Western blotting. KYN was measured to determine IDO enzymatic activity. We measured peripheral blood lymphocyte apoptosis by Annexin V following culture with PHA or IFNγ-treated eosinophils. We investigated IDO expression in lung tissue of allergic patients, and in tissues from a mouse model of allergic inflammation, by immunohistochemistry.

IFNγ-treated eosinophils expressed IDO mRNA and protein, and produced KYN. IL-3, IL-5 and GM-CSF had no effect separately but potentiated IDO induction by IFNγ-treated eosinophils. Eosinophils from atopic but not normal donors, expressed IDO constitutively. Lymphocytes cocultured with IDO-expressing eosinophils underwent apoptosis, which was blocked by the IDO inhibitor, 1-methyl-tryptophan. PHA-induced lymphocyte proliferation was inhibited by eosinophil-derived IDO. In addition, we observed extensive infiltration by IDO-expressing eosinophils in lymphoid aggregates from asthma. Additionally, eosinophils were the main IDO-expressing cells found in the lung of OVA-sensitized mouse model of allergic inflammation.

We conclude that eosinophils may play an important role in regulating T-cell function, *in vivo*, through IDO induction and thus contribute directly to the maintenance of Th2 polarization observed in asthma.

77

Human eosinophils express non-TLR receptors involved in innate immune responses

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If dendritic cells are the more potent cells of the immune system, able to activate naïve T cells, eosinophils have also recently been involved in innate immunity, able, as many other cell types, to bridge the very rapid innate system and the delayed adaptive immune responses. Indeed, eosinophils are well equipped with receptors participating in innate immunity, such as various TLRs, receptors for lipid mediators or lectin-type receptors. They also express surface receptors involved in adaptive immunity, including Fc receptors. In addition to their potential role as effector cells in parasitic infections and in allergy, eosinophils are suspected to participate in physiologic development, whereas their degranulated aspect in normal gastrointestinal tract suggests a role in immune surveillance of healthy tissues. Indeed, eosinophils can interact directly with several pathogens, such as bacteria, viruses, fungi and parasites. Our recent results indicate, that using RT-PCR, FACS analysis, immunostaining and functional assays, human eosinophils can express totally unexpected receptors, typically involved in innate immunity, and confirm the potential role of eosinophils in innate responses.

78

Comparison of Expression and Function of Toll-like Receptors in Eosinophils and Neutrophils

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The roles of eosinophils in innate immune responses have not been completely elucidated. Recently, Toll-like receptors (TLRs) were shown to play a critical role in innate immunity, but the expression profile of TLRs has not been precisely investigated in eosinophils. We investigated and compared the expression of a panel of TLRs and their functions in human eosinophils and neutrophils. The levels of TLR1~10 mRNA expression were quantitated by real-time PCR and functional activation by TLR ligands were also investigated. Eosinophils constitutively expressed TLR1, TLR4, TLR7, TLR9 and TLR10 mRNAs (TLR4 > TLR1, TLR7, TLR9, TLR10 > TLR6). On the other hand, neutrophils expressed a larger variety of TLR mRNAs (TLR1, TLR2, TLR4, TLR6, TLR8 > TLR5, TLR9, TLR10 > TLR7). Although the expression levels in eosinophils were generally less prominent compared with those in neutrophils, eosinophils expressed a higher level of TLR7. Furthermore, among various TLR ligands (Pam_3Cys-Ser-$(Lys)_4$, poly I:C, LPS, R-848, and CpG DNA), only R-848, a ligand of TLR7 and TLR8, regulated adhesion molecule (CD11b and L-selectin) expression, prolonged survival and induced superoxide generation in eosinophils. In contrast, neutrophils responded to TLR2, TLR4, and TLR7/8 ligands, which induced survival prolongation, regulation of adhesion molecule expression, and IL-8 generation in neutrophils. Stimulation of eosinophils by R-848 led to p38 mitogen-activated protein kinase (MAPK) activation, and SB203580, a p38 MAPK inhibitor, almost completely attenuated R-848-induced superoxide generation. Although TLR8 mRNA expression was hardly detectable in freshly isolated eosinophils, mRNA expression of TLR8 as well as TLR7 was exclusively up-regulated by IFN-γ but not by either IL-4 or IL-5. The up-regulation of the TLRs by IFN-γ had potentially functional significance: the extent of R-848-induced modulation of adhesion molecule expression was significantly greater in eosinophils treated with IFN-γ compared with untreated eosinophils. Although the natural ligands for TLR7 and TLR8 have not yet been identified,

our results suggest that eosinophil TLR7/8 systems represent a potentially important mechanism of a host-defensive role against viral infection and mechanism linking exacerbation of allergic inflammation and viral infection. A wide variety of functional expressions of TLR in neutrophils suggest its prominent role in host defense.

79

Signaling Effects Induced By Human Recombinant Histamine Releasing Factor in Basophils With the Hyperreleasable Phenotype.

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Rationale: We previously identified a negative correlation between histamine release to human recombinant histamine releasing factor (HrHRF) and protein levels of the phosphatase SHIP in basophils from hyperreleasable donors. Additionally, in unpublished studies, we have demonstrated prolonged kinetics of phosphorylation of the kinase ERK in anti-IgE stimulated basophils from HrHRF-responders compared to HrHRF-nonresponders. The purpose of this study was to begin characterization of signal transduction directly induced by HrHRF. Methods: Basophils were purified by negative selection. To measure cytosolic free calcium, basophils were pre-loaded with Fura-2, stimulated with several concentrations of HrHRF, or 0.5 μg/ml anti-IgE (as a positive control) and changes in calcium were quantified by digital videomicroscopy. To measure changes in ERK phosphorylation, 2 x 10^5 basophils were stimulated with several concentrations of HrHRF and Western blots were performed on cell lysates (probed with antibodies to phospho-ERK). Kinetics of ERK phosphorylation were also performed. Results: There was a biphasic calcium response to HrHRF, the magnitude of which corresponded to the magnitude of the histamine release to HrHRF. One donor who did not release histamine to HrHRF had no calcium response to HrHRF. Erk phosphorylation in response to HrHRF reached a maximum at the highest concentration of HrHRF (24 μM) that could be tested (as did histamine release) (n=4). There was no detectable ERK phosphorylation in HrHRF nonresponders (n=4). ERK phosphorylation in the four HrHRF responder donors began as early as 1 minute but unlike the antigen and anti-IgE response in nonresponders, was found elevated even at 60 minutes at levels higher than earlier time points. Conclusions: For the first time these results show that HrHRF alone stimulates signaling that has been associated with other receptor-dependent mediator release. Remarkably, the cytosolic calcium response mimics many of the characteristics observed for stimulation with anti-IgE antibody. In contrast, the kinetic behavior of Erk phosphorylation was unlike antigen or anti-IgE but also unlike non-IgE-dependent stimuli. None of these changes were observed in nonresponder basophils.

80

A specific CCR3 chemokine receptor antagonist inhibits both early and late phase allergic inflammation

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Allergic inflammation manifests as one of a number of diseases, including: asthma, dermatitis, vernal keratoconjunctivitis and systemic anaphylaxis. The diseases are often biphasic, with an early phase (occurring within the first hour after allergen exposure) driven primarily by mast cell degranulation, and a late phase (occurring 6-36 hours post-allergen exposure) characterized by leukocyte recruitment. Eosinophil recruitment is a common feature of the late phase reaction, and this is largely governed by CC chemokines that signal through the chemokine receptor CCR3. This view is supported by experiments involving in vivo antibody blockade of CCR3 or using CCR3-deficient mice. Some of these experiments also suggest that the CCR3 receptor also regulates mast cell involvement in airway inflammation. These studies have indicated that CCR3 antagonism might be effective in blocking both the early and late phase allergic reactions. We show here that administration of a single oral dose of a potent and highly specific CCR3 antagonist ablates both the early and late phase reactions in a mouse model of allergic conjunctivitis. A direct analysis of mast cells in the conjunctiva demonstrates that antagonism of the CCR3 receptor stabilizes the mast cell in vivo, thereby leading to the impaired early phase reaction. The late phase reaction is also strongly inhibited as characterized by both reduced eosinophilia and neutrophilia. These results suggest that 1) signaling from the CCR3 receptor is essential to prime conjunctival mast cells for FcεRI-mediated activation and 2) that antagonism of CCR3 has clear potential for the treatment of allergic diseases.

81

A candidate vaccine for specific immunotherapy in latex allergy: hypoallergenic mutants of the major allergen Hev b 6.01.

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Specific immunotherapy (SIT) is confirmed as efficacious in asthma and rhinitis by meta-analyses. Natural rubber latex (Hevea brasiliensis) allergy is a major cause of occupational asthma among latex glove users, associated with significant risk of anaphylaxis. Current unfractionated latex extracts are unavailable for SIT due to high anaphylactic potential. Hypoallergenic mutant recombinant latex allergens with abrogated IgE binding or T-cell epitope based peptide analogues potentially offer safer alternatives for SIT. Hev b 6.01 is one of the two major allergens in latex gloves, with sensitisation of 70% of latex glove allergic subjects. The predominant IgE reactivity of Hev b 6.01 is located in the N-terminal hevein (Hev b 6.02) domain where four disulphide bonds stabilise tertiary conformation. A panel of mutants of recombinant Hev b 6.01 and corresponding synthetic hevein (Hev b 6.02) peptides were generated, with successive disruption of the four disulphide bonds by cysteine to alanine substitutions. These mutants showed markedly decreased or ablated binding of IgE in latex-allergic patient sera in inhibition ELISA. The whole blood basophil activation test detecting CD63 surface expression by flow cytometry as a marker of basophil activation was used as a biologically relevant assay for IgE binding, showing markedly decreased activation with each successive cysteine substitution of Hev b 6.01 and 6.02. Critically, the mutants maintained their T-cell reactivity by induction of T-cell proliferation of oligoclonal CD4+ latex-specific T cells from latex-allergic donors. Our findings suggest that the Hev b 6.02 peptide with four cysteine to alanine substitutions together with the dominant T-cell epitope based peptides of Hev b 5 that we have reported previously may together offer a safe and effective preparation for SIT for latex allergy treatment and/or prevention. The use of these hypoallergenic forms of the T-cell epitope derived peptides increases the feasibility of high dose SIT for latex allergy with the concurrent induction of T regulatory cells. We have recently demonstrated the expansion of a migratory CD4+CD25+ IL-10 producing regulatory T-cell subset, expressing peripheral tissue-trafficking markers, in clinically effective house dust mite SIT. The development of high dose hypoallergenic T-cell epitope based peptide regimens with promotion of induction of T regulatory cells should allow the downregulation of both Th2- and Th1-mediated pathology and improve efficacy and safety of SIT, including that for potent allergens such as latex.

82

Specific immunotherapy with recombinant grass pollen allergens is clinically effective in the management of allergic rhinoconjunctivitis

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Background Preparations based on whole grass pollen extracts are used successfully for allergen specific immunotherapy. Recombinant DNA technology provides the possibility to create therapeutic vaccines containing only relevant allergenic proteins. Such preparations promise many advantages with respect to pharmaceutical quality, standardisation, dosage formulation etc., but their clinical efficacy has not previously been investigated.
Method A double-blind placebo-controlled clinical trial was undertaken with 62 grass pollen allergic patients suffering from rhinoconjunctivitis with or without asthma. Five recombinant pollen allergens from *Phleum pratense* were cloned and expressed in *E. coli*, purified using various chromatographic procedures and adsorbed to aluminium hydroxide. A cocktail was produced such that the maximum dose for subcutaneous injection contained 10μg Phlp1, 5μg Phlp2, 10μg Phlp5a, 10μg Phlp5b and 5 μg Phlp6. Injections of increasing concentrations were given at 7-day intervals prior to the pollen season in 2002 and maintenance injections were continued until after the subsequent pollen season.
Results Per protocol analysis included 24 active treatment and 25 placebo patients. A combined symptom-medication-score adopted as primary end-point showed a 39% improvement in the active treatment group relative to placebo (p=0.041). Symptom score alone showed an improvement of 37% (p=0.015). A validated Rhinitis Quality of Life Questionnaire registered an overall significant benefit (p=0.024) from active treatment. Significant effects were seen in 5 of 7 domains tested: activities (p=0.040), non-hay-fever

symptoms (p=0.032), practical problems (p=0.040), nasal symptoms (p=0.016) and eye symptoms (p=0.007). A conjunctival provocation test showed a favourable trend (p=0.081) with an increase in the threshold dose. Highly significant increases in allergen specific IgG1 and IgG4 were measured. Adverse events were seen with 10.4% of injections of active preparation and 5.9% of placebo, mainly as mild local reactions. Only 6 of 731 injections (0.8%) in the active treatment group were associated with systemic reactions like rhinitis or urticaria.
Conclusion Subcutaneous injection immunotherapy with a cocktail of five recombinant grass pollen allergens was shown to be clinically effective and well tolerated in the management of seasonal hay fever.

83

Inhalation allergy and desensitisation to a cysteine protease allergen

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The inhalation or intranasal administration of proteins induces mucosal tolerance. It has therefore been difficult to develop appropriate models of allergic sensitisation and to test new types of immunotherapy with defined allergens and formulations of allergens. It is shown here that the intranasal administration of low doses of papain, a potent occupational allergen and biochemical homologue of Der p 1, induces persistent and boostable Th2 - type allergic sensitisation in mice, without the need for injections or adjuvant. The mice produce IgE and, after respiratory challenge, eosinophilic infiltration, mucous production and Th2 cytokines. Only the eosinophilia and not the IgE was affected by inhibiting the cysteine protease activity, and even this was quantitative. A comparison of systemic and mucosal routes for immunotherapy of respiratory sensitisation was made. Subcutaneous injections of allergen ameliorated all aspects of respiratory sensitisation when given before or after sensitisation. In contrast, while intranasal peptides containing T-cell epitopes inhibited IgE and lung-challenge responses in mice sensitised by the injection of allergen in alum, they did not prevent sensitisation initiated by the respiratory route. The ineffectiveness was accompanied by an exacerbation of the tissue damage induce by the inhalation of allergen. The use of only the respiratory route instead of incorporating parenteral injections has thus shown that differences in the regulation of mucosal sensitisation need to be addressed by new types of immunotherapy. The ability to use defined protein formulations for respiratory sensitisation will aid the ongoing investigations to improve immunotherapeutic strategies and to test adverse as well as positive outcomes.

84

Therapy-induced alterations of the allergic response through a novel allergy vaccine based on genetically modified allergens

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Birch pollen allergy affects millions of people world-wide. A majority of the patients have IgE antibodies to the major birch pollen allergen Bet v 1. The only curative therapy of IgE-mediated allergy, allergen-specific immunotherapy (SIT), is currently performed with crude allergen extracts. In this study we describe a novel type of allergy vaccine based on genetically engineered derivatives of Bet v 1. One Bet v 1-derived vaccine consisted of an equimolar mixture of two recombinant Bet v 1 fragments (Bet v 1 1-73 and Bet v 1 74-159) and the second was a recombinant Bet v 1 trimer. Both vaccines exhibited a strongly reduced allergenic activity. A double-blind, multi-centre immunotherapy study was performed in three European centres. In total 124 birch pollen allergic patients were divided into three groups who received pre-seasonal treatment with Bet v 1 trimer, Bet v 1 fragments or placebo. We here report the immunological effects obtained from the treatment in one of the centres (Stockholm, Sweden). Blood samples from 27 patients were analysed by ELISpot for the profile of cytokine-secreting cells and for allergen-specific serum antibody levels, measured by ELISA. A strong induction of Bet v 1-specific IgG, consisting of increased IgG_1, IgG_2 and IgG_4 levels was observed after treatment with both vaccines. Interestingly, only treatment with Bet v 1-trimer led to a significant reduction of Bet v 1-reactive IL-5 and IL-13 producing cells reflecting a reduced Th2-response. In addition, a decreased number of Bet v 1-reactive IL-4- and an increase of IL-12-producing cells was noted in trimer-treated patients. Finally an improvement score was calculated based on immunological changes, reflecting a reduced Th2 response, and clinical parameters. The Bet v 1 trimer treated patients had significantly higher improvement scores than the placebo group. This is the first report demonstrating that a vaccine based on a genetically modified allergen results in a reduction of Th2-responses. Thus, this novel type of allergy vaccine leads to clear beneficial immunological changes in birch pollen allergic patients.

85

T cell peptide therapy results in improvements in objective and subjective outcome measures in asthma and rhinitis.

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Rationale: We have previously demonstrated improvements in surrogate markers of allergic disease following T cell peptide therapy. More recently, we evaluated clinical outcomes and quality of life (QOL). Additionally, effects on peripheral blood and tissue responses to allergen were further defined.
Methods: Cat allergic asthmatics were recruited into a series of studies and received incremental doses of 12 peptides from Fel d 1 allergen delivered intradermally. Cumulative doses were 41.1 or 291mcg. Outcomes included: *clinical*- magnitude of late asthmatic reaction (LAR; 2-8 hours), allergen PD_{20}, histamine PC_{20}, nasal and cutaneous allergen challenge, QOL (Juniper); *laboratory*- immunohistochemistry of cutaneous allergen challenge biopsies and T cell responses.
Results: Following 291mcg of peptides, the magnitude of the LAR was decreased in peptide-treated subjects (p=0.035; 3 month follow-up). Histamine PC_{20} significantly improved following 41.1mcg (p=0.022) but not higher doses. In nasal outcome measures (291mcg only), improvements were observed in weight of nasal secretions (p=0.04 within group *wg*; p=0.01 between group *bg*), nasal blockage (p=0.05 *wg*; p=0.001 *bg*), number of sneezes (p=0.04 *wg*; p=0.02 *bg*). Of four asthma QOL fields only *Activity Limitation* showed improvement (p=0.014 *wg*). Four of seven rhinitis fields demonstrated improvements: *Sleep* (p=0.024 *wg*), *Non nose/eye symptoms* (p=0.03 *wg*; p=0.035 *bg*), *Practical problems* (p=0.016 *bg*), *Nasal problems* (p=0.015 *wg*; p=0.015 *bg*). Analysis of 24hr skin biopsies (41.1mcg regimen), demonstrated an increase in CD4+CD25+ (p= 0.04) and CD4+IFNγ+ T cells (p=0.03) after allergen challenge. Analysis of T cell responses demonstrated a reduction in proliferation of CD4+ cells (p=0.016) and CD8+ cells (p=0.031), and modulation of cytokine responses. IL-10 production increased while IL-5 and IFNγ decreased.
Conclusions: Peptide immunotherapy is associated with improvements in a range of clinical and surrogate outcomes. Higher doses may compromise efficacy by inducing activation in certain compartments. Increased recruitment of CD4+CD25+ and CD4+/IFN γ+ cells in the skin and enhanced IL-10 production support the induction of allergen-specific regulation.

86

Inhibition of allergen-IgE binding to B cells following a successful grass pollen immunotherapy: influence of withdrawal following two years' treatment

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Grass pollen immunotherapy was highly effective in reducing seasonal rhinitis and asthma symptom scores. Clinical improvement was associated with increases in serum inhibitory activity for Phleum P allergen-IgE binding to B cells that co-purified with IgG4 (Nouri-Aria et al, J Immunol 2004). After 2 years treatment (1996-98), 13 of the 20 patients who had received the active therapy agreed to undergo a rondomised double-blind withdrawal of immunotherapy. Over 2 years (1998-2000), 7 patients continued to receive monthly maintenance injections with grass allergen vaccine (Alutard SQ, Phleum parntense. ALK Abello, Horsholm, Denmark) and 6 received matched histamine-containing placebo injections. In 2000, when compared to baseline (1996), both placebo- and actively-treated groups continued to have significant reductions (p<0.04) in combined seasonal symptom and rescue medication scores and persistence to elevated serum inhibitory

activity for allergen-IgE to B cells ($p<0.03$), In contrast, in the withdrawal group, there was a marked reduction in serum allergen-specific IgG and IgG4 ($p<0.03$) towards baseline in 2000 at a time when the serum inhibitory activity remained elevated. These data suggest that serum IgG-associated 'blocking activity' rather than immunoreactive IgG or IgG4 may be more predictive of the clinical response to immunotherapy.

87

Identification of third markers on human CD4+CD25+ T regulatory cells from peripheral blood

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BACKGROUND: The $CD4^+CD25^+$ Treg population is characterized by a constitutively high expression of the IL-2R a chain ($CD25^{high}$) found in the thymus and peripheral blood of human. These cells exhibit immunoregulatory properties and low proliferative capacity. However, the phenotypic features of human $CD4^+CD25^+$ Treg cells so far described are controversial.

PURPOSE: To investigate phenotypic markers of human $CD4^+CD25^+$ Treg cells from peripheral blood.

METHODS: Freshly isolated PBL were assessed by FACS analysis for the expression of CD4, CD25 and third markers by gating on a small population of $CD4^+CD25^{high}$ cells (on average 7% of $CD4^+$ cells). $CD4^+CD25^+$ Treg cells were purified by MACS human $CD4^+CD25^+$ Treg cell isolation kit. mRNA concentrations of third markers were measured in purified $CD4^+CD25^+$ versus $CD4^+CD25^-$ cells by quantitative RT-PCR.

RESULTS: Freshly isolated PBL were assessed by FACS analysis for the expression of CD4, CD25 and third markers. We found, CD62L, CCR4 and CCR7 were highly expressed on both $CD4^+CD25^+$ and $CD4^+CD25^-$ cells, while CD103, CCR5, CCR8 and CTLA-4 were not detectable on the $CD4^+CD25^+$ cells.
To better characterize the human $CD4^+CD25^+$ population, mRNA concentrations of Foxp3, GITR, CD62L, CD103, CCR4, CCR5, CCR7, CCR8, TGF-b and CTLA-4 were measured in purified $CD4^+CD25^+$ versus $CD4^+CD25^-$ cells by quantitative RT-PCR.
$CD4^+CD25^+$ Treg cells expressed high level of Foxp3 mRNA, whereas $CD4^+CD25^-$ cells had poor Foxp3 mRNA expression. GITR mRNA was not detectable on $CD4^+CD25^+$ or $CD4^+CD25^-$ cells. CD62L, CCR4, CCR5, CCR7 and TGF-b mRNA were detectable on both $CD4^+CD25^+$ and $CD4^+CD25^-$ cells, but they did not show significant differences between the two populations. CTLA-4 mRNA on $CD4^+CD25^+$ cells was expressed nearly two times higher than on $CD4^+CD25^-$ cells. CD103 and CCR8 mRNA were not detectable.

CONCLUSSIONS: Because Foxp3 and GITR are considered the markers of $CD4^+CD25^+$ Treg cells, mRNA concentrations of Foxp3 and GITR were measured in purified $CD4^+CD25^+$ and $CD4^+CD25^-$ cells, but GITR mRNA was not detectable in any of these subpopulations. Our findings suggest that the majority of human $CD4^+CD25^+$ T regulatory (Treg) cells are positive for CD62L, CCR4, CCR5, CCR7, TGF-b, CTLA-4, and negative for CD103 and CCR8.
Interestingly, a combinational code of CD62L and CCR7 expression is essential for entry into peripheral lymph nodes. These steps are essential for the following integrin activation, firm adhesion, and transmigration. Chemokine receptors being detectable at high levels suggest that $CD4^+CD25^+$ Treg cells may be attracted to inflamed tissues to regulate or prevent autoimmune disease. These findings will help us understand the mechanism of function of human $CD4^+CD25^+$ Treg cells.

88

REDUCED DERMAL INFILTRATION OF CYTOKINE-PRODUCING INFLAMMATORY CELLS IN ATOPIC DERMATITIS FOLLOWING SHORT-TERM TOPICAL TACROLIMUS TREATMENT

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Background: In several clinical studies, topical immunomodulators have been shown to be effective in the treatment of atopic dermatitis (AD). As calcineurin inhibitors, they target signaling pathways that control gene expression, in particular the expression of cytokines.

Objective: We examined the cellular infiltrate of skin lesions of ten AD patients and characterized the cytokine pattern expressed by the infiltrating cells before and after short-term topical therapy with tacrolimus 1% ointment.

Methods: Skin biopsies were examined for histological alterations (HE staining), composition of the inflammatory infiltrate (immunofluorescence) and cytokine expression (ELISA, immunofluorescence) one and three weeks after initiation of tacrolismus therapy. Systemic immunological effects were assessed by analyzing peripheral blood leukocytes (immunofluorescence) as well as in vitro stimulated pan-T cell cytokine production and proliferation (ELISA, lymphocyte proliferation test).

Results: All patients showed a significant improvement of their skin lesions associated with a marked regression of spongiosis, acanthosis, and of the density of the inflammatory infiltrate in the dermis. The latter was due to reduced infiltration of T cells, B cells, and eosinophils. In contrast, the numbers of mast cells did not change. Moreover, the expression of the T helper (Th) 2 cytokines interleukin (IL)-5, IL-10, and IL-13 in CD4+ T cells was reduced after therapy. Interestingly, tacrolimus therapy was also associated with a reduction of CD8+ T cells expressing the Th1 cytokine interferon-γ. Furthermore, the numbers of epidermal CD1a+ dendritic cells increased following treatment. In the peripheral blood, a decrease of granulocytes (eosinophils and neutrophils), but no changes in the distribution of lymphocyte subpopulations were noticed.

Conclusion: Topical tacrolimus treatment has anti-inflammatory effects on AD skin as indicated by reduced infiltration of cytokine expressing inflammatory cells. No evidence for drug-induced systemic immunosuppression was obtained.

89

Human manganese superoxide dismutase – a stress-inducible enzyme – can elicit IgE- and T cell-mediated reactions in atopic dermatitis

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Purpose: IgE-mediated autoreactivity of atopic dermatitis (AD) patients to human proteins suggests self antigens as decisive factor driving disease exacerbation. Here we investigated the role of human manganese superoxide dismutase (hMnSOD) – a stress-inducible enzyme – in the pathogenesis of AD.

Methods: Structurally related, recombinant fungal and human MnSOD were used to assess the differential reactivity of AD patients. Antigen specific humoral and cellular immune responses were studied ty determination of specific IgE and T cell proliferation *in vitro* and by skin prick tests (SPT) and atopy patch tests (APT) *in vivo*. Differential expression of MnSOD in inflammatory skin was determined by immunohistochemistry.

Results: hMnSOD-specific immunoglobulin E (IgE) antibodies determined by ELISA was found in sera from 29 out of 67 AD patients (36%), but not in sera of other patients or healthy controls – correlating significantly with disease activity ($R=0.783$, $p<0.0001$). In PBMC of sensitised patients, rhMnSOD induced T cell proliferation and showed *in vivo* reactivity, demonstrated by positive skin prick- and atopy patch tests. Expression of MnSOD in lesional skin of AD was stage-dependantly upregulated. Primary sensitization to hMnSOD

might be induced by cross-reacting fungal MnSOD as all patients showed positive skin reactions to fungal extracts. Moreover, rhMnSOD was able to compete for IgE binding to fungal extracts in CAP inhibition experiments.

Conclusions: These data provide strong evidence for autoreactivity against hMnSOD in a relevant subset of AD patients patients probably deriving from crosssensitization against environmental MnSOD exposure. Whether such autoreactivity against hMnSOD is a primary pathogenetic factor or just a secondary phenomenon due to disease-exacerbation needs to be further investigated.

90
Withdrawn

91
Peripheral blood mononuclear cells of nickel-allergic and non-allergic individuals display different apoptotic threshold upon exposure to nickel and metal ions released by euro coins

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Upon stimulation with metal ions antigen-specific T-cell proliferation in vitro can be observed in peripheral blood mononuclear cells (PBMC) of allergic individuals. On the other hand after inappropriate stimulation or by toxic effects, apoptosis may occur. In this study we assessed the apoptotic threshold of human PBMC in vitro upon exposure to nickel or eluates from euro coins containing nickel and copper. PBMC of 7 nickel-allergic patients (history and patch test positive) and 9 non-allergic controls were cultured for 72h in the presence of either medium, PHA, $NiSO_4$ or different dilutions of euro eluates. Apoptosis was detected by Annexin V staining followed by FACS-analysis. To further characterize and detect cells at an early stage of apoptosis, the percentage of Annexin V positive and propidium iodide negative cells was determined. In comparison to Ni-allergics, the PBMC of the control patients more easily underwent apoptosis after stimulation with PHA (19.3% vs 15.4%), $NiSO_4$ 10^{-5}M (0.66% vs 0.4%) and 3 different euro eluate dilutions (1/100, 17.7% vs 4.9%; 1/1000, 12.2% vs 2.3%; 1/10000, 6.5% vs 1.8%). Furthermore, there were marked interindividual differences with regard to apoptotic reactivity, irrespective of allergic or non-allergic status. However all except one individual were non-smokers and none had ongoing disease or systemic medication. Thus these data suggest, that PBMC of nickel allergics in general respond to PHA and the here tested metals with less cellular apoptosis than the PBMC of the controls. In addition, cell viability and apoptotic threshold differed markedly among individuals. The significance of these findings in relation to clinical reactions is under investigation.

92
Effects of cannabinoid agonists on histamine release from activated human skin mast cells

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Mast cells (MC) have been reported to express cannabinoid receptors that are utilized by endocannabinoids and cannabimimetics to alter their secretory functions. However, the data gathered so far are controversial and appear to be species- and tissue-specific. We have therefore investigated MC isolated from normal human skin (purity 90%) regarding the possible impact of palmitoylethanolamide (PEA) and the synthetic compound WIN55,212-2 on their activation process. Both agonists suppressed histamine release elicited by cross-linking of high-affinity IgE recptor in a dose-dependent fashion with significant inhibition starting at 4 µM (PEA) and 2 µM (WIN55,212-2), respectively. Pertussis toxin was able to fully abolish the effect from PEA, suggesting an involvement of G_i/G_o. Finally, various skin MC preparations, along with appropriate positive control cells, were assayed for the expression of CB1 and CB2, the two known receptors transducing cannabinoid signals. Transcripts for both receptors could be detected in skin MC, with a preferential expression of brain-type CB1. CB1 was additionally detected in a variety of other immune cell types, so that its participation in immune modulation may in fact be broader than hitherto suspected. In summary, we conclude that mast cells in human skin are responsive to cannabinoid agonists which may point at a further system by which communication between MC and nerves is accomplished. The data support the view of an antiinflammatory and immune modulatory function of the endocannabinoid system.

93
Neuroimmunological Crosstalk in Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory pruritic skin disease characterized by an influx of activated CD4+ T-lymphocytes, eosinophils and an increase of antigen presenting Langerhans-cells. Even though immunological interactions have been well described in AD the interaction between the nervous and immune system still remains unkown. Recently, neurotrophins that are produced by sensory nerves and immune cells, have been described as key players in allergic asthma supporting inflammatory conditions and neuronal hyperreactivity. As AD is characterized by increased density of sensory nerves, neurotrophins are suggested as possible mediators liniking nervous- and immunological interactions also in AD. To further reveal the functional role of neurotrophins on eosinophils as target cells of AD the following study had been assessed.
Peripheral blood eosinophils of AD patients (defined according to the criteria of Hanifin and Raijka) were purified by CD16 negative selection and stimulated with Brain-derived neurotrophic factor (BDNF), Neurotrophin (NT)-3, and Nerve growth factor (NGF) for 24 to 120 hours. Apoptotic eosinophils were investigated by Annexin V method and by determining their hypodiploid DNA peak, respectively. Chemotactic index was assessed in a modified Boyden chamber assay, respiratory burst by lucigenin-dependent chemiluminescence and BDNF production with BDNF-ELISA.
Stimulation with BDNF, NT-3 and NGF significantly inhibited the programmed cell death of AD eosinophils ($p<0.05$-0.01) at each time point (24 hours up to 120 hours). Chemotactic index was significantly increased after stimulation with BDNF and NT-3 ($p<0.05$-0.01). Respiratory burst of AD eosinophils was not modified after stimulation with BDNF, NT-3 or NGF. BDNF was detectable in AD eosinophils and BDNF levels were significantly increased compared to controls ($p<0.01$).
To summarize, neurotrophins such as BDNF, NT-3 and NGF have a functional role on peripheral blood eosinophils of AD. Moreover, eosinophils are a source of neurotrophins such as BDNF itself. Therefore, it is very likely that neurotrophins are pivotal players in the neuroimmunological interactions of AD revealing new aspects of AD pathophysiology.

94
NEW METHODS FOR QUANTITATIVE AND QUALITATIVE ASSESSMENT OF ITCH

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Itch is a major subjective symptom of allergic diseases; with its well-known psychophysiologic aspects it has substantial impact on the quality of life of patients. We investigated instruments for qualitative and quantitative assessment of the itch sensation. Objective covariates of itch and differences to pain processing were shown using imaging techniques for the CNS: a complex pattern of cerebral activation after experimental itch induction with histamine was observed in a $H_2{}^{15}O$ PET correlation study in healthy volunteers (n=6). Subtraction analysis versus control revealed significant activation of the left primary sensory cortex and motor-associated areas, predominantly left-sided activations of frontal, orbitofrontal and superior temporal cortex and anterior cingulate. Comparing to studies in a pain model, no thalamus activation, but significant activation in the insula region and differences in sensory, motor and cingulate activation were seen. Quantity and quality of perceived itch show specific characteristics in different pruritic skin diseases. The multidimensional "Eppendorf Itch Questionnaire" (EIQ) was used in patients suffering from atopic eczema (AE, n=62) and chronic urticaria (CU, n=58). The mean visual analog scale (VAS) ratings of itch intensity showed no significant difference between the two diseases (74.5 ± 2.6 % in AE vs. 75.1 ± 2.4 % in CU). In contrast, the total EIQ score was significantly higher in the AE group with 231.7 ± 11.6 vs. 175.2 ± 9.5. In 34 of 127 items, a significantly different rating was obtained, mostly with higher load for affective and some sensory items in AE. Significant differences were also seen in the description of the scratch response.

Conclusions: Itch perception in AE and CU differs on a qualitative level, influencing items relevant for quality of life. Group-specific peculiarities not measurable with VAS were mirrored by the EIQ scores. These findings can be interpreted as differences in CNS processing of a nociceptive sensation. New models to measure itch may be useful for the development of new therapeutic strategies against pruritus.

95

Expression of two neuropeptide receptors (calcitonin gene related peptide and somatostatin) in peripheral blood mononuclear cells from patients with atopic dermatitis and non-atopic controls

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There is increasing evidence that neuropeptides (NP) may be involved in the pathogenesis of atopic dermatitis (AD). Exacerbations of AD can be provoked by stress, and several studies have demonstrated changes in skin levels of NP in AD patients. The aim of this study was to evaluate the expression of NP receptors in peripheral blood mononuclear cells from patients with acute and chronic AD lesions compared with non-atopic controls.
We evaluated five patients with acute AD lesions (lesion onset fewer than 3 days before the study), eight patients with chronic AD lesions (lesion onset two weeks or more before the study) and ten non-atopic controls. PBMC were obtained and total RNA was used for cDNA synthesis. CGRPR and SSTR were amplified by semi-quantitative real time-PCR with SYBR-Green, using PBGD (Porphobilinogen-Deaminase) transcript as a reference to normalize mRNA levels. Statistical analysis was performed with the Mann-Whitney test and P values of 0.05 or less were considered significant.
We found a decrease in expression of both CGRPR (median 0.16, IR 0.23-0.072) and SSTR (median 0.86, IR 1.39-0.32) in AD patients compared with controls (CGRPR: median 0.41, IR 2.31-0.17; SSTR: median 1.32, IR 2.18-0.34), although this was only significant in the first receptor (P=0.03). Comparison between patients with acute and chronic lesions showed no differences for any of the receptors.

CGRPR in PBMC from AD patients was down-regulated compared with controls. This is in agreement with other authors who have found down-regulation of VIP receptors in AD cutaneous lesions and of Substance P receptors in PBMC. Possible explanations include: PBMC in AD may have fewer receptors, chronic exposure to NP down-regulates the PBMC receptors, or the PBMC with more receptors are located in the skin. We also found that this down-regulation does not depend on the time interval elapsed from the onset of AD, with no differences between patients with acute and chronic AD lesions. Additonal studies are underway to determine the presence of NP receptors in different memory T-cell populations in AD. These findings suggest a role for this G-protein-coupled receptor in the pathophysiology of atopic dermatitis.

96

Neuropeptide influence on cytokine production in peripheral T cells in atopic dermatitis patients with acute and chronic lesions and in controls

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Atopic dermatitis (AD) is an inflammatory skin disease whose lesions can have two stages: acute and chronic. Pathogenic T lymphocytes in AD express the cutaneous lymphocyte-associated antigen (CLA). It has recently been suggested that neuropeptides (NP) may modulate cytokine production in memory T cells, which may influence the course of the disease. The aim of this study was to compare the effect on cytokine production of different NP in patients with acute and chronic AD lesions and in controls.
We evaluated five patients with acute AD (lesion onset fewer than three days before the study), five patients with chronic AD (lesion onset two weeks or more before the study) and 10 non-atopic controls. IL-4, IL-5, IL-13 and IFN-γ in both CLA+ and CLA- subsets were analyzed by flow cytometry in peripheral T-lymphocytes with and without incubation with calcitonin gene related peptide (CGRP), somatostatin, and substance P at 10^{-8}M. P values of 0.05 or less were considered significant.
Following incubation of PBMC with the different NP, CGRP induced an increase in IL-13 production in AD patients with acute and chronic lesions and a decrease in IL-4 production in AD patients with chronic lesions and in controls; somatostatin induced an increase in IL-13 and IFN-γ production in AD patients with acute lesions; and substance P induced no detectable changes. The changes were only observed in the CLA+ subset, not in the CLA- subset.
We can conclude that the effect on cytokine production of the NP studied, CGRP, somatostatin and substance P was limited exclusively to the CLA+ subset. This effect was lower than expected, based on previous studies performed in T cell lines. Somatostatin only induced changes in AD patients with acute lesions, CGRP in patients with acute and chronic lesions and substance P induced no change. These data show for the first time a significant and preferential effect of NP on circulating CLA+ T cells in AD. These results may help clarify the interaction between the nervous and immune systems in AD.

97

Eosinophilic Esophagitis: New Clinical and Pathophysiological Insights

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Background: Eosinophilic Esophagitis (EE), an inflammatory disorder of the esophagus, is one of the leading causes of dysphagia. The diagnosis is based on a dense infiltration of the esophageal epithelium with eosinophils. So far, neither the natural course of EE nor the mechanisms, leading to the eosinophilic tissue infiltration, are clearly defined.
Aims: The intention of this study was to characterize the natural course of EE and to determine the activation patterns of the eosinophils in inflamed and non-inflamed intestinal tissue.
Methods: 30 patients with previously confirmed EE underwent, after a mean time of 7.2 years, a comprehensive follow-up examination, including endoscopy and histometric analysis of the esophageal, gastric and duodenal mucosa. Additionally, the expressions of CD25 and CD137, as well as of the T_H2 cytokines IL-4 and IL-13 were determined in esophageal, intestinal and blood eosinophils from 8 patients and 4 controls.
Results: The dysphagia persisted in 28 patients and the eosinophilic infiltration persisted in all patients, although the cell number decreased significantly. No extension of the infiltration to stomach or duodenum appeared but the inflammation led to fibrosis of the esophageal lamina propria. In patients, approximately 60% of esophageal eosinophils expressed IL-4, IL-13 and CD137, suggesting both activation of and heterogeneity among these cells. Moreover, 66% of the intestinal eosinophils expressed IL-13, indicating that the inflammatory response may not be restricted to the esophagus. In controls, 42% of intestinal eosinophils but no blood eosinophils expressed IL-13, suggesting a physiologic activation process in the digestive tract.
Conclusions: EE is a primary-chronic, esophageal-restricted inflammation, which leads to persistent dysphagia and structural alterations of the esophageal tissue. Furthermore, our data indicate heterogeneity among eosinophils, both under inflammatory and non-inflammatory conditions. We suggest the following three patterns of activation of mucosal eosinophils: 1) *Primary Activation Pattern* observed at the inflammation site and reflecting the eosinophils' pivotal involvement in the inflammatory process; *2) Remote Activation Pattern* noted far from the inflammatory process and consistent with a systemic activation; and 3) *Baseline Activation Pattern* seen in the intestinal mucosa of healthy subjects, indicating that eosinophils may be actively involved in the barrier function.

98

Histamine release for determination of systemically absorbed allergenic proteins in humans

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Introduction
The amount and quality of absorbed dietary proteins may be important in the development of food allergy. In order to determine absorbed dietary proteins in human serum we developed an immunochemical method (ELISA). However this method was not sufficiently sensitive and did not provide information about protein allergenicity. Histamine release (HR) from

sensitized human basophils is an established biological method to detect protein allergenicity. The method can be modified by passive sensitization of basophils with allergic sera eliminating the need for fresh blood.

Aim
To develop a method for detection of allergens in human blood after oral intake of highly allergenic proteins such as peanut.

Materials and methods
Buffy coats were derived from healthy donors and basophils were purified by Lymphoprep centrifugation. IgE was removed from basophils by stripping and passively sensitized by incubation (37 EC/60 min) with serum from a verified peanut allergic patient. Following sensitization, basophils were challenged with peanut allergens. HR was determined by the glass fibre method and expressed in percentage of total histamine content. A HR>10 % was considered positive.

Results
Passive sensitization and direct HR from a peanut allergic patient were equally sensitive enabling the detection of peanut allergens in concentrations in the low pg/ml-range - considerably below the detection limit of the ELISA which was 0.5 ng/ml. Dose response curves of peanut diluted in buffer or in human serum were comparable allowing the detection of peanut allergens in serum. In a subsequent clinical study, peanut allergens were detected (60-80% HR) in serum after ingestion of 5-100 g peanut by non-allergic volunteers.

Conclusions
Passive sensitization of basophils is a sensitive method for detecting allergens in serum. The method can detect food allergens after oral intake and kinetics of uptake can be established.

99
Anti-acid medication promotes oral sensitization and hyperreactivity to hazelnut: experiments and preliminary epidemiology

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Recently, we have reported that anti-acid medication promotes oral sensitization to digestion-labile fish allergens, as it prevents peptic degradation of dietary proteins. In this study, we analyzed whether the same mechanism accounts for sensitization against hazelnut. This food is associated with pollen allergy via cross-reactivity, but there have come up several studies showing that hazelnut allergy can occur independent of other sensitizations.
Interestingly, when hazelnut extract was subjected to digestion with simulated gastric fluid, all proteins were degraded within seconds. However, digestion was completely prevented by elevating the pH. The in vivo impact of this observation was examined in our mouse food allergy model. Mice were fed hazelnut with or without concomitant anti-ulcer medication. Indeed, the anti-acid treated mice produced hazelnut-specific IgG1 antibodies, whereas the control group did not. Furthermore, these mice showed positive skin reactivity to hazelnut proteins. To characterize the biologic activity of induced antibodies, we purified them from the sera of the mouse group which was fed hazelnut in combination with Sucralfate. These murine IgG1 antibodies recognized proteins in hazelnut extract at the same molecular weight range as human IgE did. When they were applied in skin tests on naive mice, passive cutaneous anaphylaxis was evoked in all animals after i.v. application of hazelnut extract. Feedings in combination with Sucralfate, therefore, induced anaphylactogenic antibodies.
In addition to these animal experiments, we screened patients of a gastroenterologic clinic (n=153). None of these patients had a positive history of allergy before, but already 3 months after taking anti-ulcer drugs, 5 of these patients had developed specific IgE and 4 of these 5 patients positive SPT to hazelnut. Moreover, two of them showed clinical symptoms like acute urticaria or OAS after ingestion of hazelnut. None of the patients in an untreated control group (n=50) did develop allergy to hazelnut. From our experimental and epidemiological data we conclude that anti-acid drugs hinder protein digestion. This is likely to induce oral sensitization and true food allergy, also to digestion-sensitive proteins like hazelnut.

100
From Diagnosis to Therapy of Fish Allergy
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Parvalbumin, a small calcium-binding muscle protein, with remarkable resistance to heat and denaturing agents, represents the major allergen for 95% of patients suffering from IgE-mediated hypersensitivity to fish. By screening a cDNA expression library from carp (*Cyprinus carpio*) muscle with the serum from a fish allergic patient IgE-reactive parvalbumin clones were isolated. Overexpression of one cDNA in *Escherichia coli* led to the production of a folded, mainly α-helical recombinant parvalbumin with immunological features comparable to its natural counterpart. The finding that the recombinant allergen contained the majority of fish-specific IgE epitopes and showed biological activity in basophil histamine release assays indicates that this molecule represents a suitable tool for diagnosis of IgE-mediated fish allergy.

With the intention to develop a strategy for a safe treatment of fish allergic patients, we aimed to design hypoallergenic variants of recombinant parvalbumin. Introduction of point mutations in the two functional calcium-binding domains significantly reduced the overall fold of the molecule and gave a parvalbumin derivative whose IgE-binding capacity was almost completely abolished. Basophil histamine release assays further revealed a profound reduction in the allergenic activity of the mutated allergen. The therapeutic potential of the parvalbumin variant was indicated by the observation that mouse antibodies raised against the mutated molecule inhibited the binding of fish allergic patients' IgE to the wild-type allergen. We thus suggest that the hypoallergenic carp parvalbumin can be used for immunotherapy of fish allergy.

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101
Oral threshold levels and in vivo basophil activation in hazelnut allergic patients during oral provocation tests
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Food allergy affects 50-80% of birch pollen allergic individuals. The affected patients often suffer from reactions to tree nuts, mainly hazelnut. Symptoms are the oral allergy syndrome (OAS), rhinitis, asthma, urticaria, angioedema and even anaphylaxis. Such reactions may be induced by small amounts of the allergen and are sometimes life-threatening. The aim of this study was to investigate the threshold levels in hazelnut allergic patients suffering from OAS. Furthermore we studied tryptase levels and CD203c expression to evaluate mast cell and basophil activation before and after the double-blind-placebo-controlled provocation tests (DBPCFC).
The recruited individuals (n=46, 30 female, 16 male) had a clinical history of birch pollen and hazelnut allergy. In all patients specific IgE for birch–pollen (43,19 ± 5,62 kU/L) and hazelnut (1,56 ± 0,35 kU/L) was detected.
After the performance of a hazelnut-free diet for 1 week DBPCFC was performed with increasing amounts of hazelnut (dosage: 0,01g - 10g). Blood samples were collected before and after the provocation tests for determination of tryptase in the sera using the Pharmacia System and basophil activation using the anti-CD203c-PE using flow cytometric analysis.

The determined oral threshold levels eliciting OAS in the study group varied from 0.01-2,0 g (0,4 ± 0,515). The measurement of the mast cell/basophil activation after cumulative hazelnut doses shows that the tryptase levels remained unchanged before (6,26 ± 0,813µg/L) and after the DBPCFC (5,60 ± 0,787µg/L), whereas the in vivo CD203c expression significantly increased from 38,14% (± 4,87) to 48,38% (± 4,62).
Our results show that despite the patients developed mild allergic symptoms after exposure to low amounts of hazelnut a systemic activation of the immune system occurred as indicated by in vivo activation of basophils during DBPCFC. Whether CD203c expression may be useful as an in vitro test for the prediction of a clinical relevant allergy will need to be determined.

102

ANTI-ULCER DRUGS PROMOTE IgE FORMATION TO DIETARY PROTEINS: AN EPIDEMIOLOGICAL STUDY

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Recently, we have demonstrated in an animal model that anti-ulcer drugs of the H2-receptor blocker and proton pump inhibitor (PPI) class promote the development of IgE-mediated food allergy. By elevating the gastric pH peptic digestion is hindered and essentially harmless, digestion-sensitive proteins acquire allergenic potency. The aim of this epidemiologic study was to examine the impact of our observations for the development of human food allergy.
We screened 152 adult patients (mean age 65.9 years) without previous history of allergic disorders, who were medicated with H2-receptor blockers or PPIs during three months due to dypeptic disorders such as dyspepsia, gastroesophageal reflux, gastritis, or ulcer (patients). 52 untreated patients, representative for the average adult population, served as controls. Serum samples for determination of specific and total IgE, cytokines, and ST2 levels were taken before, 3 and 8 months, skin prick tests were performed before and 8 months after starting therapy.
Already at the 3 months time point, 40 patients (26.3%) had developed food specific IgE towards a broad panel of regular dietary compounds, like wheat, rye flour, carrots, celery, milk, almond, potato or apple, which all were readily digested in our in vitro assay. This boost was highly significant for the patients as compared to the values 1.) before treatment and 2.) of the controls. The patients discontinued anti-acidic treatment after 3 months. Nevertheless, at the 8 months time point still 10 patients (6.6%) showed specific IgE, and 11 (7.2%) had positive skin prick reactions towards food compounds, but none of the controls. Compatible with this, we found elevated levels of Th2-markers IL 4, IL-13 and soluble serum ST2 only in the patients' sera.
The presented epidemiological data verify our previous experimental observations that anti-ulcer treatment supports the development of IgE towards dietary compounds. Importantly, these IgE can also be formed against regular constituents of the diet. Therefore, we conclude that patients who suffer from dyspeptic complaints and who are continuously acid-suppressed, are at high risk to develop type I food allergy.

103

Apple allergy: different patient allergen recognition patterns across Europe – studied by the use of recombinant allergens

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Background: In Northern and Central Europe apple allergy is related to birch pollen allergy. In the Southern Euorpean countries, apple allergy is found without exposure to birch pollen. The aim of the study was to identify the allergen recognition patterns among a considerable number of allergic patients, derived from different geographical locations across Europe.
Methods: Apple allergic patients (n=400) were recruited according to standardized questionnaire, positive case history, positive RAST and skin prick test in four different clinical centers from Central and Southern Europe. In two centers double blind placebo controlled food challenges were also performed. Purified recombinant apple allergens: Mal d 1 (Bet v 1 homologue), Mal d 2 (thaumatin-like protein), Mal d 3 (non-specific lipid transfer protein) and Mal d 4 (profilin) were used in various in vitro assays and compared to total protein extracts from apple. In addition, in vivo assays were performed with a limited number of patients.
Results: Single purified recombinant allergens proved to be useful tools for the diagnosis of apple allergy when applied in in vitro as well as in vivo assays. The patients from Central Europe were birch pollen allergic and suffered from rather mild symptoms when cosuming apples. In contrast, the apple allergic patients from the southern European countries suffered from more severe symptoms. These results were confirmed by statistical methods.
Conclusions: Different pollen exposure, consumption habits may contribute to these distinct patients' profiles. Single, purified recombinant apple allergens are useful tools for diagnosis.

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104

Pollen associated food allergy: diagnosis by recombinant allergens and CD63 expression of basophils

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Background: Basophil activation is associated with an increased CD63 expression on basophils. The aim of this study was to investigate whether incubating basophils with the recombinant allergens Bet v 1, Bet v 2, Api g 1, Mal d 1 and Dau c 1 in the basophil activation test based on CD63 expression (BAT) is a useful tool for *in vitro* diagnosis of pollen associated food allergy to celery, apple and carrot.
Methods: 30 patients with an oral allergy syndrome (OAS) induced by apple, celery or carrot and 10 controls were selected for this study. Basophils were incubated with Bet v 1, Bet v 2, Api g 1, Mal d 1 and Dau c 1. After double immunostaining with anti-IgE and anti-CD63 monoclonal antibodies CD63 expression was determined by flow cytometry. Results were compared to well established routine diagnostic methods, i.e. skin prick tests with native foods and measurement of allergen specific serum IgE by the CAP FEIA method.
Results: Although *in vivo* testing of native foods by skin prick testing showed a sensitivity of 100 % with regard to the clinical history of an OAS a combination of both *in vitro* methods - measurement of allergen specific serum IgE and the BAT - showed a sensitivity higher than 90 % for all three food allergens investigated.
Conclusion: The CD63 based BAT is a valuable new *in vitro* method for diagnosis of IgE mediated food allergy and may play a future role particularly if immediate type hypersensitivity can not be demonstrated by routine methods such as determination of allergen specific serum IgE.

105

Does sputum cell counts alter asthma exacerbations? The LOMA Study.

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Background/Aim: Patients with asthma, even on corticosteroid treatment, are at risk of exacerbations (EX) of variable severity and inflammatory response.
Objective: To compare the frequency, type and severity of EX when treatment is guided by usual clinical criteria (CS) only, vs. this plus + induced sputum (Sp) cell counts (Sp eosinophils kept #2.0%) (SS).
Methods: Multicentre, randomized, parallel group effectiveness study over 2 years. 117 adults with asthma, in whom the least treatment had not been established, were assigned to the CS or SS. Subjects were blind to the Sp results while the investigators were blind in the CS. In both strategies clinical management was by the 1999 Canadian Consensus Guidelines. EX were counted from least treatment to maintain asthma control (LT) to the end of the study.
Results: 126 EX occurred after LT. SS reduced by 49 % the risk for the first exacerbation in the SS without any increase in LT. In 120 of these events

subjects were seen at the time of the exacerbation and in 102 of these (39 SS and 63 CS) they were seen before any additional treatment was given. SS significantly reduced the frequency of eosinophilic EX (relative risk [RR] 0.19 95%CI [0.04, 0.82]) but did not alter the RR for non-eosinophilic EX. In addition, SS reduced the severity of EX; treatment with prednisone was only required in 23 (out of 126) EX, of which 78.3% were in the CS group ($p<0.001$), even though the LT was similar in SS.
Conclusion: The results suggest that when inhaled corticosteroid treatment is applied to normalize sputum eosinophils, eosinophilic EX are reduced, the overall severity of exacerbations is reduced and the majority of exacerbations are non-eosinophilic (which may be secondary to infective bronchitis and be non-steroid responsive)
Funding: Canadian Institutes for Health Research

106

Genotype Stratified Prospective Crossover trial of Regularly Scheduled Albuterol Treatment in Asthma

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Background:
Whether use of an inhaled β_2-adrenergic agonist on a regular basis worsens airflow and clinical outcomes in patients with asthma has been a subject of controversy. Retrospective studies have suggested that this may occur in patients with the Arg/Arg genotype, as opposed to the Gly/Gly genotype, at the locus corresponding to the 16th amino acid of the β_2-adrenergic receptor. However, the existence of such a genotype-dependent difference has never been tested in a prospective clinical trial.

Methods:
Patients with mild asthma were enrolled in FEV_1 matched pairs based on whether they harbored the Arg/Arg or Gly/Gly genotype. Regularly scheduled treatment with albuterol or placebo was administered, in a blinded, crossover, fashion, for 16 weeks periods. During the study, as-needed albuterol use was discontinued and ipratropium bromide was used as-needed. AM peak flow was the primary outcome indicator.

Results:
During the run-in period, when albuterol use was minimized, patients with the Arg/Arg genotype had a 23 L/min (P=0·0162) increase in AM peak flow and there was no significant change in patients with the Gly/Gly genotype. During randomized treatment, patients with the Gly/Gly genotype had an increase in AM peak flow during treatment with regularly scheduled albuterol as compared with placebo. In contrast, patients with the Arg/Arg genotype had an increase in AM peak flow during treatment with placebo (when albuterol use was minimized) as compared to treatment with albuterol. The genotype attributable treatment difference, i.e., the effect of treatment in Arg/Arg patients less that observed in Gly/Gly patients, was −24 L/min (−37- −12, P=0·0003). Similar genotype-specific effects were observed in FEV_1, symptoms, and supplementary reliever medication use.

Conclusion:
Genotype at the 16th amino acid locus of the β_2-adrenergic receptor affects the long-term response to albuterol use. These data suggest that bronchodilator treatments avoiding albuterol may be appropriate for patients bearing the Arg/Arg genotype.

107

Probability of persistent wheeze and early childhood asthma increases with increasing specific IgE antibody levels

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Background. Sensitisation to dust mite and cat allergen is a strong risk factor for asthma in adults and older children in the UK. However, in most of the studies IgE mediated sensitisation is considered only as a dichotomous variable, i.e. individuals are assigned as either sensitised or not. There is a growing interest in the potential validity of the quantitative analysis of IgE antibody responses in predicting the presence and severity of childhood asthma.
Aim: Within the context of a population-based birth cohort study, we investigated whether the level of IgE antibodies to dust mite and cat is associated with persistence and severity of wheeze in 5 year-old children.
Methods: Children (n=483) were followed from birth to age 5 years, when questionnaires were administered and lung function (specific airway resistance-sR_{aw} by whole body plethysmography) was performed. Specific serum IgE (mite and cat) was measured using UniCAP^{TM} assay. According to parentally-reported history of wheeze at the follow-ups at age 3 and 5 years, children were assigned as persistent wheezers if they had at least one episode of wheezing during the first three years of life and reported wheezing in the previous 12 months at age 5 years (n=70). Children were classified as asthmatic if they had parentally-reported wheezing in the previous 12 months at age 5 years and poor lung function (sRaw>1.3kPa/s; n=48).

Results: The predicted probability of persistent wheeze and asthma increased markedly and significantly with the increasing summated mite and cat IgE antibody level (OR 1.5, 95% CI 1.28-1.76, $p<0.0001$ and OR 1.42, 95% CI 1.19-1.69, $p<0.0001$; persistent wheeze and asthma respectively). Predicted probabilities (and 95% CI) for persistent wheeze and asthma in relation to the increasing summated mite and cat IgE antibody level is presented in Figures A and B.

108

21 Year Longitudinal Study of Healthy Infants – Correlates with Atopy, Atopic Disease and Asthma (The RIFYL Study)

Maree Gleeson, Robert Clancy, Karla Lemmert

This presentation summarises the outcomes of a 21 year prospective study of 263 healthy term children born into an Australian coastal suburban population, with a focus on parameters that influence the evolution of atopy, atopic disease and asthma.

The initial 5-year prospective study (1979-84) obtained comprehensive records of feeding, demography, immunization and health events, including the onset of asthma and allergic diseases, as well as determining the pattern of normal development of mucosal immunity using saliva markers. Subsequent studies in 1985, 1991, 1993 and 2001 correlated clinical assessments of atopic disease and asthma, skin prick test (SPT) atopic status, bronchial hyperreactivity, induced sputum cell counts and cytokine patterns with clinical outcomes of allergic disease and asthma.

The 1985 study concluded that there was no relationship in pre-school children, aged 2-4 years, between SPT atopy and asthma (23% atopic asthmatics). The 1991 study (aged 6-9 years) indicted that the transient absence of SIgA in the first year of life was associated with increased bronchial hyperreactivity, a lower incidence of atopy, but no relationship with asthma. In 1993, aged 9-13 years, 36% of the cohort had asthma, 63% of whom were atopic. Sputum eosinophil counts correlated with bronchial hyperreactivity and asthma.

The 2001 study evaluated clinical outcomes over the 21 years for associations with risk factors in the first year of life for atopic disease and asthma. Environmental exposure in infancy had a time-dependent effect on clinical outcomes – an issue not considered in previous studies. The findings indicated that: (1) Older siblings protected against allergic disease, (2) Exposure to birds was linked to less allergic disease in adulthood. (3) Early introduction of cow's milk was associated with an early expression of SPT atopy, but was protective against the later prevalence of atopic disease. (4) Early introduction of solids was associated with a higher prevalence of allergic disease that did not express until school age. Distributions of specific cytokine-containing cells, assessed in the 2001 study, showed correlations with asthma.

It is concluded that correlations between induction mechanisms, risk factors and clinical outcomes, vary over time, which needs to be recognized in epidemiological studies.

109

Angiogenesis and remodeling of airway vasculature in asthma

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It has long been recognized that, in fatal asthma, the airway mucosa is edematous and contains dilated, congested blood vessels. An early study showed that vascularity (the percentage of lamina propria occupied by vessels) but not the number of vessels was increased in the small airway mucosa of postmortem fatal asthmatic lung. In another study of fatal asthma, an increase in numbers of vessels and vessel area in the cartilaginous airways was characterized, suggesting enlargement of existing vessels. Histological examination of biopsies from mild asthmatics obtained during bronchoscopy has highlighted the presence of increased vessel numbers and vascularity in the submucosa when compared to control subjects. We hypothesized that certain cytokines may play an important role in airway remodeling, particularly in angiogenesis associated with bronchial asthma. To investigate the development of angiogenesis in asthmatic airways, we examined the levels of several angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and angiogenin protein immunoreactivity in endobronchial biopsy specimens obtained from asthma and control subjects. Asthmatic subjects exhibited higher VEGF and bFGF, and angiogenin immunoreactivity in the submucosa than controls. Significant correlations were detected between the vascular area and the numbers of these angiogenic factors within the asthmatic airways. Furthermore using *in situ* hybridization methods, VEGF and its receptor were not only overexpressed in asthma but also correlated with decline of FEV_1 and with airway hyperresponsiveness. Recently, we have demonstrated that stromal cell-derived factor-1 (SDF-1), one of the CXC chemokine, also induces angiogenesis in asthma. Angiogenesis may lead to produce enlarged congested mucosal blood vessels which contribute to the increased airway wall thickness. These findings provide evidence that angiogenesis can be proposed to contribute to the airway remodeling process in patients with asthma.

Figure A: Persistent wheeze
Sum of specific IgE, mite and cat

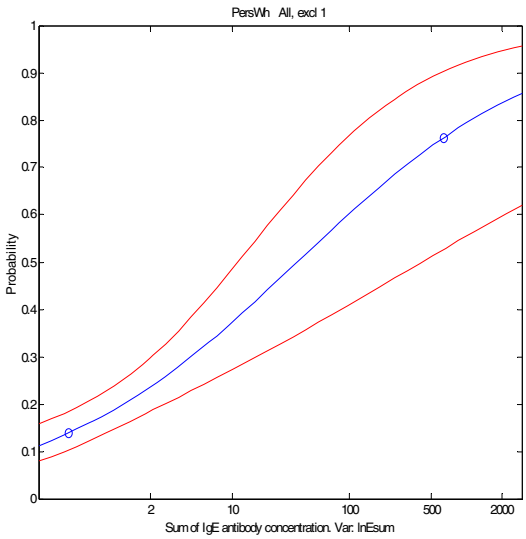
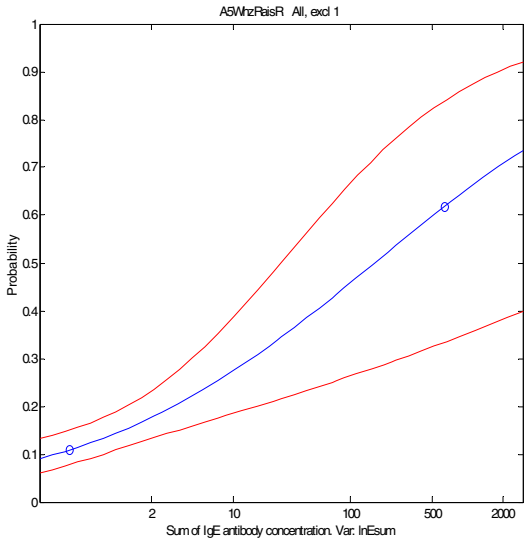


Figure B: Asthma
Sum of specific IgE, mite and cat



Conclusions: IgE mediated sensitisation is not a yes/no phenomenon. The probability of persistent wheeze and early childhood asthma increases with increasing specific IgE antibody levels.

110

Immediate adverse reactions to cephalosporins. Study of in vitro crossreactivity by determining specific IgE to different betalactams

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Following penicillins, cephalosporins are the betalactam most increasing in consumption and in induction of IgE mediated allergic responses. The in vivo and in vitro methods to evaluate these reactions are not very sensitive and because high cross-reactivity with penicillins is assumed, only penicillin derivatives are in many cases recommended to evaluate these reactions. We examined the importance of different cephalosporin and penicillin determinants in the in vitro detection of specific IgE antibodies in the sera of patients with immediate reactions to a cephalosporin derivative.
Nineteen patients with a clinical history of immediate allergic reactions to different cephalosporins and with skin test positive to the culprit drug were evaluated. The drugs involved were cefaclor (4), cefonicid (1), cefotaxime (2), ceftazidime (2), ceftriaxone (3) and cefuroxime (7). All patients had specific IgE antibodies, as determined by radioimmunoassay (RAST), to the culprit cephalosporin. Cross-reactivity with penicillins and other cephalosporins was studied by RAST to different betalactams: benzylpenicillin, amoxicillin, ampicillin, cefaclor, cefoxitin, cefuroxime, cefadroxil, ceftazidime, cephalexin, ceftriazone, cefotaxime, cefminox, cefepime, cefonicid.
RAST showed that of the 19 patients, 12 (63.15%) were only positive to the cephalosporin inducing the allergic reaction and six (31.58%) had a cross-reaction with a cephalosporin having a similar side chain to that inducing the reaction. IgE antibodies to a cephalosporin with a different side chain to the culprit drug were detected in just one patient. No patient was positive to penicillin derivatives.
We can conclude that there is high cross-reactivity in vitro between cephalosporins sharing the same or a similar side chain, although further studies are needed to determine the clinical value of this in vitro crossreactivity. Nevertheless, we detected no cross-reactivity between penicillins and cephalosporins in the patients studied, which could imply that this is not as high as is thought. Thus, to detect specific IgE antibodies in patients with immediate allergic reactions to cephalosporins it is not enough to use just penicillin derivatives; it is also necessary to use at least the culprit cephalosporin.

111
Withdrawn

112
Lack of Availability of Epinephrine for First-Aid Treatment of Anaphylaxis Worldwide
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Background: The prevalence of anaphylaxis is increasing globally, and this medical emergency now occurs more commonly in the community than in a health care setting. Epinephrine (adrenaline), the initial drug of choice in anaphylaxis, can be life-saving if injected promptly by patients themselves or, for children, by their caregivers. Epinephrine is listed by the World Health Organization as an essential drug. We hypothesized that, worldwide, availability of epinephrine auto-injectors for use in the first-aid treatment of anaphylaxis might differ from country to country.
Methods: This hypothesis was tested through a validated, cross-sectional survey administered to members of the World Allergy Organization 2003-2005 House of Delegates. Responses were tabulated by country.
Results: Completed surveys were received from representatives of 38 countries. At the time of the survey, epinephrine auto-injectors suitable for use in adults were available in only 55% (confidence intervals [CI] 39-71%) of these countries. Epinephrine auto-injectors containing a dose suitable for use in children were available in only 45% (CI 30-61%) of the countries. Epinephrine auto-injectors containing a dose suitable for use in infants were not available in any country. In 91% (CI 78-100%) of countries where epinephrine auto-injectors were available, a doctor=s prescription was required for them. Costs ranged from $30.00 (U.S.) to $110.00 (U.S.). Patients paid the total cost of the epinephrine auto-injector, without financial assistance from government or private insurance, in 29% (CI 9-48%) of the countries. Epinephrine was available **only** in the form of 1 mL ampules supplied with a syringe and needle in 44% (CI 30-61%) of the countries. Epinephrine was not available at all for first-aid use in one country.
Conclusion: This study raises concerns about lack of availability and affordability of epinephrine auto-injectors worldwide, especially lack of age- and weight-appropriate epinephrine auto-injectors for children and infants. International allergy organizations, manufacturers, government agencies, and humanitarian agencies should work together to improve availability of epinephrine auto-injectors at reasonable cost worldwide.

113
Indications of a systemic inflammatory response to Hymenoptera venom immunotherapy
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Background: Whether specific immunotherapy induces systemic inflammation has been hardly investigated. We looked for this during Hymenoptera venom immunotherapy (VIT).

Methods: 42 consecutive patients (22 males, 20 females) with Hymenoptera sting anaphylaxis due to honeybee venom allergy (n = 10) or vespid venom allergy (n = 32) were included. Patients received rush VIT, and blood samples were taken before start of VIT, after reaching the maintenance dose (day 3 to 6), and one week thereafter. We assessed blood eosinophils (numbers per volume), circulating immune complexes (CIC), the complement components C3 and C4, C-reactive protein (CRP), IL-6, IL-10, sICAM-1, sIL-2R, sCD14, and mast cell tryptase (MCT).

Results: Compared with baseline levels, 36 patients who tolerated VIT without systemic anaphylactic reaction (SAR) exhibited a significant decrease of CIC (p=0.014) and significant increases of eosinophils (p=0.018), CRP (p=0.004), IL-6 (p=0.002), IL-10 (p=0.008), sICAM-1 (p=0.005), and MCT (p=0.01) when they had reached the maintenance dose. After one further week, these changes were no longer demonstrable except a still significant elevation of eosinophils and MCT. No significant changes of the other parameters were found. 6 patients who developed SAR due to VIT exhibited a similar pattern of parameters as those without SAR, except that MCT was no longer significantly increased at the last assessment.

Conclusion: Not only “allergy-associated” parameters (eosinophils, IL-10, MCT), but also “unspecific” parameters of inflammation (CIC, CRP, sICAM-1, IL-6) change during VIT. The clinical relevance of these findings with regard to efficacy and side effects of specific immunotherapy needs to be further investigated.

114
Modular antigen translocation molecules (MAT): a new concept for the development of efficient vaccines

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According to the present knowledge antigens entering the body are taken up and processed by antigen presenting cells (APC) depending on the route of entry. $CD4^+$-T cells recognize peptides bound to MHC class-II molecules on the surface of APC. Assembly of the α and β chains of MHC class-II complexes and their association with the invariant chain (Ii) begins in the endoplasmic reticulum (ER). The functional domains of Ii include an N-terminal cytoplasmic domain, a domain that occupies the peptide-binding groove of the MHC class-II complex and a C-terminal trimerisation motif. Ii is translocated into the ER where trimerisation occurs, followed by association with the MHC class-II complex. Ii protected MHC class II-complexes are directed to lysosmes where the Ii chains are degraded, the complex loaded with antigenic peptides and directed to the APC surface. Our approach was to generate modular antigen translocation molecules (MAT) to increase MHC class-II-mediated antigen presentation. MAT consists of a $[His]_6$ purification tag, an arginine rich peptide for protein delivery into cells, the Ii N-terminal domain and a multiple cloning site allowing accommodation of any antigen. The Ii domain should assist specific targeting of cytoplasmatically delivered fusions to the ER through heterotrimerization with endogenous Ii. Incorporation of Ii-antigen fusions into MHC class-II complexes, thereafter selectively transported to lysosomes, where antigen loading of MHC complexes with degraded antigen fragments occurs, should result in an increased presentation of selected antigen fragments on the APC surface. We evaluated the utility of MAT in T-cell proliferation experiments using different MAT fusions including Bet v 1, Der p 1 and PLA_2. In all cases a stronger T-cell proliferation at 10-100x lower antigen concentrations than the proliferation obtained with recombinant allergens was obtained. Immunization of CJ/2B mice with MAT-PLA_2 fusions showed a complete suppression of the IgE production paralleled with an increased production of IgG2a at concentrations where recombinant PLA_2 induces strong IgE production. We conclude that MAT molecules represent potent allergy vaccines deprived of side effects because MAT proteins are produced as unfolded molecules and thus unable to bind IgE.

115
Human Regulatory T-cell-lines Cultures Maintained with CD3/CD28 Expander Beads

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RATIONALE: $CD4^+CD25^+$ T-cells have been shown to play a central role in regulating normal immune responses and might be the key to understanding abnormal responses in allergic and autoimmune diseases. These cells however are difficult to study because of a limited cell number. Here we describe a new way to culture $CD4^+CD25^+$ T-cells making regulatory T-cell-lines without loss of inhibitory function.
METHODS: $CD4^+CD25^+$ and $CD4^+CD25^-$ T-cells were separated from blood of healthy donors and activated using CD3/CD28-T-cell-expander-beads under influence of different exogenous cytokines and irradiated feeder cells.
RESULTS: The ability of $CD4^+CD25^+$ T-cell-lines but not $CD4^+CD25^-$ T-cell-lines to inhibit proliferation of target cells (autologous $CD4^+CD25^-$ T-cells activated for 24h before co-culturing) was persistent even after several generations of culture. Regulatory T-cell-lines cultured in presence of IL-2 and IL-15 possessed the strongest inhibitory ability. The cell-lines did not differ in their cytokine profile when comparing IL-4, IL-5, IL-10 and IFN-γ in a resting stage. After activation a higher IFN-γ production was detectable in $CD4^+CD25^-$ T-cell-lines on protein and mRNA level. 4-5 days after removal of the CD3/CD28-T-cell-expander-beads, the expression of CD25 remained high on $CD4^+CD25^+$ T-cell-lines, whereas $CD4^+CD25^-$ T-cell-lines expressed a significant lower level of CD25 even after four generations of culture under influence of IL-2.
CONCLUSIONS: $CD4^+CD25^+$ T-cell-lines may be cultured using CD3/CD28-T-cell-expander-beads for activation. $CD4^+CD25^+$ T-cell-lines but not $CD4^+CD25^-$ T-cell-lines inhibit the proliferation of $CD4^+CD25^-$ Target-cells after several generations of culture. CD25-expression seems unchanged

in $CD4^+CD25^+$ T-cell-lines does not reach a comparable high level in $CD4^+CD25^-$ T-cell-lines.

116

Allergen-specific immunotherapy is effective in atopic dermatitis

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Background Sensitisation to house dust mite allergens which is detectable with specific IgE-tests is very common in patients suffering from atopic dermatitis. Specific immunotherapy is an effective therapy in respiratory IgE-mediated allergic diseases and has been described to be effective in atopic dermatitis in some open clinical trials. "Classical" specific immunotherapy has not been investigated in double-blind placebo-controlled trials in atopic dermatitis so far.

Methods In a multi-centre, double-blind, randomised trial we treated patients with atopic dermatitis, IgE-mediated sensitisation against house dust mites and a SCORAD-score greater than 40 points with subcutaneous specific immunotherapy. Patients were randomised into 3 dose groups with maintenance doses of 20 ("active placebo"), 2000 and 20000 SQ-U ("active treatment") Dermatophagoides pteronyssinus/farinae extracts for a 1year treatment.

Findings 79 patients with at least one SCORAD assessment (equivalent with treatment for 2 months up to 1year) could be evaluated as full analysis set (intention-to-treat). The SCORAD-score declined in all three study groups. The differences in the SCORAD between baseline and the last 3 months of treatment were significantly greater in patients actively treated (2000 and 20000 SQ-U) compared to patients with active placebo (20 SQ-U). The application of topical corticosteroids in the two active treatment groups was significantly reduced compared to active placebo. The final assessment of the physician confirmed a better skin condition of patients with active treatment compared with active placebo at the end of therapy.

Interpretation Here we show for the first time in a double-blind placebo-controlled trial that "classical" allergen-specific immunotherapy with house dust mite extracts is effective in patients with atopic dermatitis who are sensitised to house dust mite allergens and may be valuable in the treatment of this chronic skin disease.

117

Allergy prophylaxis by DNA vaccination inhibits specific IgE response and lung pathologic parameters in a mouse model

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More than 20% of the western population suffer from symptoms of type I allergy. Often inhalent allergens induce hypersensitivity and lead to the development of asthma. We wanted to determine the efficacy of prophylactic DNA vaccination using the major pollen allergen Phl p 5b of timothy grass (*Phleum pratense*) as a model allergen. BALB/c mice were preimmunized intradermally with the Phl p 5b plasmid or the vector (mock control) before being intraperitoneally sensitized with the Phl p 5b protein. As a control mice were only treated with allergen protein. Immunologic changes were monitored by determination of the specific immunoglobulins (IgE and IgG1 versus IgG2a and IgG2b) and by histologic parameters of the lung. The results showed that specific IgE and IgG1 production was significantly inhibited when mice were immunized with the Phl p 5b-plasmid. In contrary IgG2a and IgG2b were increased. Interestingly, the plasmid vector alone partially also inhibited the IgE/IgG1 antibody response, probably as an innate immune response directed against the CpG motifs. Concerning lung morphology, both groups of mice revealed no pathologic alterations in contrast to the Phl p 5b-sensitized mice that showed goblet cell hyperplasia, peribronchial inflammation, eosinophilia and mucus production. The results demonstrate that vaccination with the Phl p 5b-plasmid can effectively inhibit IgE synthesis and pathologic alterations in the lung and thus can serve for allergy prophylaxis. Now we are in progress to treat Phl p 5b-sensitized mice with the plasmid to study, whether it is possible to cure them from an ongoing allergy.

118

'Blocking IgG' induced by specific immunotherapy is reduced following treatment termination

Larsen JN, Aalberse RC, Ipsen H, Wachholz P, Durham SR.

Successful allergen immunotherapy is accompanied by a rise in allergen specific IgG, i.e. 'blocking IgG'. Early studies failed to demonstrate a direct correlation between improvement in clinical outcome and the concentration of 'blocking IgG'. Assays designed to address the inhibitory capacity of the IgG-allergen interaction showed dependency on duration of and dose applied in allergen immunotherapy, indicating that a more nuanced assessment of the IgG antibody response including concentration as well as avidity, might improve serological models for the mechanism of immunotherapy. In this study we addressed the fate of the 'blocking IgG' following treatment termination.

Grass pollen allergic hay fever patients received immunotherapy for 24 months in a double-blind placebo-controlled trial (Walker et al., 2001). Patients from the active group were then randomized to receive continued therapy or placebo for another 24 months.

Compared to placebo patients in active treatment had improvement in symptom scores of 49% and reduction in medication use of 80% after two years. Withdrawal of treatment had no effect on clinical parameters after four years. Sera were analysed for Phl p 1 and Phl p 5 specific antibody responses, respectively. There was a statistically significant increase in total IgG as well as IgG4 to both allergens, although the response to Phl p 5 was more pronounced. There was no change in IgE. After four years IgG in the withdrawal group had returned to pre-treatment levels, whereas IgG remained elevated in the group receiving continued treatment.

The inhibitory capacity of IgG was measured in a direct binding immunoassay as well as in allergen-IgE complex binding to CD23 on antigen presenting cells. Both assays showed a reduction in IgE binding in sera from the active group when using grass pollen extract as antigen, however, reduction in the withdrawal group was gradually abolished when measured by direct immunoassay but remained reduced when measured in the CD23 assay.

Two years after termination of immunotherapy the blocking activity of allergen specific IgG when measured in direct immunoassay is abolished. IgE-allergen complex binding to CD23, however, remain reduced in agreement with the persistence of the clinical improvements.

119

Fel d 1 mutants – new candidates for treatment of cat allergy

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Proteins from the domestic cat (*Felis domesticus*) are one of the most common causes of allergic disease worldwide. The dominating cat allergen is Fel d 1 which elicits IgE responses in 90-95% of patients with cat allergy. Fel d 1 is a tetramer composed of two heterodimers of protein chain 1 and chain 2. The allergen is produced by sebaceous glands and squamous epithelial cells and is transferred to the pelt by licking and grooming.
A soluble and correctly folded recombinant Fel d 1 molecule would be useful for diagnosis and treatment of cat allergy. For that purpose, we have recently produced a recombinant Fel d 1 molecule by direct fusion of the Fel d 1 chain 2 to chain 1, with molecular and biological properties similar to the natural counterpart. Circular dichroism analysis showed identical folds of natural and recombinant Fel d 1. Furthermore, the recombinant Fel d 1 reacted specifically with IgE, induced expression of CD203c on basophils and lymphoproliferative responses in cat allergic patients.
To obtain a rationale framework for design of treatment for cat allergy, we have lately determined the crystal structure of the recombinant monomeric Fel d 1 to a resolution of 1.85-Å. Fel d 1 is an all-helical protein and consists of

eight helices. The structure of Fel d 1 presents a striking similarity to that of uteroglobin, a steroid-inducible cytokine-like molecule.
Based on our knowledge of the Fel d 1 molecule we have now generated hypoallergenic mutants by disrupting the three-dimensional structure of Fel d 1 using site directed mutagenesis. To retain the T-cell reactivity of the modified allergen, a short sequence within two of the T-cell epitopes of Fel d 1 was duplicated. Three out of twelve different constructs proved to be hypoallergens with 400-1000 times lower IgE binding capacities than Fel d 1 in ELISA inhibition experiments, and with clearly reduced IgE binding capacities when evaluated in basophil-activation experiments. In addition, the hypoallergenic mutants displayed similar or stronger ability to induce T cell proliferation than Fel d 1. The results indicate that the Fel d 1 hypoallergens may be useful tools to obtain a safer treatment of cat allergy.

120

Inhibition of rBet v 1-induced histamine release from human basophils by specific immunotherapy-patient IgG is generally not mediated by Fc RIIB

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RATIONALE: Specific immunotherapy (SIT) induces allergen-specific IgG, termed "blocking" antibodies since they inhibit allergen binding to IgE. Basophils and mast cells express the low-affinity IgG receptor FcγRIIB which contains an immuno-receptor tyrosine-based inhibitory motif (ITIM). It has previously been shown that cross-linking the high affinity IgE receptor (FcεRI) and FcγRIIB on basophils and mast cells lead to inhibition of FcεRI-mediated histamine release. It has been proposed that complexes containing allergen, specific IgE and specific SIT-induced IgG bind to FcγRIIB thereby cross-linking it with FcεRI and downregulating histamine release, as a mechanism of SIT. The purpose of this study was to investigate whether SIT-patient IgG inhibits basophil histamine release by FcγRIIB activation and inhibition of FcεRI signalling, or by acting as "blocking" antibodies inhibiting the allergen IgE interaction.
METHODS: Serum samples were collected from birch pollen-allergic patients treated with SIT for 1 to 5 years. In receptor binding experiments ^{125}I -labelled rBet v 1 was pre-incubated with IgE-depleted SIT-serum and added to FcγRII expressing Raji cells. In basophil histamine release experiments, rBet v 1 was pre-incubated with IgE-depleted SIT-serum as above and added to washed leukocytes from birch pollen-allergic patients with or without prior a-FcγRII Fab incubation. Histamine release was measured in supernatants by ELISA.
RESULTS: Binding of rBet v 1-complexes to Raji cells could be detected with IgE-depleted serum from 8 out of 22 SIT-patients. This binding could be completely inhibited by Fab fragments of a FcγRII-specific blocking antibody. Pre-incubating rBet v 1 with IgE-depleted SIT sera from the 8 patients where IgG-rBet v 1 binding to Raji cells were detected, reduced rBet v 1-induced basophil histamine release from birch pollen-allergic patients by up to 95% compared to preincubation with negative serum. Inhibition of release with two of these SIT-sera samples could be partially reversed by blocking FcγRIIB, however for the 6 other sera inhibition was not affected.
CONCLUSIONS: Our data suggest that FcγRIIB activation is significant in only a fraction of SIT-patients in IgG-mediated inhibition of histamine release from human basophils. The main contributor to the inhibitory effect of allergen-specific IgG is most likely blocking of the allergen-IgE interaction.

121

Quality of life and compliance in patients allergic to grass and rye pollen during a three-years treatment with specific immunotherapy (the LQC study)

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Quality of life and compliance are important paraneters for the success of a 3-years specific immunotherapy (SIT). We investigated the disease-specific and general quality of like and compliance of patients during routine treatment with SIT. The open, uncontrolled study included 106 allergists and 1257 patients with rhinoconjunctivitis to grass pollen treated with SIT (ALK-depot SQ) for 3 years. Patients retrospectively completed the Rhinitis Quality of Life Questionnaire (RQLQ) and a general quetionnaire ("Alltagsleben") for the grass pollen seasons before and during a 3-years SIT. Compliance was assessed according to treatment protcols and discontinuations of treatment.

The average score overthe 6 RQLQ-domains (scale 0 to 6) improved for 2.91 before SIT to 1.05 after 3 years ($p>0.0001$) For patients impaired more severely (30.8% with a score ≥4) to the general quality of life (scale 1 to 5) improved from 3.60 ± 0.34 before SIT to 4.43 ± 0.45 after 3 years. SIT was discontinued by 3.3% of the patients due to compliance problems and by 18.7% due to other reasons, 8.2% did not return.

A 3-years SIT improves the disease-specific and general quality of life in routinely treated patients with rhinoconjunctivitis. Compliance with SIT applied by specialized allergists is very high.

122

DEVELOPMENT OF AN ORAL ALLERGEN IMMUNOTHERAPY TARGETING M-CELLS

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Since the advantages of an oral immunotherapy are evident, we aimed here to generate a formulation that 1.) serves as a vehicle for orally delivered allergens and 2.) allows targeting to M-cells, which are the most effective sites for antigen uptake and transfer into Peyer´s Patches of the intestine. Several gastrointestinal pathogens, like Salmonella, are specifically recognized by M-cells, and subsequently induce strong Th1-type responses. We investigated in a murine model, whether allergen uptake via enterocytes *versus* M-cells, may be decisive for modulation of an ongoing Th2-response.

Microspheres of the PLGA type were loaded with birch pollen proteins (BP), and specifically targeted to enterocytes or to M-cells. BALB/c mice express different carbohydrates on these two cell-types. To target the alpha-L-Fucose on M-cells we functionalised allergen-loaded microspheres with Aleuria Aurantia lectin (AAL) from an edible mushroom, and to target the sialyl residues on murine enterocytes with Wheat Germ Agglutinin (WGA). BALB/c mice were first sensitised with BP to induce an allergic phenotype. Subsequently, they were fed with BP-loaded particles functionalised with AAL, WGA, or non-functionalised. Exclusively mice which were fed with AAL-particles developed BP-specific IgG2a ($p<0.048$), but no IgG1 or IgE. As expected, their splenocytes proliferated specifically with BP in a ^{3}H-Thymidin assay. Importantly, AAL-mice produced significantly more Interferon-gamma ($p<0.002$) and Interleukin-10 ($p=0.037$) than the WGA- and control group.

We conclude that in oral allergen immunotherapy, M-cell targeting is a necessity to achieve an allergen-specific immune modulation towards the Th1 type.

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123

Epithelial immunoexpression of transforming growth factor-beta, eotaxin and stem cell factor in allergic rhinitis, and their relationship to epithelial mast cell accumulation in naturally occurring disease

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Background and objectives: Allergic airway disease is characterized by the epithelial accumulation of cells, in particular mast cells and eosinophils. Whilst attention has focused on eosinophil chemoattractants, there is little information relating to the chemotaxins responsible for mast cell epithelial accumulation. We have thus investigated the expression of known mast cell chemoattractants in tissue sections from biopsies in naturally-occurring allergic rhinitis.

Methods: We have investigated of the expression of selected mast cell chemoattractants and their receptors using specific immunohistochemical staining of thin sections of inferior turbinate biopsies from 10 patients with perennial allergic rhinitis, 10 with seasonal (in-season) allergic rhinitis, and for comparison, from 10 non-atopic non-rhinitic healthy volunteers. The chemotaxins investigated included transforming growth factor-beta (TGF-β) isotypes 1, 2 and 3, stem cell factor (SCF) and eotaxin as well as their respective receptors; TGF-β receptors (TGF-βR) I, II and III, c-kit and C-C chemokine receptor 3 (CCR-3).

Results: There was significantly increased epithelial immunoreactivity for TGF-β1, TGF-β2, TGF-β3, TGF-βRI, TGF-βRII, TGF-βRIII in the perennial ($p<0.05$) and seasonal (in season) ($p<0.05$) allergic rhinitis compared with normal non-atopic non-rhinitic healthy controls. Epithelial immunoreactivity for eotaxin was significantly increased ($p<0.05$) in the perennial group and elevated nasal lavage levels of eotaxin were evident in both seasonal and perennial disease, as compared to that in the healthy controls ($p<0.05$). There were no disease-related differences found in relation to CCR-3 or SCF immunoreactivity. There were significant correlations ($p<0.05$) between both TGF-β1 and TGF-β2 epithelial immunoreactivity and the number of epithelial mast cells in allergic rhinitis.

Conclusions: In view of the known potency of TGF-β as a mast cell chemoattractant and the significant correlations identified in this study in naturally occurring disease, between epithelial TGF-β expression and mast cell numbers at this site, these findings would support the concept that the epithelial expression of this growth factor is an important contributor to the epithelial accumulation of mast cells in allergic rhinitis and is likely to be a fundamental process underlying clinical disease expression.

124

Phenotypical and Functional Characterization of Distinct Populations of Human Lung Macrophages

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Macrophages are components of the mononuclear phagocyte system, a complex cellular network widely distributed in several organs and tissues. These cells acquire distinct morphological and functional features as they mature in different environments and may have distinct roles in inflammatory and immune responses. Density gradient separation of human macrophages purified (>95%) from the lung parenchyma of patients undergoing thoracic surgery, yielded two distinct populations of cells: high density macrophages (HDM: $1.065<d<1.078$) and low density macrophages (LDM: $1.039<d<1.052$). LDM represented $31.2 \pm 5.5\%$ of total lung macrophages, whereas HDM accounted for the remaining $58.8 \pm 5.5\%$. Density of each population remained stable during cell culture up to 36 h. Flow cytometry analysis of a wide panel of surface markers (CD11c, CD14, CD16, CD40, CD45, CD63, CD64, CD71, CD86, HLADR) revealed that both populations consisted of mature macrophages. LDM expressed higher levels of the activation markers (CD63 and CD64), whereas HDM expressed higher levels of the T cell costimulatory molecules (CD40 and CD86). Significant differences between LDM and HDM were also found in their functional responses to stimulation with LPS. HDM produced significantly higher quantities of proinflammatory (IL-6 and TNF-α) and immunomodulatory (IL-10 and IL-12) cytokines than LDM. RT-PCR analysis of the molecules involved in the binding and early signaling of LPS revealed that the expression of Toll-like receptor 4, MD-2 and MyD88 were similar in HDM and LDM. In contrast, western blot experiments showed that LPS-induced activation of p38 and ERK1/2 kinases was delayed in LDM as compared to HDM. Thus, human lung parenchyma contains at least two populations of macrophages distinct on the basis of their density, surface marker expression and functional response to LPS. These results suggest that specialized populations of macrophages may play a differential role in immune and inflammatory responses in the human lung.

125

Inflammation-Associated Block of Apoptosis by Survivin in Neutrophils

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Survivin has received great attention due to its expression in many human tumors and its potential as a therapeutic target in cancer. Survivin expression has been described to be cell-cycle-dependent and restricted to the G_2-M checkpoint, where it inhibits apoptosis in proliferating cells. In agreement with this current view, we found that survivin expression was high in immature neutrophils, which proliferate during differentiation. In contrast to immature cells, mature neutrophils contained only little or no survivin protein. Strikingly, these cells re-expressed survivin upon GM-CSF or G-CSF stimulation *in vitro* and under inflammatory conditions *in vivo*. Moreover, survivin-deficient mature neutrophils were unable to increase their life span following survival factor exposure. Taken together, our findings demonstrate that: (i) Overexpression of survivin occurs in primary, even terminally differentiated cells and is not restricted to proliferating cells; and (ii) Survivin acts as an inhibitor of apoptosis protein (IAP) in a cell-cycle-independent manner. Hence, survivin plays distinct and independent roles in the maintenance of the G_2-M checkpoint and in apoptosis control, and its overexpression is not restricted to proliferating cells. These data provide new insights into the regulation and function of survivin, and have important implications for the pathogenesis, diagnosis, and treatment of inflammatory diseases and cancer.

126

Two forms of human CD8: possible implication of a novel 52 kDa form of CD8 on myeloid cells in allergic disease

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Originally characterized as T cell restricted, CD8 has since been described on some NK cells and dendritic cells, as well as rat macrophages, and mast cells. Ligation of CD8 on rat alveolar macrophages (AM) and mast cells induces release of TNF and nitric oxide (NO). In rat models of autoimmune disease (e.g. arthritis, multiple sclerosis) it was discovered that CD8+ cells believed responsible for pathology may actually be monocytes and macrophages. Similarly correlations of CD8+ T cells with disease severity in experimental allergic alveolitis and with asthma death may be partly due to CD8+ myeloid cells. Unfortunately, it is unknown whether human macrophages or mast cells express CD8.

CD8 is currently considered a 32 kDa O-glycosylated protein. Our previous studies of rat mast cell and macrophage CD8 suggested CD8 structure was modified on these cells because only two of three anti-CD8 monoclonal antibodies (mAb) detected CD8. The nature of the unusual structure of CD8 on myeloid cells and its implications for CD8 ligand binding has not been elucidated.

Using RT-PCR we demonstrated expression of CD8 mRNA by human monocyte (THP-1) and mast cell (HMC-1) cell lines. Flow cytometry of human AM from bronchoalveolar lavage, and peripheral blood monocytes demonstrated expression of a form of CD8 that bound three of five anti-CD8 mAb. Western blot analysis showed expression of two forms (32 kDa and 52 kDa) of CD8 by HMC-1 mast cells and peripheral blood monocytes. Intriguingly, only the 52 kDa form of CD8 could be detected among monocyte (THP-1) plasma membrane proteins purified after biotinylation on an avidin column. This suggests the 52 kDa form of CD8 accounts for binding of anti-CD8 mAb by myeloid cells. Anti-CD8 mAb could block binding of MHC class I tetramers to monocytes and augment monocyte TNF production. CD8 on human monocytes, AM, and mast cells may be involved in allergic disease.

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127

Development of immune responses to allergens and clinical allergy in relation to microbial stimulati

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Background: Estonia is a post-socialist country in rapid transition and traditionally a low prevalence of allergic disease. This offers a unique opportunity for clinical studies on the development of immune responses to allergens and manifest allergic disease.
Objective: To compare the development of immune responses to allergens through the first 5-10 years of life in relation to appearance of clinically manifest allergic disease.
Methods: Two cohorts of children have been followed from birth up to 5 and 10 years respectively. Data about symptoms of allergy, infections and use of antibiotics were obtained by questionnaires. Clinical examination, skin prick tests (SPT) with food and inhalant allergens, and blood sampling for IgE analyses and allergen-stimulated cytokine production and expression by blood mononuclear cells were carried out at 3 and 6 months and at 1, 2, 5 and 10 years of age. Faecal samples were obtained from subgroups of the children.
Results: In the birth cohorts born 1993 and 1997/98, the point prevalences of positive SPTs were 7-9% until 2 years. In the first cohor, born in the Soviet Union, the prevalence dropped to 3% at 5 years, while in the later born cohort it increased to 14%. In contrast, circulating IgE antibodies to allergens were common, i.e. 13-15%, 26-36%, 31-36% and 47% respectively. Postnatal increase in allergen-stimulated IFNγ, IL-5, -10 and-13 was less prominent in Estonian than in Swedish infants and they had more infections ($p<0.001$). The gut flora contained less bifidobacteria and less diversity in infants who developed allergic disease and such children more often had received antibiotics with a broad antimicrobial spectrum.
Conclusion: The prevalence of allergic manifestations and positive SPT were as high in Estonia as in Scandinavia during the first 2 years of life and then declined. In contrast, the prevalence of IgE antibodies during and positive SPTs was lower in Estonian infants, while circulating IgE antibodies were more common and often present without any clinical significance. Allergen-induced cytokine secretion was less pronounced in Estonian infants. The findings might be related to a high microbial pressure and an altered microbial microenvironment affecting immune regulation.

128

Successful mucosal immunotherapy in asthma induces tolerance and *M. vaccae* potentiates IL-10 related tolerance in a murine asthma model

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In the induction of healthy immune response to aeroallergens entering the mucosal route, IgA antibody production and development of tolerance represent most important steps. Compared to healthy children, we observed decreased levels of specific IgA antibodies to house-dust-mite (HDM) in atopic children with asthma. These low IgA levels were normalized by sublingual immunotherapy (SLIT) after 6-12 months of administration.
Interestingly, in an experimental murine model of chronic asthma, the protective effect of intranasal immunotherapy improved substantially by additional administration of *M. vaccae* before establishment of the asthmatic state. In particular, changes in cytokine profile of cells towards a suppressive T cell phenotype and of the lung histopathology were observed. BALB/c mice were pretreated with either OVA alone or additionally with *M. vaccae* three times on each alternate days, before chronic asthma was established by seven intraperitoneal administrations of OVA, followed by three challenges with intratracheal OVA. Two days after the last OVA instillation, splenocytes were cultured with PHA, OVA and *M. vaccae* for cytokine determination and the lungs were histopathologically examined.
Evaluation of the lung histopathology revealed that airway epithelium, basement membrane, subepitelial smooth muscle thickness and goblet cell numbers in the small, medium and large airways were significantly increased in the chronic asthma model. All these parameters were downregulated both in mice pretreated with intranasal immunotherapy alone or in conjunction with *M. vaccae* to the values observed in control mice.
The comparison of histopathological parameters in OVA-immunized, *M. vaccae* pretreated and nontreated mice revealed that epithelial and smooth muscle thickness in small and large airways, and the number of hyperplasic goblet cells in large airways were the same in *M. vaccae* pretreated mice and healthy controls.
The secreted levels of IFN-γ and IL-10 from PHA-stimulated splenocytes were significantly higher in OVA pre-immunized mice compared to those with chronic asthma.
In contrast, compared to the controls, OVA pre-immunized mice revealed significantly higher spontaneous IL-10 and IFN-γ levels induced in splenocytes by *M. vaccae*. Moreover, mice pre-immunized with OVA and pretreated with *M. vaccae* produced higher IL-10 and decreased IL-5 and IFN-γ levels.

Thus, we conclude that intranasal immunotherapy appears to be a potential strategy for primary prevention of histopathological changes in asthma.The simultaneous administration of *M. vaccae* in mice upregulates IL-10 production and down-regulates both Th1 (IFN-γ) and Th2 (IL-5) cytokine secretion by splenocytes. This suggests that *M. vaccae* treatment potentiates the induction of IL-10-secreting regulatory T cells and development of mucosal tolerance.

129

Alpha-toxin induces proliferation and secretion of IFN-γ in T cells of atopic donors

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Background: S. aureus is known as an important trigger factor of atopic dermatitis. Besides exotoxins with superantigenic properties, alpha-toxin is secreted by S. aureus. A wide range of human cells has been shown to be susceptible to lysis by this exotoxin. In this study we aimed to investigate the effect of low concentrations of alpha-toxin on CD4+ T cells known as important effector cells of atopic inflammation.
Methods: Patients with atopic dermatitis were investigated for colonization with alpha-toxin-producing S. aureus strains. CD4+T lymphocytes were incubated with alpha toxin. Proliferation was assessed with the Vibrant™ CFDA SE Cell Tracer Assay. IFN-γ was measured by ELISA. Induction of IFN-γ on the mRNA level was determined by efficiency controlled real time RT-PCR. CD4 + T cells were assessed for the induction of t-bet translocation by alpha-toxin with the EMSA.
Results: Alpha-toxin producing S. aureus strains could be isolated from lesional skin of 22 / 64 adult patients with atopic dermatitis who presented without signs of cutaneous infection. Alpha toxin was detected in skin sections of patients colonized with alpha-toxin producing strains by staining with polyclonal anti-alpha-toxin antibodies. Low (i.e. pg-ng) concentrations of alpha-toxin induced massive proliferation of CD4+ T cells derived from atopic patients and healthy controls. Moreover IFN-γ was induced on the protein and the mRNA level. These effects were more pronounced when PBMC instead of CD4+ cells were used. The expression of IFN-γ could also be shown by microarray analysis of stimulated CD4+ cells. Stimulation of PBMC with alpha-toxin resulted in nuclear translocation and DNA binding of t-bet, known as a transcription factor involved into primary TH1 commitment.
Conclusion: We show here for the first time that alpha-toxin is able to activate CD4+ T-cells in low concentrations. Our results indicate that alpha-toxin may represent a factor relevant for the induction of a Th1 like cytokine response in atopic inflammation thus facilitating the development of chronic eczema.

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130

Intrinsic defect in T cell production of interleukin (IL) 13 in the absence of both IL-5 and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma

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Interleukin-(IL)-5 and IL-13 are thought to play key roles in the pathogenesis of asthma. Although both cytokines utilise eotaxin to regulate eosinophilia, IL-13 is thought to operate a separate pathway to IL-5 to induce airways hyperreactivity (AHR) in the allergic lung. However, identification of the key pathway(s) employed by IL-5 and IL-13 in the disease process is confounded by the failure of anti-IL-5 or anti-IL-13 treatments to completely inhibit the accumulation of eosinophils in lung tissue. By utilizing mice defiecient in both IL-5 and eotaxin (IL-5/eotaxin$^{-/-}$) we have abolished tissue eosinophilia and the induction of AHR in the allergic lung. Notably in mice deficient in IL-5 and/or eotaxin the ability of CD4$^+$ T helper 2 lymphocytes (Th2 cells) to produce IL-13, a critical regulator of airways smooth muscle constriction and obstruction, was significantly impaired. Moreover, the transfer of eosinophils to IL-5/eotaxin-/- mice overcame the intrinsic defect in T cell IL-13 production. In IL-5 Tg mice Th2 cells produced exaggerated levels of IL-13. We also show that IL-18 derived from eosinophils palyed a key role in the modulation of cytokine production. Thus, factors produced by eosinophils may either directly or indirectly modulate the production of IL-13 during Th2 cell development. This mechanism may have evolved to promote the expulsion of parasites from the intestinal mucosa. Eosinophils loaded with parasitic antigens may enhance production of IL-13 by T-celss in gut-associated lymphoid tissue, which subsequently promotes the expulsion of the pathogen by increasing gastrointestinal motility by amplifying cholinergic responsiveness and enhancing mucus secretion. Our data shows that IL-5 and eotaxin intrinsically modulate IL-13 production for Th2 cells and that these signaling systems are not necessarily independent effector pathways but may also be integrated to regulate aspects of allergic disease. Furthermore eosinophils play and intimate role in the regulation of Th2 type immunity.

131
Withdrawn

132
Asthma provoked by inhalation of T cell peptides
A.B. Kay

It was previously shown that intradermal administration of T-cell peptide epitopes to atopic asthmatics induced late asthmatic reactions (LARs) which appeared to be independent of IgE and mast cells. In the present study we show that peptides can produce LARs when given by the inhaled route and that this is associated with airway hyperresponsiveness (AHR) and local bronchial mucosal T cell infiltration and increased TARC expression. Overlapping peptides derived from the major cat allergen, Fel d 1 with reduced ability to cross link IgE as shown by basophil histamine release assays, were synthesised and delivered by aerosol inhalation to cat allergic asthmatics. Using a randomised, placebo-controlled, crossover study design we performed bronchial biopsies and bronchoalveolar lavage in 12 subjects who developed LARs (greater than 20% reduction in FEV_1) 6 hr after Fel d 1 peptides inhalation (responders) and 12 subjects who showed no clinical response (non-responders). Histamine PC_{20} was performed at baseline and 1 week after each challenge. Peptide-induced LARs were associated with elevations in bronchial mucosal CD4+ (p=0.034) and CD3+ cells (p=0.005) and TARC expression (p=0.005). There were no significant differences (responders vs non-responders) in eosinophils, mast cell tryptase, histamine, LTC_4, LTE_4, PGD_2 or PGF_2 in BAL fluid. However, there was a significant increase, 7 days after peptide challenge, in AHR in the responders (p=0.002), but not in non-responders. We conclude that inhalation of T-cell peptides epitopes induces isolated LARs which are associated with bronchial infiltration of CD4+ T-cells and increased AHR. These observations support the concept that there is a T cell component to the asthma process which is independent of mast cells and IgE and partly under control of the CC chemokine receptor ligand TARC.

133
Protease-Activated Receptor-2 Enhances Allergen-Induced Airway Inflammation and Hyperresponsiveness
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Serine proteinases such as mast cell tryptase, trypsin-like enzymes, and various allergens are important in the pathogenesis of asthma. These proteinases can activate the Proteinase-Activated Receptor-2 (PAR-2), which has been shown to be up regulated in asthmatic airways. We hypothesized that PAR-2 activation in the airways leads to enhanced allergen-induced airway inflammation and airway hyperresponsiveness (AHR).

Male Balb/c mice (6-8 weeks old) were sensitized intraperitoneally on days 1 and 6 with ovalbumin (OVA) and then challenged intranasally with OVA (50 μg) on days 12 and 14. AHR was evaluated in conscious animals by plethysmography. The mice were then sacrificed and bronchoalveolar lavage (BAL) was performed. Cells from BAL were used to make cytospins for differential counts.

Using immunohistochemistry we localized PAR-2 to the nasal mucosa and to alveolar macrophages, epithelium, endothelium, and smooth muscle in the lower airways of OVA sensitized mice. Mice sensitized to OVA were administered intranasally the PAR-2 activating peptide (PAR-2ap) SLIGRL-NH_2 (100 μM) or the PAR-2 control peptide (PAR-2cp) LSIGRL-NH_2 (100 μM) alone or in conjunction with OVA twice on alternate days and evaluated the day after the last challenge. PAR-2ap did not induce airway inflammation or AHR in mice that had not been challenged with OVA. When administered with OVA, PAR-2ap enhanced airway inflammation and AHR when compared to OVA administered alone or with the PAR-2cp. Enhanced airway inflammation consisted of increased eosinophil and mononuclear cell numbers in the BAL. AHR enhancement persisted for 5 days after the last challenge while the enhancement to airway inflammation did not. To define the mediators leading to enhanced AHR and inflammation we performed RNAse protection assay with RNA isolated from the lung of OVA-challenged mice. Mice receiving PAR-2ap showed increased levels of IL-4, IL-6, TNF, MIP1α, MIP2 and MCP1 mRNA in the lungs compared to mice receiving OVA alone or OVA with PAR-2cp. This increase was evident 2-4h after PAR-2ap administration and dissipated by 8h.

In conclusion, PAR-2 activation enhances allergen-mediated airway inflammation and AHR through the upregulation of pro-inflammatory cytokines and chemokines and may therefore play an important role in the pathogenesis of asthma.

134
Glucocorticoids promote T regulatory cells in asthma and in vitro by increasing FOXP3 expression
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T regulatory (T_{reg}) cells play a key role in balancing immune responses and constitute peripheral tolerance against antigens, including autoantigens and allergens. Glucocorticoids (GC) are used to control inflammatory diseases, but their impact on T_{reg} cells is currently unknown. The present study investigates local and systemic GC treatment and its impact on asthmatic patients' T_{reg} cells *in vitro* and *in vivo.* CD4$^+$ T cells from healthy donors and 50 asthmatic, GC-treated patients were isolated and mRNA expression of FoxP3, a crucial T_{reg} marker, along with IL-10 and TGF-β_1 was determined. Furthermore blood was taken before and 3h after systemic GC treatment for FoxP3 mRNA expression analysis, and for quantitation of T_{reg} cells by FACS. FoxP3 and IL-10 mRNA expression correlated with each other and FoxP3 was significantly increased in asthmatic patients receiving inhalative or/and systemic GC treatment. In contrast, TGF-β_1 expression was not increased. No correlation of FoxP3 mRNA expression was observed in relation to a known microsatellite polymorphism. Increased FoxP3 expression was observed *in vivo* within 3h following GC administration, as well as in *in vitro*-culture of CD4$^+$ T cells. The current data show that GCs are promoting the Tr1 phenotype of T_{reg} cells by a FoxP3 dependent mechanism.

135

The phenotype of steroid dependent severe asthma is not associated with increased lung levels of TH-2 cytokines. Sally E. Wenzel, M.D. Silvana Balzar, M.D., Hong Wei Chu, M.D. and Meghan Cundall, M.S. National Jewish Medical and Research Center, Denver, CO

The phenotype of severe, steroid dependent asthma represents a small, but costly percentage of the asthma population. The mechanisms for development of this phenotype remain poorly understood, but uncontrolled TH-2 inflammation could contribute. We hypothesized increased levels of interleukin (IL)-4 or 13 would be found in severe asthma, as compared to mild- moderate asthma and normal control lungs. To investigate this, bronchoalveolar lavage (BAL) cell and tissue biopsy specimens were collected from 16 normal controls, 13 mild-moderate asthmatics, on no or low dose steroids and 19 severe steroid dependent asthmatics. The BAL cell pellet (macrophages, lymphocytes and granulocytes) was analyzed at baseline and after 24 hrs in ex vivo culture for IL-4 and IL-13 mRNA by quantitative real-time RT-PCR. The supernatant was collected for analysis of IL-4 and IL-13 protein by enzyme linked immunosorbant assay (ELISA). Total RNA from endobronchial tissue was isolated using Trizol (Invitrogen) for analysis of IL-4 and -13. IL-13 protein was evaluated in lysed tissue by ELISA. BAL and biopsy cell differentials were also obtained. Data were log-transformed. IL-4 mRNA was not detected in any compartment, while IL-4 protein was at the level of sensitivity of the high sensitivity assay, and not different between groups. IL-13 mRNA and protein levels in nearly all compartments and all subjects were low. Although severe asthma subjects had the highest BAL lymphocyte numbers ($p=0.05$), they had the lowest levels of BAL cell IL-13 mRNA after 24 hrs in culture ($p=0.008$). BAL cell IL-13 mRNA was present in significantly higher amounts in mild-moderate than severe asthma. The IL-13 BAL cell supernatant protein and mRNA levels from biopsies of mild-moderate subjects also tended to be the highest ($p=0.10$ and 0.13, respectively), while in every instance, severe asthmatics had the lowest mRNA or protein levels. IL-13 protein was not detected in biopsies. Correcting for numbers of BAL or tissue lymphocytes did not alter the results. In asthma, 24 hr BAL cell IL-13 mRNA correlated with tissue eosinophils ($r^2=0.23$, $p=0.02$), but not with lymphocyte percentages in the BAL cell pellet ($p>0.7$). These results suggest that although TH-2 cytokines may have a role in mild-moderate asthma, they are not contributing factors in most severe asthma patients. Other mechanisms for severe asthma should be entertained.

136

The Three Mediator Paradigm in Bronchoconstriction

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Although airway inflammation involves a great number of cells, mediators and cytokines, strong evidence is accumulating to support that bronchoconstriction in asthma can be explained in terms of the actions of three main classes of mediators, and with the mast cell as the master cell in the orchestration of the reaction. For example, both the early and the late phase allergen-induced airway obstruction in atopic asthmatics is predominantly mediated by histamine and cysteinyl-leukotrienes (CysLTs), with a residual small response presumably accounted for by the mast cell mediator prostaglandin (PG) D_2. We have recently characterised the mediators of allergen-induced bronchoconstriction in the isolated perfused and ventilated guinea-pig lung (IPL), a model that has a sensitivity to mediators of bronchoconstriction that is very similar to human airways. Intravascular challenge of IPL from actively sensitised guinea pigs, with cumulatively increasing (10-10,000 μg) doses of ovalbumin (OVA), resulted in dose-dependent and reproducible reductions in lung conductance. The antihistamines mepyramine (1 μM) and metiamide (1 μM), the leukotriene antagonist zafirlukast (0.1 μM), or the cyclooxygenase enzyme (COX) inhibitor diclofenac (10 μM), each administered alone caused a distinct parallel and rightward shift in the dose-response relation for OVA, providing evidence for contributions of histamine, cysteinyl-leukotrienes and COX products to the OVA-induced bronchoconstriction in the IPL. However, when all three drugs were combined, there was a complete abolishment of the response to OVA and 10,000 times higher doses of OVA could be administered without a significant bronchoconstrictive response. In contrast the bronchoconstrictive action of methacholine was unaffected by this combination of drugs, supporting the specific mode of action by antagonising the major mast cell derived mediators. Combinations of leukotriene biosynthesis inhibitor, antihistamine and TP antagonist gave similar complete inhibition of the OVA response. The findings in this model, that previously has been documented to have a pharmacology closely resembling that of human airways, suggest that combined antagonism of histamine, cysteinyl-leukotrienes and PGD_2 might be an effective alternative treatment of bronchoconstriction in asthma.

137

T-cells and eosinophils in bronchial smooth muscle cell apoptosis

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Background: Chronic inflammatory diseases of the lung like asthma and chronic obstructive pulmonary disease (COPD) are associated with pathological remodeling of airway tissues, disrupting their proper structure and function. Bronchial smooth muscle cells (SMC) are an active cellular component capable to proliferate, express adhesion molecules and secrete cytokines. The structural changes in bronchial SMC refer to the size, mass and number of the cells with remarkably increased death index.

Aim of the study: Investigation of the mechanisms and factors responsible for the bronchial SMC death process in asthma and COPD.

Methods: Cultured primary bronchial SMC were stimulated with in vitro differentiated Th1 and Th2 cell supernatants, activated eosinophil and neutrophil lysates, IFN-γ, TNF-α and soluble Fas-ligand (sFasL). Viability, expression of apoptosis receptors, apoptosis-indicating enzymes and othermolecules and morphologic features of SMC were investigated by flow cytometry and immunohistology.

Results: Bronchial SMC death was demonstrated characteristic morphological features of apoptosis. The viability of SMC, assessed by ethidium bromide uptake was markedly decreased in comparison to the control cells after 3-6 days of incubation with the death-inducing cytokines (IFN-γ, TNF-α, sFasL) and activated Th1 and Th2 cell supernatants. Whereas IL-5-stimulated eosinophils induced apoptosis, SMC were resistant to GM-CSF- and IFN-γ- activated eosinophil lysates as well as to IFN-γ-activated neutrophil lysates, Death receptor expression on SMC was regulated. Unstimulated SMC expressed the death receptors: TNFR 1&2, Fas, TRAIL 1&2 and membrane Fas-Ligand (55.1%, 16.6%, 88.6%, 64.4%, 73.4%, 62.3%, respectively). IFN-γ and TNF-α appeared to be the effector molecules rendering the bronchial SMC susceptible to apoptosis. They upregulated TNFR1, TNFR2 and Fas as well as the membrane FasL expression on SMC after 48h of co-incubation (IFN-γ: 77.8%, 21.2%, 92%, 81.7%; TNF-α: 90.3%, 42.3%, 90.9%, 95.1%). TNF-α upregulated both TRAIL 1&2 receptors (84.4%, 89.7%) and soluble FasL upregulated TNFR2 (83.3%).

Supporting these findings intracellular caspase-3 activation in SMC significantly increased by IFN-γ (22.9%), sFasL (20.6%), TRAIL (23.2%), Th1 and Th2 supernatants (17% and 8.25%) compared to un-stimulated SMC (2.5%).

Conclusion: Bronchial SMC appears as an essential target of the inflammatory attack by T-cells and eosinophils These data demonstrate an important pathogenetic event leading to morphological changes and functional contraction/relaxation disorder in bronchial wall in asthma and COPD.

138

Autoimmunity and Allergy: a bioinformatic approach
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Previously we have critically reviewed the performance of the current guidelines proposed by Food and Agriculture Organization (FAO) and World Health Organization (WHO) for allergenicity prediction based on protein sequence and proposed a new strategy based on sequence motifs identified from a new allergen database. We concluded that our proposed motif based prediction is a superior alternative to the current method for use in the decision tree approach for allergenicity assessment. The motif based prediction allows also data mining in allergen sequences for the search of not yet identified allergens. With this approach new allergens have been identified previously only known based on their organism of origin. Even more interestingly it is now possible to create simple

schemes of potential cross reactivity between allergens.
Our bio-informatic approach has now to be veryfied by wet lab procedures. We will present data concerning consensus sequences between three human autoantigens and their corresponding cross reacting allergens. Thus a bioinformatic approach may share light on common epitopes involved in the ontogeny of allergic and autoimmune disease.

139

Expression and Function of Inhibitory and Activatory Receptors on Mast Cells and Eosinophils: New Mechanisms in Mast cell-Eosinophil Cross-Talk

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Background: Evidences indicate that a cross-talk between mast cells (MC) and eosinophils (EOS) exists that might perpetuate and aggravate allergic-inflammation. This cross-talk, due to release of specific mediators, results in enhancement of survival and activation of these cells.
Although MC and EOS express several cell surface activatory and/or inhibitory receptors, they were not thought to be directly involved in the allergic scenario. Expression and function of inhibitory/activatory receptors on human MC and human EOS has not yet been thoroughly addressed, and particularly not in the context of MC-EOS interactions.

Aim: To examine whether the expression and/or function of inhibitory and activatory receptors contribute to mast cell-eosinophil interactions.

Methods and Results: MC from cord blood mononuclear cells (CBMC) and human lung were obtained using standard procedures. Human peripheral blood EOS were purified from mildly atopics/normals (MACS). Expression of cell surface inhibitory (i.e IRp60) or activatory (i.e CD2-subfamily) receptors on MC or EOS respectively was assessed by FACS. MC were incubated for 5 days with chimeric IgE (mouse/human), then activated using goat anti-mouse IgG in 96 well plates pre-coated with the cross-linker sheep anti-mouse, and mouse anti-human IRp60. EOS were activated by incubation with the cross-linker and one of the antibodies recognizing activatory receptor(s). As activation markers β-hexosaminidase, tryptase for MC, and eosinophil-peroxidase (EPO) for EOS, were determined using enzymatic-colorimetric assays. IFN-γ and IL-4 were determined by ELISA.

CBMC and human lung MC express the inhibitory receptor IRp60. EOS display a variety of CD2-subfamily receptors. Cross-linking of IRp60 with FcεRI inhibited β-hexosaminidase, tryptase and IL-4 release from CBMC. Cross-linking of 2B4, CD48 or NTB-A caused EPO release. Cross-linking of 2B4 on EOS induced IFN-γ and IL-4 release and phosphorylation of ERK 1/2. Co-culture of EOS and MC induced MC activation. Neutralization of 2B4 on EOS or CD48 on CBMC abrogated the activatory effect. Also, eosinophil major basic protein (MBP) down-regulated expression of IRp60 on MC.

Conclusions: **The demonstration that activatory receptors expressed on EOS play a role in MC-EOS cross-talk, and that MBP can down-regulate inhibitory receptors on MC suggests a new pathway to regulate the inflammatory responses coordinated by these cells.**

140

Regulation of MITF Transcriptional Activity in Mast Cell by Two Endogenous Suppressors.

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The network by which MITF transcriptional activity is regulated in mast cells is complex and unique, but comprehension of this complexicity could be critical for our understanding of how mast cells function and therefore could also have tremendous therapeutical implications.
We have previously identified two MITF-interacting proteins using the yeast two hybrid system with the bHLH-Zip domain as a bait: PKCI/Hint and PIAS3, the STAT3 inhibitor protein. These two MITF associated proteins were shown to be repressors of MITF transcriptional activity.

We have found that lysyl-tRNA synthetase (LysRS) is also associated with MITF and forms a multi-complex with MITF and Hint. We have also found that Ap_4A, which is known to be synthesized by LysRS, is accumulated intracellularly in activated cells. We have shown that Ap_4A then directly binds to Hint, liberates MITF and thus leads to the activation of MITF dependent gene expression (1). This implies that a key role of LysRS and Ap_4A as a regulators of MITF transcriptional activity.
Additionally, we found that phosphorylation of MITF on serines in positions 73 and 409 plays an important role in its association with PIAS3. This effect was profound with phosphorylation on ser409, which significantly reduced the inhibitory affect of PIAS3 on MITF and also modulated MITF's transcriptional activity (2). Thus, phosphorylation of MITF could be considered as a fine and alternative tuning of its transcriptional machinery.

1. Yu-Nee Lee, Hovav Nechushtan, Navah Figov and Ehud Razin. Immunity 2004 in press.
2. Carmit Levy, Amir Sonnenblick, Ehud Razin. Molecular and Cellular Biology 23:9073, 2003

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