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Oral Abstract Session 1

Atopic dermatitis: from mechanisms to innovative management

1
Polyprenol with atorvastatin could improve management of atopic dermatitis

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Background: Atorvastatin (As) is an inhibitor of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase, a key enzyme in mevalonic acid (MVA)-dependent signaling. Recent data suggest that statins exhibit inhibitory effects on cysteinyl leukotrienes and IgE-dependent histamine release in human mast cells.

Polyprenol (Pp) is a substitute of Dolichyl Phosphate Cycle and the rate limiting factor in N-glycosylation. Recent data suggest that Pp could prevent cell-mediated cytotoxicity against skin fibroblasts in atopic dermatitis (AD) and main side effects of statins *in vitro*. The purpose of the present study was to evaluate the mechanism of action and efficacy of As with Pp as a treatment for patients with AD.

Methods: SCORAD index was used to measure the severity of the disease and to evaluate the effect of treatment in 42 adult patients. Evaluation of erythema, induration, excoriation, lichenification, scaling, erosion were scored on a 0–3 scale each week A 30% decrease in total score was considered clinically significant. Leukotriene E4 and dolichol (Dol) were assayed in urinal excretion, immunoCAP total IgE levels were measured in serum, dolichol phosphate N-acetyl-glucosamine-1 phosphate transferase (GPT) activity was defined in dermal fibroblasts by metaboling labeling (ML) method with [2-(3)H]-mannose. As (10 mg/day, *per os*) with Pp (5 mg/day, *per os*) were given in a randomised, double-blind, placebo-controlled study. The effect of the treatment was evaluated weekly up to 6 months.

Results: Initially patients with AD were found to have a statistically significant increase in leukotriene E4 (4-fold) and Dol (6.2-fold) excretion, total Ig E level and GPT activity in fibroblasts in comparison to controls. The normalisation up to 90% of Dol excretion was achieved after 2 weeks of treatment, IgE and E4 in 3 weeks, GPT after one month in 70% of patients with remission for more than 1 year. Significant difference in AD scores between As with Pp and placebo ($P < 0.01$) was recognised.

Conclusion: The present study demonstrates alleviation of AD with the use of the As with Pp. The activity of this combination involves the main links of AD pathogenesis. As with Pp could present novel therapeutic options in the management of atopic dermatitis.

2
Expression of histamine-synthesizing enzyme, histidine decarboxylase, by keratinocytes and its regulation by cytokines and factors relevant to atopic skin disease

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Background: Atopic eczema is a common chronic skin disease where both genetic and acquired disturbances lead to the inflammation. At the immunological level both the accumulation of immune cells and reduced skin barrier function has been observed; the cross-talk at the interface seems to be critical for the disease pathogenesis. Histamine has been long known to be an abundant inflammatory mediator accumulating in the skin of the patients where it is thought to be primary immune cell-derived. Very little is known about the capability of keratinocytes to express the histamine-synthesizing enzyme, histidine decarboxylase (HDC) or factors that regulate its expression.

Method: Both immortalized and primary human keratinocytes were investigated for the expression of HDC at mRNA (qPCR) and protein level (WB and ICC). Expression of the enzyme and the histamine-dependent negative feedback loop was also investigated in organotypic epidermal models (IHC). Furthermore, we have assessed keratinocyte expression of HDC under stimulation of calcium as well as cytokines and factors relevant to atopic eczema.

Results: We have detected low levels of HDC at the mRNA level. However, expression of the protein was abundant, especially in HaCaT keratinocytes, but also in primary cells. While we did not observe differential expression under low/high calcium conditions, histamine downregulated HDC in a stratified epidermis model. Furthermore, we found that exposure of keratinocytes to IL-4, IL-13, TSLP, TNF-α as well as LPS and HDM extract enhanced expression of HDC in cultured cells.

Conclusion: Keratinocytes express HDC, an exclusive enzyme responsible for histamine synthesis. While calcium dependence is unlikely in human cells, environmental factors as well as cytokines implicated in allergic skin inflammation promote the expression of the enzyme, suggesting the possibility that histamine could be actively secreted by keratinocytes under inflammatory states and beyond. This finding therefore presents new point of therapeutic intervention for atopic eczema patients.

3
Dieckol, a phlorotannin of *Ecklonia cava* suppresses spontaneous dermatitis in NC/Tnd mice, a model for human atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterised by pruritic, dry and eczematous skin lesions with an abnormal immune response, in particular, over activation of the Th2 pathway and over production of immunoglobulin E (IgE). The immunological capacities of *Ecklonia cava* known as a brown seaweed and its phlorotannin, dieckol on the antiinflammatory, immunomodulatory, and anti-allergic reactions have been reported. However, little is known its action on the symptoms of AD in NC/Tnd mice.

Methods: We investigated the therapeutic effect of dieckol on the spontaneous dermatitis in conventional NC/Tnd mice. Mice were orally and daily administrated with dieckol (62.5 and 125 μg/mouse) for 4 weeks from 12 weeks of age (mean

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clinical skin score: 6). The clinical skin score, scratching duration and frequency, and trans-epidermal water loss (TEWL) were analyzed every week. The histological analysis and the infiltration of inflammatory cells were evaluated by H&E, toluidine blue, and congo red staining assays. The mRNA expression and protein production levels of cytokines and chemokines were evaluated by real-time RT-PCR, luminex multiplex cytokine analysis and immunohistochemistry. The serum levels of total IgE were examined by an ELISA.

Results: Administration of dieckol (125 μg) significantly reduced the skin dermatitis severity, scratching duration and frequency and TEWL, whereas the 62.5 μg of dieckol did not affect. In addition, dieckol greatly improved the epidermal hyperplasia and inhibited the dermal infiltration of inflammatory cells including mast cells and eosinophils in the affected skins. Moreover, the suppression of dermatitis by dieckol significantly was accompanied by the decreased mRNA expression and protein production levels of Th2 cytokines such as IL-4, IL-5, IL-6, IL-10, IL-31 and/or Th1 cytokine, IFN-γ and chemokines such as MDC and/or TARC in skins, spleens and draining lymph nodes as well as those of IL-25, IL-33 and TSLP known as the initiators of AD symptoms in skins. Furthermore, dieckol significantly reduced the higher serum level of total IgE in conventional NC/Tnd mice, whereas it was maintained in control group.

Conclusion: These results suggested that dieckol may be the natural agent with beneficial potentials for the treatment of AD.

4
Characterisation of soluble markers of apoptosis during diet therapy in children with atopic dermatitis

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Background: To estimate the dynamics of soluble apoptosis markers in infants with atopic dermatitis for updating mechanisms of immunopathogenesis and improvement of diet therapy.

Method: We observed 66 bottle-fed infants aged 1.5–12 months old (boys – 47, girls – 19) with atopic dermatitis (AD). The sensibilization to cow milk protein was revealed in all 66 infants. Detected allergen-specific IgG and IgE antibodies to cow milk protein, its fraction and goat milk protein were the reason to include infants into the 1st group and feed with hydrolyzed formula (27 infants). Thirty-nine infants in the 2nd group, who were not sensibilized to goat milk protein, were fed by goat milk-based formula. Serum levels of soluble apoptosis markers (sCD153, caspase-8, sFas-L, caspase-9 and annexin-5) were measured by immunoenzyme method (ELISA).

Results: The activation of signal apoptosis systems in infants with AD with increased levels of sFas-L è sCD153 was revealed. Levels of caspase-8 and caspase-9 were significantly lower than in control group, and reflected the impaired elimination of modified immmunocompetent cells. The level of annexin-5 was significantly lower in infants with AD than in control group. The estimation of the dynamics of investigated parameters during diet therapy showed significant increase of caspase-9 level in both groups. The level of caspase-8 was increased only in infants who were fed by goat milk formula. Levels of sFas-L, sCD153 and annexin-5 durind diet treatment did not differ significantly between groups.

Conclusion: The results showed that sCD153, caspase-8, sFas-L, caspase-9 and annexin-5 play a role in the realisation of allergic inflammation in infants with AD. The diet therapy with goat milk formula promotes more physiological repair of the effectory component of the apoptosis.

5
Longitudinal analysis of atopic dermatitis in ALSPAC shows that known genetic variants are associated with early-onset or persistent atopic dermatitis, but not late-onset disease

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Background: Several genetic variants have been identified which predispose to atopic dermatitis (AD). However, most of these have been discovered in genome-wide association studies that have used relatively crude case definitions. But, AD is a heterogeneous condition in which some individuals demonstrate a transient itchy erythematous rash only in childhood, whilst others follow a chronic, relapsing/remitting course persisting into adulthood. We used the Avon Longitudinal Study of Parents & Children (ALSPAC) to identify different longitudinal patterns of AD and investigate associations with known AD genetic variants between the different disease courses.

Method: We analysed rash questionnaire data at 12 time-points through childhood (6 months – 16 years) using latent class analysis in Mplus to identify longitudinal patterns of AD in approximately 9000 children. Genetic variants that had previously shown association with AD (in/near Filaggrin (*FLG*), *IL4/KIF3A, OVOL1, ACTL9, C11orf30* & *TNFRSF6B*) were available. Phenotype data from at least 6 of the time-points and genetic data were available for 6273 children. Immunoglobulin E (IgE) levels and skin prick response to common airborne (grass, cat & dustmite) and food (peanut, tree nut & egg) allergens were measured at 7 years of age.

Results: Four classes were identified that showed different patterns of association with genetic variants; 'early-onset transient', 'early-onset persistent', 'late-onset transient' & 'never/infrequent'. *FLG* genotype showed the strongest association with the 'early-onset persistent' class ($\beta = 1.14$, $P = 1 \times 10^{-18}$), a weaker association with 'early-onset transient' ($\beta = 0.65$, $P = 7 \times 10^{-6}$), but no association with 'late-onset' ($\beta = 0.083$, $P = 0.79$). A similar pattern was seen for the other genetic variants. Airborne allergen sensitisation was associated with all three AD classes. However, IgE levels and nut allergen sensitisation were only associated with 'early-onset transient' and 'early-onset persistent' AD, whereas the 'late-onset' class showed no association.

Conclusion: Our results indicate that there are distinct clinical sub-groups of AD with different genetic aetiology. The strongest associations with known AD variants were seen for the 'early-onset persistent' class, whereas the 'late-onset' class was not associated with any variants. This will be informative for future epidemiological and gene-finding studies of AD and may help to clarify the different pathogenic mechanisms in this heterogeneous disease.

6
Infants who develop eczema at 12 months have a deficient T regulatory cell response to microbial stimuli at the time of birth

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Introduction: Regulatory T cells (Treg) cells may play an essential role in regulating early immune development and shaping

the immune response toward a pro-allergic or tolerant state. Pro-inflammatory cytokines may be associated with tolerance induction.

Objectives: We evaluated cord blood T regulatory and proinflammatory responses to microbial and non-microbial stimuli in infants at high risk of allergic disease. We investigated whether variations in these responses are associated with development of allergic disease in the first year.

Methods: Cord blood mononuclear cells of 72 neonates at high risk allergy whose mothers participated in a prenatal probiotic eczema prevention study were cultured with TLR2 ligands – lipoteichoic acid (LTA) and heat-killed *Lactobacillus rhamnosus* GG (HKL); TLR4 ligand – lipopolysaccharide (LPS); ovalbumin (OVA); anti-CD3 or media for 48 h. Numbers of Treg, proinflammatory and regulatory cytokines were compared between children who developed eczema at any time and atopic sensitisation during the first year of life and those who did not.

Results: Infants who developed eczema ($n = 24$) had reduced percentages of Fox-$P3^{hi}CD25^{hi}$ Treg cells in response to LTA ($P = 0.01$, adj $P = 0.005$) and HKL ($P = 0.04$, adj $P = 0.02$), reduced IL-6 ($P = 0.03$), increased IL-10 ($P = 0.049$) and a trend towards reduced TGF-β1 ($P = 0.07$) production following HKL stimulation as compared to those without eczema ($n = 48$). No differences in Treg numbers or pro-inflammatory responses following stimulation with LPS, OVA, or anti-CD3 were seen. Infants who developed sensitisation had lower percentages of Treg following TLR stimulation (but not other stimuli) compared to non-sensitised infants.

Conclusions: Children who develop allergic disease in the first year of life have deficient Treg numbers and pro-inflammatory responses to microbial stimuli but not allergen from the time of birth, which may contribute to failure of immune tolerance development in infancy.

6B

Influence of the potential hydrogen on skin barrier function and immune responses in NC/Tnd mice, a model for human atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic, relapsing and pruritic skin disease. Excessive T helper type 2 (Th2) signaling and skin barrier dysfunction play key roles in the pathogenesis of AD. The stratum corneum (SC) of mammalian skin normally shows acidic pH. The acidic pH of the SC regulates antimicrobial function and epidermal functions that are involved in permeability barrier homeostasis and SC integrity/cohesion. Increased pH of SC adversely induces the abnormality of epidermal functions. However, the influence of the potential hydrogen (pH) on skin barrier function and immune responses in AD is not clear.

Method: Skin pH and transepidermal water loss (TEWL) in both SPF and conventional NC/Tnd mice, a model for human AD, were measured. In the skins of 5, 7, 9, and 12 week-old NC/Tnd mice, the expression levels of pH-sensitive serine proteases (Kallilrein 5), lympho-epithelial Kazal-type-related inhibitor (LEKTI), protease activated receptor-2 (PAR-2) and thymic stromal lymphopoietin (TSLP) were analyzed by using a real-time PCR, western blot analysis, and immunohistochemistry.

Results: Skin pH and TEWL in conventional NC/Tnd mice from 7 weeks of age were significantly increased when compared with age-matched specific pathogen free (SPF) NC/Tnd mice. Protein levels of Kallikrein 5 and PAR-2 in epidermis of conventional NC/Tnd mice were significantly increased during the disease progression when compared to age-matched SPF NC/Tnd mice. However, protein levels of LEKTI in conventional and SPF NC/Tnd mice were not altered. In addition, mRNA levels of TSLP in conventional NC/Tnd mice were time-dependently increased from 5 to 12 weeks of age. Kallikrein 5 and PAR-2 were co-localized in the epidermis of conventional NC/Tnd mice.

Conclusion: Increase in pH of the SC was obvious in the mouse model for AD. Kallikrein 5, which is inactivated at acidic pH, was increased in the affected skin, indicating the association of the pH elevation with Kallikrein 5 activation. Unregulated Kallikrein 5 is reported to activate PAR-2 and induce overexpression of TSLP, that is a pro-Th2 cytokine. Therefore pH elevation of the SC may be responsible for the early development of AD by the subsequent activator, Kallikrein 5.

Oral Abstract Session 2

Clinical studies in allergen specific immunotherapy

7

The European survey on adverse systemic reactions due to allergen immunotherapy: the 'EASSI' pilot survey

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Background: At present, there is no European report on clinically relevant systemic reactions due to the regular use of subcutaneous or sublingual allergen immunotherapy (SCIT and SLIT, respectively) outside clinical trials. Using an electronic survey and a 'harmonised terminology' according to MedDRA we aimed to prospectively collect systemic adverse reactions due to (allergen immunotherapy) AIT from real life clinical settings.

Methods: Under the framework of the EAACI, a team of European specialists in AIT, pharmacovigilance, epidemiology and drugs regulation, set up a survey to be conducted, first as a pilot, in four countries (France, Italy, Germany and Spain). A designated 'country coordinator' is responsible for following specific country ethics requirements and to select at least 30 doctors per country. Patients have been recruited the same day they received their first dose of either SCIT or SLIT. The survey started on 01/09/12 and is planned to be finished on 31/01/14. Patient inclusion criteria are: adults and children, with IgE mediated pollen and/or house dust mite, *Alternaria*, and/or animal dander respiratory allergies who will initiate AIT. The Survey Monkey® online survey instrument is used by participant doctors to submit information directly into a central base via a blinded coded programme which allows to follow any adverse reaction due to AIT without compromising patients' and doctors' anonymity. A list of symptoms and their definition was selected from the MedDRA dictionary in order to report all adverse reactions in this survey.

Results: Three questionnaires have been generated: the Doctor Questionnaire, the Patient Questionnaire and the Reaction questionnaire; as well as a handbook and a mistake report form. A list of 30 terms for single symptoms has been selected to report adverse reactions. As of Jan 15th 2013, a total of 144 doctors have been recruited, 1233 patients included and 15 adverse reactions due to AIT have been reported.

Conclusion: This is the first report in Europe collecting systemic adverse reactions due to AIT in a prospective real life setting. This survey will bring interesting data to the scientific community and could provide the platform for a future pan-European registry on AIT safety.

8

ERS/ATS joint statement definition of moderate asthma exacerbation operationalised for use in a randomised, double-blind, placebo-controlled trial (MITRA trial) of the house dust mite allergy immunotherapy tablet

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Background: The house dust mite (HDM) allergy immunotherapy tablet (AIT) (ALK, Denmark) is being investigated for improved control of HDM-induced allergic asthma in the randomised, double-blind, placebo-controlled MITRA trial. The primary analysis is estimating the relative risk of asthma exacerbations by time-to-event methodology. Asthma is not included in the 'guideline for development of products for immunotherapy', and immunotherapy is not included in the 'guideline for clinical investigation of products for treatment of asthma', leaving a regulatory gap for immunotherapy trials in patients with asthma. Previous trials in asthma have largely focused on severe exacerbations, but the aim in the MITRA trial was to capture asthma exacerbations at an earlier stage. The clinical definition of a moderate asthma exacerbation in the MITRA trial is based on the ERS/ATS 2009 joint statement (Reddel et al. 2009).

Method: In the ERS/ATS joint statement a moderate asthma exacerbation is an event that 1: results in a temporary change in treatment (not including systemic corticosteroid); 2: includes one or more of the following for at least 2 days: deterioration in symptoms, deterioration in lung function, or increased rescue bronchodilator use; 3: has a magnitude of change that differs depending on each patient's baseline variation. This was operationalized into clinical measures applicable to a large, multi-site, phase III clinical trial.

Results: For the MITRA trial, a moderate exacerbation is defined as at least one of the following criteria fulfilled and leading to a change in treatment:

1. nocturnal awakening(s) due to asthma requiring SABA for 2 consecutive nights or increase of $\geq$0.75 from baseline in daily symptom score on two consecutive days;
2. increase from baseline in occasions of SABA use on two consecutive days (minimum increase of 4 puffs/day);
3. $\geq$20% decrease in PEF from baseline on at least two consecutive mornings/evenings or $\geq$20% decrease in FEV_1 from baseline;
4. visit to the emergency room/trial site for asthma treatment not requiring systemic corticosteroids. The individual baseline values are symptom use, SABA use or PEF during 14 days before randomisation.

Conclusion: This definition of a moderate asthma exacerbation was endorsed by the EMA Scientific Advice Working Party in February 2011. The MITRA trial is currently on-going, and results on the usefulness of the endpoint and on the efficacy of HDM AIT will be available late 2013.

9
Generation of a safe and efficacious allergy vaccine for birch pollen and associated food allergies

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Background: In the Northern Hemisphere, Bet v 1 from birch pollen is the main sensitiser for tree pollen allergy. People allergic to birch pollen also often develop adverse reactions to structurally related allergens from fruits, nuts, and vegetables, resulting in the oral allergy syndrome (OAS). Specific immunotherapy (SIT) is the only curative approach to meet the disease. Therapy-induced IgE-mediated side-effects in conventional SIT can be diminished by replacing pollen extracts with hypoallergenic recombinant molecules.
Method: With the intention of simultaneously curing birch pollen allergy and related adverse reactions to apple and hazelnut, a hybrid hypoallergen was designed by combining parts of the three parental allergens. Further, a mutation to alter the structure of the molecule (MBC4) was introduced. The hybrid protein, as well as parental allergens were produced recombinantly, purified to homogeneity, and characterised physico-chemically. To examine the IgE-binding properties of MBC4, ELISA and mediator release assays were performed with sera of allergic patients. Moreover, a mouse model was established to monitor the immunologic behavior of MBC4 *in vivo*.
Results: The new hybrid molecule showed alternation in secondary structural elements characteristic for Bet v 1 -like allergens. Moreover, it was less reactive with human IgE than its parental allergens but still able to induce a strong cross-reactive IgG response in mice. Upon restimulation of spleenocytes of MBC4 immunised animals with parental allergens, high numbers of IFN-γ secreting cells were induced indicative for a strong cross-reactive Th1 immune response. However, IL-4 or IL-5 producing cells induced by MBC4 immunisation did not cross-react upon restimulation with parental allergens.
Conclusion: MBC4 was proven to react as hypoallergen as determined by ELISA as well as mediator release assays. Significant changes in the protein structure of MBC4 had no negative influence on its immunogenicity. The hybrid molecule induced high levels of cross-reactive IgG antibodies in a mouse model. Therefore, MBC4 represents a promising candidate for a protein-based vaccine to simultaneously treat birch pollen and associated food allergies.

10
Sublingual immunotherapy with a five grass allergen extract on reduces local inflammation as measured by exhaled breath temperature in grass pollen allergic patients

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Background: Pollinosis is a classical atopic condition in which allergen immunotherapy has been proven to decrease in-season complaints and to change the natural course of the disease. However, clinical studies rely on assessment of subjective symptoms and substantial placebo effect has been documented in double blind studies. Our previous data suggest that exhaled breath temperature (EBT) increases during the pollen season in subjects with pollinosis. The aim of our study was to explore whether sublingual immunotherapy (SLIT) would influence the increase of EBT during the grass pollen season.
Method: We recruited 56 subjects (33 men, mean age 30 ± 12 years) with grass pollen allergy for this double-blind placebo-controlled clinical trial. All subjects had positive skin prick tests restricted to grass pollen allergen extracts. Spirometry was performed using Schiller SP 10 equipment (Switzerlznd). EBT was assessed with portable hand-held device at controlled (22–23°C) indoor temperature (X-halo, Delmedica Investments Ltd, Singapore). Blood samples were collected to measure eosinophil counts. Evaluations were performed before the grass pollen season, at the peak of it and about 2 months after the pollen counts have subsided. Subjects were randomised to be treated with five component grass allergen solution or with placebo applied sublingually for at least 3 months before, and during the pollen season.
Results: Out of 56 recruited patients, 51 completed the study visits before and during the pollen season, 45 returned for the post-seasonal control visit. Overall, EBT increased from 34.32 ± 0.08°C to 34.71 ± 0.05°C, $P < 0.001$. In placebo group EBT changed from 34.26 ± 0.10°C to 34.80 ± 0.07°C (increase of 0.54°C, $P < 0.001$) and in the SLIT group, the EBT changed from 34.40 ± 0.12°C to 34.61 ± 0.71°C (increase of 0.21°C, $P = 0.052$). The difference between active (0.54 ± 0.09°C) and placebo (0.21 ± 0.10°C) was statistically significant ($P = 0.03$). EBT of all subjects returned to pre-seasonal levels after the grass pollen season. Spirometry values and blood eosinophil counts did not change significantly at any of the visits, nor between the treatment arms.
Conclusion: Our study provides evidence for the effectiveness of SLIT with grass pollen allergen extracts on the basis of EBT, an objective measure allegedly reflecting the level of airway inflammation.

11
Efficacy of house dust mite sublingual tablets in an environmental exposure chamber study of patients with house dust mite-associated allergic rhinitis

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Background: House dust mite (HDM) sublingual immunotherapy (SLIT) tablets have proven efficacious in treating HDM-associated allergic rhinitis in a natural field study. This phase II study assessed the dose-response relationship by testing several doses in an environmental exposure chamber (EEC).
Methods: In this DBPC study, adults with HDM-associated allergic rhinitis for at least 1 year, a positive prick skin test, and mites-specific IgE antibodies ≥0.7 kU/l were randomised 1:1:1:1 to receive a daily sublingual tablet containing either placebo or HDM allergens at a dose of 100IR, 300IR or 500IR (IR = Index of Reactivity) for 6 months. Participant selection and efficacy evaluation were based on rhinitis total symptom scores (RTSS) upon exposure to *D. pteronyssinus* over 4 h in the EEC during screening and after 6 months, respectively. The areas under the curve of RTSS corresponding to all time points ($RTSS\_AUC_{0–4\ h}$) and the last 2 h ($RTSS\_AUC_{2–4\ h}$) of each EEC were determined. Changes from baseline (ChBl) to the end of treatment in these values served as the primary ($ChBl\_RTSS\_AUC_{0–4\ h}$) and secondary ($ChBl\_RTSS\_AUC_{2–4\ h}$) efficacy endpoints. Differences between placebo and active groups were analyzed by ANCOVA. Safety was assessed by means of adverse event (AE) reporting.
Results: Three hundred and fifty-five participants were randomised: Placebo ($n = 87$), 100IR ($n = 89$), 300IR ($n = 86$), and 500IR ($n = 93$). The least squares mean differences vs placebo for the 100IR, 300IR and 500IR groups on the primary efficacy endpoint indicated reductions in symptom severity of 20%, 29% and 33%, respectively. The corresponding relative

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reductions over the last 2 h of the 6 month challenge were 31%, 41%, and 42%, respectively. The incidences of AEs were similar across the active treatment groups with throat irritation, oral pruritus, and mouth oedema the most frequent treatment-related AEs.

Conclusion: After 6 months of treatment, a dose effect of sublingual tablets of HDM allergen extract was observed in this allergen challenge study. The 500IR dose was associated with the greatest reduction in symptom score. The efficacy observed over the last two hours of exposure reinforces the robustness of the effect. All doses were well-tolerated.

12

Grass pollen carbamylated monomeric allergoid sublingual tablets for the treatment of allergic rhino-conjunctivitis: a prospective, randomised, controlled, double-blind dose-finding study in 155 patients

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Background: The efficacy, the safety, and tolerability of sublingually administered, monomeric carbamylated allergoids have been documented in several DBPC trials. Purpose of this study was to confirm the optimal dose of the current marketed product in a conjunctival provocation test (CPT).

Method: Patients 18–70 year-old with a history of 2 or more years of moderate to severe allergic rhino-conjunctivitis due to grass pollen with/without controlled asthma and a positive skin test were recruited to receive tablets containing grass carbamylated allergoid with a daily dose of 300UA, 600UA, 1000UA or 2000UA for 84 consecutive days between January and April 2012. Primary endpoint of this prospective, randomised, double-blind, controlled trial was the improvement of the reaction to CPT challenge with grass-pollen concentrations of 100 SQ, 1000 SQ and 10000 SQ evaluated by the investigators on a rating-scale according to Riechelmann. Secondary endpoints were patients' rating of treatment satisfaction and investigators' rating of efficacy (0–3), their rating of tolerability (0–3), and frequency of adverse reactions.

Results: Data of 151 of the 155 patients included in the treatment phase were analyzed in the ITT population (mean age 40.3 year, 54% males, mean disease duration 21 year). Their reaction to conjunctival challenge significantly improved after 3 months of treatment in all four treatment groups (70.4%, 62.9%, 76.7%, and 66.7% of patients resp.), consistent with investigators' judgement of efficacy (mean scores 2.11, 1.95, 2.37, 2.03). Improvement under 1000 UA daily dose was rated significantly better ($P < 0.03$) as compared to that of the other doses. Thirty-eight treatment-emergent adverse events occurred in 27 of the patients (17.4%) again with the lowest incidence in the 1000 UA group. All these were classified as mild or moderate with an equal distribution of local and systemic side-effects. Only one patient experienced a local side effect after the first application. Of 98.7% of patients rated the tolerability 'very good' or 'good'. No serious adverse events nor drop-outs due to safety-reasons occurred.

Conclusion: Carbamylated monomeric allergoid tablets of grass pollen are well tolerated and effective in improving the allergic condition when taken sublingually for 3 months. Of 1000 UA daily dose demonstrated the best results in the efficacy and safety analysis.

Oral Abstract Session 3

Genetic and epigenetic markers for food allergy

13
A role for disrupted DNA methylation at key immune genes in the pathogenesis of IgE food allergy

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Background: IgE-mediated food allergies are thought to arise through complex gene-environment interactions occurring during critical periods of immune development. Epigenetics may be one important mechanism through which such gene-environment interactions are mediated. DNA methylation is a well-characterised epgienetic mark that plays a central role in normal immune development, and is sensitive to environmental Disruption. Variations in DNA methylation and loss of gene regulation has been described in a number of immune-mediated diseases, and previous studies have indicated maternal exposures can epigenetically program the risk for disease at key immune genes in the developing fetus.
Method: This study is a longitudinal case-control design from an Australian cohort of children with IgE food allergy and age-matched healthy controls ($n = 60$). CD4+ cells were collected at birth and at the 12-month time of diagnosis and DNA methylation was measured genome-wide using high density microarrays together with parallel genome-wide expression measurements.
Results: A total of 240 DNA methylation differences were identified between allergics and non-allergics. A large proportion (65.8%) of these were found to overlap annotated SNPs which we believe are in linkage with polymorphisms. The top pathway enriched in these disease-associated markers was 'Intestinal immune network for IgA production' and included the CD80, TNFRSF17, IL5RA. Eighty-two loci were not SNP associated and these epigenetic variants occurred calcium signaling genes CACNA1A1, CACNA1B1, KCNN3. Comparisons of birth and 12-month samples revealed a proportion of disease-associated methylation variants were also present at the fetal stage suggesting they are persistent marks of fetal origin.
Conclusion: This study provides evidence that disruption in DNA methylation may be an important component to the risk for food allergy. We have demonstrated that several disease-associated variants are present prior to the clinical manifestation of disease and are therefore programmed before birth. Functional studies are now needed to ascertain the effects of these variations on immune regulation.

14
Relationships between genetic polymorphisms of TSLP, IL-10, IL-13, CD14, SPINK5, SCCA and STAT6 molecules which are effective in allergic inflammation and phenotypic features in children with egg allergy

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Background: Even though lots of studies have investigated the genetics of asthma and other allergic diseases, there is limited information regarding the genetic basis of food allergy. Our aim is to investigate whether there is a relationship between genetic polymorphisms of molecules related to Th2 immune response and phenotypes of egg allergy in children.
Method: DNA samples were taken from IgE mediated egg allergic children and healthy children. The diagnosis of egg allergy was made in the presence of positive skin prick test, specific IgE $\geq$0.35 kU/l with history of egg related symptoms or positive egg challenge tests. We investigated the most common polymorphisms of TSLP, IL-10, IL-13, CD14, SPINK5, SCCA and STAT6 molecules.
Results: DNA samples were analyzed in 240 children with egg allergy and 230 healthy controls. The frequency of rs11466749 AA genotype in 4th exon of TSLP gene was significantly increased in children with egg allergy (0.682) compared with healthy controls (0.545) ($P = 0.003$). Eosinophil count was significantly higher in healthy children with rs11466749 AA genotype in TSLP gene compared to those with the AG+GG genotype [182 (100–300) vs 113 (100–214) ($P = 0.032$)]. Similarly eosinophil count was significantly higher in egg allergic children with rs11466749 AA genotype in TSLP gene compared to those with the AG+GG genotype [600 (371–1100) vs 450 (300–900) ($P = 0.033$)]. Additionally initial egg sIgE level was higher in egg allergic children with rs11466749 AA genotype in TSLP gene compared to those with AG+GG genotype [4.2 kU/l (1.4–13.5) vs 1.9 kU/l (0.9–8.5) ($P = 0.021$)].
Conclusion: This study showed that TSLP gene rs11466749 AA genotype was more frequent in egg allergic children than healthy controls and it was associated with high eosinophil count and initial egg sIgE in children with egg allergy. Our results suggest that thymic stromal lymphopoeitin (TSLP) may be critical molecule of inflammation in the development of IgE-mediated egg allergy.

15
The role of indoleamine 2,3-dioxygenase (IDO) gene polymorphisms and tryptophan/kynurenine pathway in food allergic and tolerant children

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Background: Indoleamine 2,3-dioxygenase (IDO) is the rate-limiting enzyme in degradation of tryptophan (Trp) to kynurenine (Kyn). Tryptophan/Kynurenine pathway is effective in immune tolerance process by virtue of modulating regulatory T cell generation and function. However, the role of this pathway in food allergy has not elucidated yet.
Method: The diagnosis of IgE-mediated food allergy was made with positive SPTs, sIgE > 0.35 kU/l in conjunction with a consistent history and food challenge tests. Serum Trp and Kyn concentrations were determined by HPLC. In order to evaluate the effect of diet on the level of serum Trp and Kyn a list concerning food consumption of each child for the last 24 h was checked for the amount Trp intake. Ten

polymorphisms in IDO 1 and 2 genes were determined.

Results: Kyn/Trp quotient that indicates the activity of IDO enzyme was higher in healthy children [48.7 umol/mmol (40.6–59.9)] than food allergic children [42.7 (35.5–55.8)] ($P = 0.005$). When food allergic group was categorised as 'reactive' and 'tolerant', the results revealed that Kyn/Trp quotient was higher in tolerant group [44.8 (38.2–56.2)] than reactive children [42.2 (34.7–54.1)]. In order to determine the effect of food intake, we performed the same analyses with the sera which were collected after 8–12 h fasting, and the results were found compatible with the initial results. General linear model analysis disclosed that dietary protein and tryptophan intake in the diet of the children had no influence on Trp, Kyn concentrations and Kyn/Trp quotient. The frequency of polymorphisms in IDO 1 and 2 genes were similar in food allergic and healthy children. In the group of food allergy, the polymorphism rs2955903 GG genotype was significantly more frequent in food tolerant children (0.600) than reactive ones (0.327) ($P = 0.011$). In the group of healthy children Kyn/Trp quotient was higher in children with rs11992749 AA+AC genotype [51 (41–59)] than children with CC genotype [41 (38–53)] ($P = 0.027$). There was no relationship between Trp, Kyn, and Kyn/Trp quotient and IDO genotypes in food allergic children.

Conclusion: Our results show that IDO enzyme activity is higher in healthy controls than food allergic children. Moreover IDO activity is higher in food tolerant children than reactive ones. Our study suggests that the increased activity of Tryptophan/Kynurenine pathway may be related with the development of tolerance in food allergy.

16

The association between serum vitamin D and food allergy is modified by polymorphisms in vitamin D metabolism pathway genes

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Background: Emerging evidence suggests that low vitamin D is associated with allergen sensitisation, and that this association may be modified by genetic factors. We have recently found that infants of Australian-born parents with vitamin D insufficiency (VDI; <50 nM/l) were more likely to be food allergic (aOR 3.08, 95% CI 1.10, 8.59, $P = 0.032$) than those with adequate vitamin D levels. No previous studies have examined whether the relationship between VDI and challenge proven food allergy is modified by genotype. Using data from the HealthNuts population-based infant cohort, we aimed to examine whether the association between VDI and food allergy is modified by polymorphisms in vitamin D metabolism pathway genes.

Method: A population sample of 5276 1-year-old infants underwent skin prick test (SPT) to peanut, egg and sesame. All those with a detectable wheal, and a random sample of SPT negative participants, attended a hospital-based food challenge. Blood samples were available for 577 infants (344 with challenge-proven food allergy; 74 sensitised but tolerant to food challenge; 159 negative on SPT and challenge). Serum 25(OH) D levels were measured using liquid chromatography tandem mass spectrometry. DNA was genotyped for 28 single nucleotide polymorphisms (SNPs) in five genes involved in the vitamin D metabolism pathway (*VDR*, *GC*, *CYP24A1*, *CYP2R1* and *CYP27B1*). Associations between these SNPs and food allergy, and interactions between VDI and SNPs, were examined using logistic regression.

Results: The *CYP24A1* intron variant rs2244719 was associated with an increased odds of food allergy among sensitised infants (OR 2.0, 95% CI 1.2–3.3, $P = 0.007$).

The association between VDI and food allergy was modified by *CYP24A1* and *CYP2R1* genotype. Significant interactions were identified between VDI and four SNPs in these genes. For *CYP24A1*, VDI was associated with food allergy only among infants with the minor allele (T) in rs2426496 (OR 1.8) and rs6068821 (OR 1.7). For *CYP2R1*, VDI was associated with food allergy only among infants with the genotypes CC in rs1562902 (OR 7.7) and GG in rs12794714 (OR 3.1).

Conclusion: The *CYP24A1* intron variant rs2244719, previously associated with serum vitamin D levels, was associated with challenge-proven food allergy. There is also evidence that the effect of VDI on food allergy is modified by genotype. These findings provide support for a causal relationship between vitamin D and food allergy.

18

Does interleukin 33 play a role in peach food allergy?

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Background: Interleukin-33 (IL-33) is a member of the IL-1 cytokine family, which includes IL-1 and IL-18, and is considered to be important for host defense against nematodes by inducing Th2 cytokine production via the IL-33 receptor. IL-33 receptor is a heterodimer of IL-1RL1 and IL-1RAcP. On the other hand, excessive and/or inappropriate production of IL-33 is considered to be involved in the development of various disorders, such as allergic and autoimmune diseases.The aim of the study was to know if there is any difference in the level of IL-33 in normal, allergic and peach food allergic patients.

Method: We measured IL 33 in pg/ml in normal ($n = 21$), allergic patients (rhinitis and/or asthma; $n = 27$) and peach food allergic patients ($n = 20$). All patients were without corticosteroids therapy.

Results: The level of IL 33 was significantly different ($P < 0.0005$) in normal (mean = 15.94 pg/ml) allergic (mean = 39.96 pg/ml) and peach food allergic patients (mean = 579.6 pg/ml).

Conclusion: IL 33 seems to be an important role in the development of peach food allergy.

Oral Abstract Session 4

Diagnosis of venom allergy

19

Quantification of bee allergen Api m 1 in bee venom by high-performance liquid chromatography

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Background: Bee (*Apis mellifera*) venom is an important source for insect venom allergies. Compared to respiratory and food allergen sources, the protein composition of bee venom is relatively simple. One relevant allergen in bee venom is Api m 1 or more commonly known as phospholipase A2. A reversed-phase high-performance liquid chromatography (HPLC) method was developed and tested for its suitability to quantify Api m 1.
Method: Samples: Api m 1 standard was purified from bee venom. Bee venom raw material and bee venom drug product (containing albumin) were tested along with the standard on HPLC.

HPLC: Reversed-phase HPLC was performed on an Agilent 1200 system. The samples were analysed on an YMC column (YMC Co. Ltd. Kyoto, Japan) and Xbridge™ C_8 column combined with UV detection at 210 nm. A gradient was applied in presence of acetonitrile and trifluoracetic acid.
Results: The HPLC chromatogram of the Api m 1 standard showed two peaks. The first peak accounted for 85–90% of the total peak area. The two Api m 1 peaks were better separated with the YMC column than with the Xbridge column. The total peak area was related to the Api m 1 concentration. A linear relationship was observed in the protein concentration range of 12.5–600 μg/ml. The repeatability was shown to be good (CV ≤ 5%). An HPLC chromatogram of bee venom raw material showed that the Api m 1 peaks were well separated from the other protein peaks. Based on the HPLC chromatograms, 12% of the total protein in bee venom was determined to be Api m 1 (w/w). Humane serum albumine which is added as a stabiliser to the bee venom drug product did not interfere with the Api m 1 peaks in the HPLC chromatogram.
Conclusion: The developed reversed-phase HPLC method is a suitable method to quantify relevant allergen Api m 1 in bee venom raw material and drug product.

20

Serologic evaluation of mastocytosis-associated hymenoptera venom allergy using a panel of six molecular components from *Apis mellifera* (Api m 1–4) and *Vespula vulgaris* (Ves v 1, Ves v 5)

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Background: Anaphylaxis caused by hymenoptera venom allergy may be associated with systemic mastocytosis (SM) and/or elevation of baseline serum tryptase (BST). Up to now, no information has become available on single venom allergen sIgE reactivity in SM patients with a history of a previous systemic insect sting reaction. We aimed to address the component-resolved sIgE sensitisation pattern in hymenoptera venom-allergic patients with SM and/or elevated BST levels using six allergens on a widely used IgE immunoassay platform.
Method: Sera ($n = 58$) from SM patients with a history of a systemic reaction after an insect sting and elevated BST levels (mean 35.5 μg/l; range 4.63–250 μg/l) were analysed for sIgE to bee venom (BV) extract (i1), vespid/yellow jacket venom (VV) extract (i3), rApi m 1 (phospholipase A2), rApi m2 (hyaluronidase), rApi m 3 (acid phosphatase), sApi m 4 (mellitin), rVes v 1 (phospholipase A1), and rVes v 5 (antigen 5) on Immulite3g (cut-off: 0.1 kU/l). All recombinant allergens were produced in Sf9 cells. Api m 4 was made by peptide synthesis. All allergens lacked core fucosyl CCDs. Controls ($n = 27$) were tested in parallel and consisted of patients with confirmed hymenoptera venom allergy without SM and a normal BST level.
Results: Twenty-eight patients (48%) were positive to VV extract only, 4 (7%) to BV extract only, 17 (29%) to both BV and VV, and 9 (16%) were negative to both BV and VV. Component testing revealed sIgE sensitisation frequencies for the individual allergens in the extract-positive groups as follows: rApi m 1, 67% (14/21); rApi m 2, 76% (16/21); rApi m 3, 67% (14/21); sApi m 4, 14% (3/21); rVes v 1, 89% (40/45); rVes v 5, 98% (44/45). Of the nine extract-negative patients, 4/9 (44%) were further resolved. 3/9 patients showed reactivity to rVes v 5, and one patient was double positive to rVes v 1 and rVes v 5. Thus, only 5/58 (9%) remained serologically negative (improving the diagnostic sensitivity from 84% to 91%). The component-resolved sIgE allergen reactivity in the control patient group was not significantly different from the SM/elevated BST group.
Conclusion: Hymenoptera venom allergic patients with SM and/or elevated BST display a complex sIgE sensitisation pattern which is quite similar to the one found in hymenoptera venom-allergic patients without SM and/or elevated BST. The use of molecular components may further increase the diagnostic sensitivity in the SM/elevated BST patient group.

21

IgE reactivity to a broad panel of CCD free bee venom allergens reveals diverse sensitisation profiles in bee venom allergic patients

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Background: Additional diagnostic value of IgE detection to CCD-free, species-specific recombinant (r) Hymenoptera venom allergens, such as rApi m 1, rVes v 1 and rVes v 5, has recently been demonstrated. In wasp venom (WV) allergic patients, the diagnostic sensitivity of a combination of the currently available WV allergens rVes v 5 and rVes v 1 has been reported to be as high as 96%, while the frequency of sensitisation to rApi m 1 in honey bee venom (BV) allergic patients appears to be lower, ranging from 58% to 80%. This suggests that sensitisations to additional BV allergens may be of relevance in the pathogenesis and diagnosis of BV allergy. Here we address this issue in a large population of bee venom allergic patients.
Method: Diagnosis of bee venom allergy ($n = 144$) was based on history, skin test and sIgE to bee venom extract (Immuno-

CAP, i1). IgE reactivity was additionally analysed to wasp venom extract (i3), rApi m 1 (i208), rVes v 1 (i211), rVes v 5 (i209) and to a panel of CCD free BV allergens rApi m 2, rApi m 3, nApi m 4, rApi m 5, and rApi m 10 coupled to ImmunoCAP research prototypes (Thermo Fisher Scientific, Uppsala, Sweden).

Results: IgE reactivity (≥0.35 kU_A/l) to the commercially available rApi m 1 (i208) was detected in 72.2%, to rApi m 2 in 47.9%, to rApi m 3 in 46.5%, to Api m 4 in 22.9%, to Api m 5 in 22.9% and to Api m 10 in 61.8%. Positive results to at least one allergen were detected in 94.4% of all BVA patients. Positive results to at least one of the BV specific allergens (Api m 1, 3, 4, or 10) were detected in 89.6% of the patients. In wasp venom allergic patients no IgE reactivity was detected to rApi m 1, rApi m 2, rApi m 3, nApi m 4 and rApi m 10 and only in 1/40 to Api m 5. The majority of BV allergic patients was sensitised to more than one allergen (69.4%) and only a minority (5.9%) to all allergens tested. IgE reactivity to Api m 3, Api m 10 or both was detected in 100 (69.4%) of the patients, and eight patients displayed IgE reactivity only to Api m 3, Api m 10 or both. This is of particular interest since these allergens have been demonstrated to be absent or underrepresented in therapeutic SIT extracts.

Conclusion: The analysis of a large panel of CCD free bee venom allergens improves the diagnostic sensitivity and precision particularly in BV and WV double sensitised patients. In addition it allows the identification of distinct sensitisation profiles that may be of relevance for the outcome of BV immunotherapy.

22
IgE recognition of multiple novel Api m 10 isoforms evaluated by protein array technology

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Background: Until now, two splice variants of the honeybee venom allergen Api m 10 have been described. Variant 2 exhibits IgE reactivity with approximately 50% of honeybee venom sensitised patients. Our proteomic analysis of honeybee venom gave an indication for the existence of additional Api m 10 isoforms. As such, further research was needed to identify these isoforms and investigate the impact of protein heterogeneity on IgE recognition.

Method: Novel isoforms were searched by sequence analysis of cloned RT-PCR amplicons. All isoforms were produced as synthetic peptides or aglycosylated recombinant proteins and were spotted on nitrocellulose-coated glass slides. The colorimetric protein array technology was used to test IgE reactivity of the complete panel of isoforms using sera of 22 Api m 10 sensitised patients.

Results: Nine additional Api m 10 isoforms were found, which derive from the same genomic locus by a complicated alternative splicing. Some truncated isoforms are produced by frameshifts which introduce an alternative stop codon. All 11 variants were obtained as synthetic peptides or purified bacterial recombinants. The colorimetric protein array showed differential IgE reactivity of different Api m 10 isoforms and allowed to predict an IgE epitope.

Conclusion: Differential IgE reactivity of eleven Api m 10 isoforms was investigated using the colorimetric protein array technology. This approach shows some major benefits for testing IgE reactivity of a broad antigen panel. First, compared to many other technologies, only very low amounts of serum (25 μl) are needed. Second, in contrast to fluorescent arrays, the colorimetric signal detection allows the use of much cheaper scanners.

23
Polistes species venom is devoid of carbohydrate-based cross-reactivity and allows interference-free diagnostics

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Background: *In vitro* diagnosis of venom allergy and thus choice of the adequate immunotherapy is heavily affected by cross-reactive carbohydrate determinants (CCDs). A high percentage of cross-reactivities to honeybee and yellow jacket venom can be attributed to IgE directed against the causative structure of CCDs, α1,3-linked core fucose residues. In addition to honeybees and yellow jackets paper wasps (*Polistinae*) represent a major challenge for diagnostic approaches. Hence, in this study we addressed CCD-based cross-reactivity of *Polistes* venom.

Method: CCD-reactivity of American as well as European *Polistes* venom was assessed by different immunological approaches. General presence of glycosylation of *Polistes* venom was analyzed applying *Galanthus nivalis* agglutinin (GNA). Moreover, CCD-based reactivity was addressed using HRP antiserum, specific for α1,3-core fucosylation as well as by CCD-reactive sera of hymenoptera and grass pollen allergic patients and compared with honeybee and vespid venom.

Results: Applying GNA in AlaBlots™ with *Polistes ssp*. (i4), *A. mellifera* (i1), and *V. vulgaris* (i3) venom demonstrates the presence of several glycoproteins in all three venoms. Using HRP antiserum revealed pronounced α1,3-core fucosylation for honeybee as well as for yellow jacket venom. In contrast, *Polistes ssp*. venom which is a mixture of the venoms of different *Polistes* species did not exhibit any CCD-based cross-reactivity. These data were corroborated by ELISA analysis. Moreover, the analysis of CCD-reactive sera from hymenoptera and pollen allergic patients in ELISA and ImmunoCAP® measurement confirmed the absence of immunolocially detectable CCDs in *Polistes* venom.

Conclusion: In summary, this study for the first time demonstrates the absence of CCD-based cross-reactivity from American and European *Polistes* venom preparations and provides evidence that *Polistes* venom preparations constitute a highly beneficial and easy to interpret tool for diagnostic decisions.

24
Bee and wasp venom allergen standardisation and characterisation

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Background: Life-threatening anaphylactic reactions to bee or wasp stings occur in 0.8–5% of the general population. Treatment of this IgE-mediated allergy with specific immunotherapy is highly effective. Two candidate formulations have been investigated, a bee venom therapy collected directly from the bee by electro-stimulation and a wasp therapy extracted from the venom sack. A number of methods have been developed to better understand the role of total allergenicity and specific allergen function in the bee and wasp formulations.

Methods: Several complimentary methods have been developed to determine allergen functionality in bee and wasp product formulations. Protein and allergen profiles were analysed by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western blotting. Presence of bee and wasp relevant allergens have been successfully demonstrated using

proteomics methods. The presence and enzymatic activity of the relevant allergens are demonstrated using gel based methods. In addition, ELISA methods have been developed to measure total allergenicity. The excipient make-up of the bee and wasp formulations has been analysed by complimentary methods.

Results: Bee and wasp product formulations have been characterised to gain further insight to the role of relevant allergens in product functionality. Development of methods for proteomics style analysis has allowed assessment of specific allergens for the standardisation of products for bee and wasp immunotherapy.

Conclusion: A novel battery of complimentary analytical methods has been used to fully characterise products for use in bee and wasp immunotherapy.

Oral Abstract Session 5

Mechanisms of transcription in allergy

25
Lipoxin B_4 promotes the resolution of experimental allergic rhinitis

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Background: Allergic Rhinitis (AR) is an important clinical problem with excess morbidity. Lipoxins (LXs) are endogenous counter-regulatory mediators that decrease inflammation in the lower respiratory tract. Therefore, we determined if LXs could promote the resolution of AR.
Method: To induce AR, Balb/c mice were first systemically sensitised to chicken ovalbumin (OVA) by intraperitoneal injection on day 0 and 7. Starting on day 14, mice were challenged twice a day by OVA intranasal instillation for 2 weeks until day 27. Maximal allergic inflammation (AR) was present 24 h after the last OVA challenge. To determine their impact on AR resolution, LXB_4 (100 ng), dexamethasone (100 ng) or vehicle (EtOH) were given intravenously for three consecutive days. Mice were euthanized 24 h later for quantitation of inflammation in nasal mucosa (NM), cervical lymph nodes (CLN) and serum. For mechanism-based assays of LXs on specific cell types, bone marrow (BM) derived mast cells (MC) and eosinophils (Eos) were prepared for IgE-dependent degranulation of MC and eotaxin-dependent chemotaxis of Eos, respectively.
Results: Relative to vehicle control mice, LXB_4 significantly decreased allergic responses in the NM, including total inflammatory cell numbers and mucus secretion in the lumen of the nasal cavity. LXB_4 also reduced total inflammation in the CLN with an increase in the percentage of $Fc\varepsilon RI^+$ cells. OVA-specific IgE levels were significantly decreased by LXB_4. Of note, LXB_4 was equipotent with dexamethasone in reducing tissue inflammation and more effective at decreasing IgE levels. Relative to control, LXB_4 and LXA_4 significantly reduced *in vitro* IgE-mediated degranulation of MC by 20–30% and eotaxin-dependent chemotaxis of Eos by 50%.
Conclusion: Exogenous administration of LXB_4 led to the more rapid resolution of experimental AR and had direct actions to limit MC and Eos activation. LXB_4's pro-resolving actions for AR were even broader than the antiinflammatory agent dexamethasone, suggesting LXB_4 or related pro-resolving mediators as a potential new therapeutic approach for AR.

27
Forkhead box P3 in human airway regulatory T cells is regulated by suppressor of cytokine signaling

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Background: In persistent upper airway inflammation, the number of forkhead box P3 $(Foxp3)^+$ T regulatory (Treg) cells is reduced but the regulation of Foxp3 expression in Tregs is poorly understood.
Objective: We investigated the interaction between SOCS3 and Foxp3 expression in the airway mucosa.
Methods: Expression of SOCS3 and Foxp3 was measured in CRSwNP tissue and control tissue. Co-expression of SOCS3 and Foxp3 was evaluated in PBMC and in CRSwNP tissue. We also switched off and overexpressed of SOCS3 in CRSwNP tissues and in PNAC-1 cell lines and examined the effect on Foxp3 expression.
Results: SOCS3 gene and protein expression was up-regulated in inflammatory cells in airway mucosa, whereas Foxp3 gene and protein expression was down-regulated. Mucosal Treg cells co-expressed both proteins. Switching off the expression of SOCS3 in human airway mucosa resulted in Foxp3 up-regulation, whereas inducing it in PANC-1 cells led to Foxp3 down-regulation. We also found that phosphorylation of STAT3 was decreased in inflamed mucosa, and we hypothesised that SOCS3 was responsible. Phosphorylation of STAT3 increased upon silencing SOCS3 expression in inflamed mucosa and decreased upon SOCS3 plasmid transfection in PANC-1 cells.
Conclusion: We demonstrate for the first time that SOCS3 and Foxp3 are co-expressed in Treg cells in human nasal mucosa, and that SOCS3 negatively regulates Foxp3 expression in human airway mucosa, possibly by phosphorylation of STAT3. Hence, SOCS3 could be a potential target for restoring Foxp3 expression in Treg cells in persistent mucosal inflammation.

28
Early growth response 1 and dual specificity protein phosphatase 1 transcription factors are involved in down-regulation of allergic responses

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Background: The airway epithelium is accepted as an active player in immune responses. Besides its role as physical barrier towards invading pathogens and irritants, epithelium also affects the outcome of the immune response by the production of various proinflammatory mediators. We have previously shown that nasal epithelial cells are able to respond to exposure to house dust mite (HDM) allergen and that this response is different for epithelial cells isolated from healthy or from allergic individuals. Expression profiling in allergic individuals relative to healthy ones reveals genes that are permanently activated (e.g. NFKB-1, FOSL-1 and JUN) and genes that fail to be up-regulated (e.g. DUSP-1, EGR-1). As EGR-1 and DUSP-1 have been implicated in the down-regulation of inflammatory responses, we hypothesise that failure of up-regulation of DUSP-1/EGR-1 after exposure to HDM in allergic individuals could be responsible for the sustained activation of the allergic response.
Method: We characterised regulatory responses triggered by allergen and viral stimulation in airway epithelium and the contribution of EGR-1 or DUSP-1 to these responses. The parent human bronchiolar cell line (NCI-H292) together with two mutant cell lines with silenced EGR-1 or DUSP-1 were exposed to HDM or poly (I:

C) in a time course of 96 h. Expression levels of selected transcription factors and cytokines were quantified by the real-time PCR and ELISA.

Results: Knock down of EGR-1 significantly enhanced and sustained the production of cytokines (e.g. IL-6, IL-8) after both HDM and poly (I:C) stimulation. The DUSP-1 knock down resulted in enhanced and sustained cytokines production after HDM stimulation. Additionally, in the wild type cell line, we observed a two-phase temporal response after HDM exposure, with EGR-1, DUSP-1, ATF-3 induced rapidly and reaching maximal expression not later than 1 h after stimulation, while other genes and cytokines reached their maximal expression 4 h after induction. This early indication of EGR-1, DUSP-1 and ATF-3 is compatible with the notion of an allergen c.q. viral-induced negative feedback loop of the inflammatory response. Furthermore, the high degree of overlap between the poly (I:C) and the HDM response suggests a potential mechanism of viral induced allergic exacerbations.

Conclusion: Failure of EGR-1 or DUSP-1 up-regulation in allergic individuals could be responsible for the prolonged activated state observed *in vivo*.

29
Splicing regulation of glucocorticoid receptor isoforms in lymphocytes with glucocorticoid resistance

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Background: Glucocorticoid treatment is effective to patients with allergic diseases and autoimmune disorders. However, there are some subjects who respond poorly to glucocorticoid, indicating the existence of glucocorticoid resistance. We previously demonstrated that nuclear factor-κB (NF-κB) involved in the modulation of glucocorticoid sensitivity by altering the expression ratio of glucocorticoid receptor (GR) isoforms in lymphocytes; however, the detailed mechanism was unclear. Focusing on the splicing factor serine-arginine rich (SR) proteins, contribution of NF-κB in the regulation of GR isoforms was examined.

Method: Activation of NF-κB in Raji cells was inhibited with a NF-κB inhibitor or siRNA against NF-κB p65, and then the expression levels of splicing factors such as SRp20, SRp30c, SRp40, and ASF/SF2 were detected by RT-PCR and a western blot analysis. SRp30c in Raji cells was suppressed by introducing specific siRNA with an electroporation technique. Before and after the SRp30c silencing, the expression levels of GR isoforms, binding of SRp30c to pre-mRNA of GR, and sensitivity to dexamethasone were evaluated by RT-PCR, a RNA chromatin immune precipitation assay, and a BrdU incorporation test.

Results: NF-κB inhibition diminished the expression of SRp30c in Raji cells. Knockdown of SRp30c increased the expression level of a functional GR isoform, GRα, and decreased that of a dominant negative GR isoform, GRβ. A RNA chromatin immune precipitation assay confirmed that SRp30c bound to pre-mRNA of GR in Raji cells, and silencing of SRp30c reduced the binding. Although dexamethasone had little effect on original cells, the proliferation of SRp30c-suppressed Raji cells was effectively inhibited by dexamethasone.

Conclusion: Activated NF-κB caused overexpression of SRp30c, accelerating the binding of SRp30c to pre-mRNA of GR. Since SRp30c promotes the splicing from pre-mRNA of GR to GRβ, glucocorticoid resistance may develop in lymphocytes. Therefore, a drug that interferes SRp30c activity may have beneficial effects in combination chemotherapy for treatment of allergic and autoimmune disorders with glucocorticoid resistance.

30
Atopic dermatitis, STAT3- and DOCK8-hyper-IgE syndromes differ in IgE-based sensitisation pattern

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Background: Elevated serum IgE levels are hallmarks of allergic diseases but not unique to these entities. Hyper-IgE syndromes (HIES), primary immunodeficiencies due to monogenetic defects such as in genes *DOCK8* and *STAT3,* overlap clinically with atopic dermatitis (AD) regarding eczema, eosinophilia and elevated serum IgE levels, while type I allergy is not present in all HIES subforms.

Method: To characterise the IgE sensitisation pattern of AD and HIES patients, clinical data, genotype, skin prick tests, specific IgE sensitisation pattern and T helper (Th) subpopulations were assessed in seven AD patients, 13 HIES patients and nine healthy controls.

Results: Total serum IgE levels were similar and significantly elevated in STAT3-HIES, DOCK8-HIES, and AD patients compared to healthy controls. The ratio of aeroallergen-specific IgE to total IgE was highest in AD, whereas DOCK8-HIES patients showed the highest IgE-levels against food allergens. The specificity of IgE in STAT3-HIES patients remained unknown in >99% of total IgE. Allergic manifestations and skin prick test findings correlated significantly to the in-vitro IgE results in all groups. Th2 cells were significantly elevated in DOCK8-HIES and AD patients. AD patients showed significantly higher nTreg cell counts compared to all other conditions. STAT3-HIES patients had decreased Th17 cell counts, whereas all other T cell subsets were normal.

Conclusion: Though total serum IgE is elevated in HIES and AD patients, all disease groups show a different and specific IgE-based sensitisation pattern correlating with specific clinical disease and Th cell subset phenotypes. A key role of DOCK8 in the development of specific IgE and clinical allergy was identified.

Oral Abstract Session 6

Pathogenesis of hereditary angioedema

31

DX-2930, a fully human monoclonal antibody, specifically and potently inhibits both plasma kallikrein-mediated generation of bradykinin and direct B2R activation by plasma kallikrein

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Background: Patients with hereditary angioedema (HAE) have deficient levels of functional C1-INH, which leads to attacks of painful edema via dysregulated plasma kallikrein (pKal)-mediated bradykinin generation. PKal inhibition is a viable therapeutic option for HAE, as demonstrated by the efficacy of ecallantide, a specific pKal inhibitor, for treatment of acute attacks of HAE. There remains an unmet medical need for a non-intravenous, long-lasting prophylactic treatment for HAE. Using antibody phage display we have discovered DX-2930, a potent and specific, fully human antibody inhibitor of plasma kallikrein. The pharmacokinetic (PK) properties of DX-2930 in monkeys following subcutaneous injection indicate that the antibody exhibits a high bioavailability (66%) and a half-life (12.5 days) that allometrically scales to approximately 28 days in humans.
Methods: The biochemical potency and binding properties of DX-2930 were determined using *in vitro* enzyme inhibition, ELISA, and surface plasmon resonance methodologies. The ability of pKal to activate the bradykinin 2 receptor (B2R) was investigated using a stable CHO cell line stably expressing B2R with a beta-lactamase reporter system (Invitrogen). *In vivo* efficacy and PK properties of the antibody were determined in rats and cynomolgus monkeys.
Results: DX-2930 is a potent antibody inhibitor of active pKal (Ki = 0.14 nM) in solution and does not bind prekallikrein or any other serine protease tested. DX-2930 also binds pKal bound to endothelial cells (HUVECs) via high molecular weight kininogen, which may be the form of the enzyme responsible for the HAE attack localisation. We demonstrate that pKal activates cell surface B2R via a bradykinin-independent mechanism that involves proteolytic receptor cleavage. *In vivo* efficacy of DX-2930 was demonstrated by the dose dependent reduction in carrageenan-induced paw edema in rats.
Conclusion: A potent and potentially long acting inhibitor of pKal activity is expected to be an effective prophylactic treatment option for pKal-mediated diseases, such as HAE.

32

Activation of the ficolin-lectin pathway during attacks of hereditary angioedema

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Background: The activation of the plasma enzyme systems is insufficiently controlled in hereditary angioedema caused by the deficiency of functional C1-INH (HAE-C1-INH), a disorder characterised by recurrent subcutaneous and/or submucosal edematous attacks. Recently, a few studies suggested that it is not the MBL-lectin pathway, but the ficolin-lectin pathway (ficolin-LP), which might play a role in the pathomechanism of HAE-C1-INH. As the role of ficolin-LP in the development of edematous attacks is still enigmatic, we analyzed its activity during such episodes.
Method: Thirty-five HAE patients, who have experienced severe edematous attacks on 112 occasions, were enrolled. We analyzed blood samples drawn during attacks, and 39 samples obtained from the same patients during symptom-free periods. The serum concentrations of ficolin-3, ficolin-3/MASP-2 complex, antigenic C1-INH, C4, as well as the extent of ficolin-3 mediated activation of the lectin pathway (F3-TCC) were measured using in-house methods. Commercially available kits were used to quantify C1-INH activity, as well as C4d, and C3a levels.
Results: The level of the ficolin-3/MASP-2 complex was elevated ($P = 0.0224$), whereas F3-TCC was lower ($P = 0.0002$) during attacks, compared with the symptom-free period of the same patients. During symptom-free periods, the extent of F3-TCC was significantly related to the concentrations of ficolin-3 ($R = 0.2778$, $P = 0.0022$), antigenic C1-INH ($R = 0.3152$, $P = 0.0006$), and C4 ($R = 0.5307$, $P < 0.0001$), whereas the ficolin-3/MASP-2 complex level correlated significantly with the C4d ($R = 0.8571$, $P = 0.0107$) concentration. During attacks, the level of the ficolin-3/MASP-2 complex correlated with ficolin-3 ($R = 0.5319$, $P = 0.0025$), functional C1-INH ($R = 0.5391$, $P = 0.0066$), and C3a ($R = -0.4981$, $P = 0.0096$) levels. The level of the ficolin-3/MASP-2 complex was stable in consecutive symptom-free samples ($P = 0.8614$), whereas it varied significantly ($P = 0.0183$) in the samples collected during edematous attacks of the same patients.
Conclusion: The fluctuation of the level of the ficolin-3/MASP-2 complex during attacks, as well as its strong association with C1-INH activity suggest that ficolin-LP undergoes activation during edematous attacks in HAE-C1-INH. We presume that the ficolin-3 mediated activation of LP may contribute to the consumption of the small reserve of functional C1-INH and thus, it can lead to uncontrolled activation of the plasma cascade systems, and thereby to edema formation.

33

Mutation in factor XII gene in Brazilian families associated with hereditary angioedema with normal C1 inhibitor

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Background: Hereditary Angioedema (HAE) type III is a rare condition of familial occurrence, described mostly in women, and influenced by estrogen exposure. Normal C1-inhibitor (C1-INH) levels and activity are found in patients with HAE type III. Mutations of the gene coding for

Coagulation Factor XII (*F12*) have been identified in some patients with HAE type III, however functional implications of these mutations remain controversial. Our aim was to investigate *F12* mutations in patients with clinical characteristics of HAE type III from Brazil, and to use molecular modeling tools to gain insights into the role of these mutations in clinical disease.

Methods: Four families with index cases of female patients, who presented history of recurrent angioedema episodes with normal C1-INH and C4 levels, were evaluated for HAE type III by genetic analysis. Genomic DNA was isolated from whole blood from patients and relatives with recurrent angioedema attacks. PCR was carried out with 50 ng DNA, and sequencing of exon 9 from *F12* was performed. Modeling of Factor XII protein with and without mutation was performed by molecular dynamics simulations in pure water using the Gromacs v4.5 program.

Results: Patients′ symptoms include episodes of subcutaneous angioedema, abdominal pain, and dyspnea, triggered by trauma, stress, and oral contraceptives. After diagnosis, symptoms were controlled with withdrawal of contraceptives and/or Danazol or Tranexamic acid treatment. Genetic analysis revealed a previously identified missense mutation located in exon 9 of *F12,* c.983C > A, also designated as p.Thr309Lys, in index cases of three of four families. Relatives with history of episodes of angioedema in two of these families also bear the *F12* mutation. Comparison of predicted structures of native and mutated protein revealed that the p.Thr309Lys mutation affects stability and dynamic behavior of the structure. One possible explanation would be that this mutation occurs in a region of previously identified O-linked glycosylation site.

Conclusion: This study describes the presence of a mutation in the gene coding for Factor XII as a likely cause of HAE type III in three families in Brazil. Molecular modeling suggests that this mutation could disrupt significantly the structure of Factor XII molecule, in a way that it may alter its role in kinin pathways.

34

A deletion in the factor 12 gene analysed in two Turkish families with hereditary angioedema with normal C1 inhibitor (HAE type III): a Turkish *F12* mutant

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Background: In patients and families with hereditary angioedema with normal C1 inhibitor (HAEnCI) coming from West European and other countries, the missense mutations p.Thr328Lys and p.Thr328Arg in the coagulation *F12* gene have been found to be associated with the disease. To investigate whether in two Turkish families with HAEnCI a mutation in the gene coding for the coagulation factor XII could be identified.

Method: Family members coming from two unrelated Turkish families were investigated for recurrent angioedema, for C1 inhibitor activity and C4 in plasma, for mutations of the *F12* gene, and for the cosegregation of angioedema and mutation. Sequencing of all exons, the exon/intron boundaries, and the 3' and 5' flanking regions of the *F12* gene locus was performed.

Results: Four women, two women in each of the two families, had clinical symptoms of HAEnCI. In three women the symptoms started or exacerbated after beginning to take oral contraceptives. One woman had never received any estrogens. All four women had the novel deletion mutant in the *F12* gene causing the deletion of 72 base pairs (c.971_1018+24del72). The deletion leads to the loss of the 3' end of exon 9 and the 5' region of intron 9 of the *F12* gene. In a control group of 71 healthy individuals of Turkish origin the deletion was not present.

Conclusion: A novel large deletion in the *F12* gene was found in two Turkish families with HAEnCI. A founder effect for this 'Turkish' *F12* gene mutation is likely. The deletion are localised in the same *F12* gene region as the point mutations associated with HAEnCI of West European countries.

35

Kinin catabolism and disease severity in hereditary angioedema with *F12* mutation

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Background: Bradykinin (BK) angioedema (AO) is characterised by recurrent, self-limiting attacks of cutaneous and/or mucosal swelling. The etiopathological process depends on BK accumulation onto the endothelium, as consequence of an overproduction of BK or a failed catabolism, or both associated. BK AO is primarily associated with *SERPING1* gene mutations and the C1Inhibitor (C1Inh) deficiency, and recently with the mutation 983A/G on the exon 9 of the *F12* gene, conferring a gain-of-function of factor XII. Difficulty of diagnosis of the mutation carrying families is attributed to the highly variable penetrance of the disease. The aim of this study was to explore the enzymes of the BK catabolism and their relationship to the severity of the disease.

Method: Our analysis was based on retrospective reports of patients affected by hereditary AO associated with *F12* gene mutation compared with healthy donors (2007–2012).We measured BK forming activity (based on the amidase activity on the PFR*p*NA substrate) and activities of the three major BK catabolism enzymes (Angiotensin-I converting enzyme, ACE; Aminopeptidase P, APP; Carboxypeptidase N/M, CPN) from citrated plasma.We developed a logistic regression analysis from the enzymatic data to predict severity of AO symptoms. Severity score was established according to frequency of AO attacks, age of disease onset, localisation of attacks (Freiberger T. et al *Scand J Immunol* 2011:*74*, 100–106).

Results: We analyzed 64 symptomatic patients in 37 unrelated families (representing 184 individuals) and 200 healthy donors. Twenty-five patients suffered from mild severity disease, 20 with intermediate severity and 19 with severe AO symptoms. The severity was found significantly increased with high amidase activity ($P < 0.0001$), with decreased ACE ($P < 0.01$) and CPN ($P < 0.02$) activities, but not correlated with APP activity ($P = 0.09$).

Conclusion: These results support the observation that, in addition to the increased BK production, severe patients exhibit a low BK catabolism. The severity of disease in the *F12* mutation carriers is well correlated to amidase, ACE and CPN activities, in contrast to C1Inh deficient patients where the severity is mainly correlated to APP activity. This suggests a higher responsibility of the enzymes of the BK catabolism in AO with *F12* mutation than those of the *des*Arg9-BK catabolism.

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36
Evaluation of icatibant for the repeated treatment of hereditary angioedema attacks across three phase III open label extension studies

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Background: The For Angioedema Subcutaneous Treatment (FAST)-1, -2, and -3 trials were double-blind, randomised, controlled multicenter phase three studies of icatibant 30 mg in patients with hereditary angioedema (HAE) type I or II. All studies had open-label extensions (OLEs) and included patients who were previously treated in or eligible for the controlled phase, but whose first attack occurred after completion of the controlled phase. A retrospective analysis was conducted on the prevalence and efficacy of ≥1 icatibant injection on attacks in the OLE phase.

Method: The subset of HAE attacks treated in the OLEs was analyzed to assess icatibant use by the number of injections ever and per attack, time between injections, and time to onset of and almost complete symptom relief. Time to onset of symptom relief was calculated from the time of first drug administration to the first of three consecutive patient-assessed measurements with ≥50% reduction in the pretreatment composite visual analog scale (VAS) score. Time to almost complete symptom relief was calculated from the time of first drug administration to the first of 3 timepoints where all VAS scores were <10 mm.

Results: Of 1149 attacks in 208 patients (female: 139; HAE type I: 181) were analyzed. Of all attacks, 91.5% (n = 1051) were treated with one injection, 8% (n = 92) with two injections, and 0.5% (n = 6) with 3. Regardless of attack location (n = 1147), >91% of attacks were treated with one injection (abdominal 91.1%; cutaneous 92.0%; laryngeal 91.8%). Most moderate and severe attacks were treated with one injection (92.4%, n = 415; 90.7%, n = 594). Median time to first injection after attack onset was 2.5 h. The median time between first and second injection was 24.3 h, and 81.6% of all second injections were given ≥12 h after the first injection. Median time to onset of symptom relief was 2.3 h with one injection (n = 935). Median time to almost complete symptom relief occurred within 5.5 h of first injection.

Conclusion: The majority of attacks treated in the OLEs of the FAST studies only required one injection of icatibant, independent of attack severity and location. We observed a similar finding by attack location and severity, ≥90% of attacks were treated with one injection.

Oral Abstract Session 7

Pediatric allergy

37

Early regular egg exposure in infants with eczema: a randomised controlled trial

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Background: Observational studies suggest that early regular ingestion of allergenic foods may reduce the risk of food allergy.
Methods: A double-blinded randomised controlled trial to determine if early regular oral egg exposure will reduce subsequent IgE-mediated egg allergy in infants with moderate to severe eczema. Infants were allocated to one teaspoon of whole egg powder ($n = 49$) or rice powder ($n = 37$) daily from 4–8 months of age. Cooked egg was introduced to both groups after an observed feed at 8 months. The primary outcome was IgE-mediated egg allergy at 12 months defined by observed pasteurised raw egg challenge and skin prick tests.
Results: Within the first week of exposure, 14/49 (29%) allocated to egg had an allergic reaction and did not continue powder ingestion. The intention to treat analysis found a lower (but not significant) incidence of egg allergy in the egg group (33%) compared to the control group (51%; relative risk [RR] 0.65; $P = 0.11$). The per-protocol analysis (infants who continued the intervention beyond the first week) revealed significantly lower egg allergy in the egg compared to the control group (3% vs 48%; RR = 0.07; $P < 0.001$). Egg-specific IgG4 levels were significantly ($P < 0.001$) higher in the egg group at both 8 and 12 months. At 4 months of age, prior to any known ingestion of egg, 36% (24/67) infants already had egg-specific IgE >0.35 kU_A/l. There were no differences in egg-specific IgE levels between the groups at any time point.
Conclusion: Induction of immune tolerance pathways and reduction in egg allergy incidence can be achieved by early regular oral egg exposure from 4 months of age in some infants with moderate to severe eczema provided the infant tolerates their first few exposures to egg. Caution needs to be taken when these high-risk infants are first exposed to egg as many have already developed sensitisation and clinical reactivity by 4 months of age. This points to much earlier events in the initiation of food sensitisation, well before the introduction of complementary feeding.

38

Is partial intake of hen's egg associated with early tolerance of hen's egg allergy?

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Background: Children, who are diagnosed as hen's egg allergy (HEA), are forced to strictly avoid food containing hen's egg. However, some of HEA patients can eat a little of hen's egg without any symptom. We retrospectively analyzed whether a partial intake of hen's egg contributed oral tolerance induction in HEA subjects or not.
Methods: We enrolled subjects who had a history of egg allergy, and passed the oral food challenge to a heated egg yolk with contamination of 1/20 egg white (E1) from 2005 to 2010. Heated egg yolk with contamination of 1/20 egg white was simply made separating raw egg yolk from egg white and then heated. We defined the subjects passed E1 at 0 to 1 years of age (y) as 'early phase group' (EP), and at 2 to 3 y as 'late phase' (LP) group. After the subjects passed E1, they were advised to eat a heated egg yolk with contamination of 1/20 egg white at home. After certain period of time, they underwent the oral food challenge to half a heated egg (E2). When they passed E2, we defined heated hen's egg tolerance. We compared the ratio of heated hen's egg tolerance at 4, 5, and 6 y between EP group and LP group. We used logistic regression analysis to investigate associations between timing at start of a partial of hen's egg intake and hen's egg tolerance at 5 y adjusted for egg white specific IgE at 1 y.
Results: The number of EP group was 59 subjects, and that of LP group 132. Median age of a partial of hen's egg introduction was 18.6 months in EP group and 35.0 months in LP group. Median egg white specific IgE at 1 y was 7.7 in EP group and 15.4 in LP group ($P = 0.000$). The number of analysis subject was 167 in 4 y, 145 in 5 y, and 103 in 6 y. The ratio of heated hen's egg tolerance between EP group and LP group was 84% (37/44) vs 38% (47/123) at 4 y ($P = 0.000$), 96% (29/30) vs 67% (77/115) at 5 y ($P = 0.001$), and 100% (19/19) vs 83% (70/84) at 6 y ($P = 0.028$). In logistic regression analysis, subjects in EP group more likely tolerated hen's egg at 5 y than subject in LP group (adjusted odds ratio, 10.3 [95%CI, 1.3 to 80.3]; $P = 0.026$).
Conclusion: This retrospective study suggests a partial intake of hen's egg contributes early tolerance induction in HEA subjects.

39

Predictive value of component-resolved diagnostics on the outcome of oral food challenge in children with egg allergy. Ovomucoid predicts development of tolerance

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Background: In children with egg allergy, consecutive oral food challenges are the method of choice to evaluate clinical threshold and development of tolerance. The aim of this study was to investigate if component-resolved diagnostics can improve the specificity of allergy testing and help to predict the outcome of oral food challenges.
Method: A total of 130 children (aged 8 months to 8 years, mean 38 months) with suspected egg allergy were challenged with raw egg and specific IgE to egg white (f1), ovomucoid (Gal d1), ovalbumin (Gal d2), conalbumin (Gal d3), lysozyme (Gal d4) and egg yolk was measured (Thermo Fisher, Sweden). Children with a positive

oral food challenge were re-challenged and had specific IgE measured with regular intervals.

Results: Of the 130 included children 31 had a negative initial oral egg challenge. The 99 children with a challenge proven egg allergy underwent a total of 265 oral egg challenges. During a mean follow up period of 32 months (range 6–104 months) 34 children developed tolerance, 48 were persistent egg allergic and 17 were not re-challenged.

Based on the initial 130 challenges, the receiver operating characteristic (ROC) curve analysis for egg white (f1), ovomucoid, ovalbumin, conalbumin, lysozyme and egg yolk revealed an AUC of 0.83, 0.79, 0.81, 0.79, 0.63 and 0.78, respectively, with optimal cutoff points for f1, ovomucoid and ovalbumin of 1.23, 1.01 and 0.61 kU/l, respectively. Food challenge symptom score was not correlated neither to threshold nor to the level of IgE to any component. At baseline children who became egg tolerant had significantly lower specific IgE to f1 (5.1 vs 11.2 kU/l; $P = 0.048$), ovomucoid (4.1 vs 9.2 kU/l; $P = 0.044$) and ovalbumin (4.3 vs 9.2 kU/l; $P = 0.07$) compared with children who were persistent egg allergic. Longitudinally, IgE to ovomucoid but not to any other component nor f1 was significantly correlated to threshold in the individual patient and was the superior denominator for prediction of development of tolerance.

Conclusion: Based on our data, component-resolved diagnostics are not significantly better than f1 in predicting the outcome of the first oral food challenge with raw egg. However, longitudinally, on an individual basis a decrease in ovomucoid is associated with an increase in threshold and may predict development of tolerance.

40

6 months sublingual immunotherapy in children with cow's milk allergy

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Background: Sublingual immunotherapy (SLIT) could be an option in long lasting cow's milk (CM) allergy, but controlled studies need to confirm its efficacy.

Objective: To investigate the efficacy of CM SLIT compared to the exclusion diet alone.

Method: After a baseline double-blind, placebo-controlled CM challenge (DBPCFC), measuring the CM cumulated reactive dose, patients were randomised 2:1 to SLIT or exclusion diet. A DBPCFC performed 6 months later was compared with baseline. Skin prick testing and measurement of CM specific IgE and IgG subclasses were done at each DBPCFC.

Results: Fifty one subjects with CM allergy aged 5–12 years were enrolled. Adherence to treatment was excellent. No side-effects were reported at home. After 6 months, 7 of 35 SLIT subjects (20%) and 3 of 16 exclusion diet subjects (20%) passed the 200 ml CM challenge. The number of subjects whose CM cumulated reactive dose increased at M6 was significantly higher in the SLIT group, 31/35 vs 9/15 in the exclusion diet group, $P = 0.02$. Despite an identical clinical pattern in both groups at entrance, IgE and IgG4 showed a discrepancy, both exhibiting higher levels in the exclusion diet than in the SLIT group.

Conclusion: This study failed to prove a real efficacy of SLIT in CM allergy. However, considering the increased of cumulated reactive dose in most treated patients, the absence of side-effects and the good acceptation of the technique, SLIT might be relevant relevance as a first line treatment in patients reacting to low CM doses.

41

Comparison between two maintenance feeding regimens after successful cow's milk oral desensitisation

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Background: Cow's milk allergy is common in infancy, and total avoidance of this food is the only effective approach. In alternative, oral immunotherapy has been proposed to achieve tolerance. Once desensitisation is achieved, daily intake of milk is recommended to maintain it, but this may be impractical for children/parents. We assessed if a twice weekly maintenance regimen is effective.

Methods: Children who were successfully desensitised with oral immunotherapy were randomised to two maintenance regimens for 1 year: group A had to eat 150–200 ml milk daily, group B had to eat 150–200 ml milk twice weekly. Both regimens were associated to a totally free diet. Maintenance of tolerance and adverse events were recorded during 1 year. Specific IgE, IgG4 and prick-by-prick to milk were carried out before immunotherapy (T0), before maintenance (T1) and after 1 year (T2).

Results: Recorded episodes included asthma, oral itching, urticaria, rhinitis, abdominal pain variously combined, usually associated with concomitant illness or exercise. The episodes were eight in group A and nine in group B, with no difference. None of the children discontinued the feeding maintenance. Specific IgG4 increased at T1 and remained high at T2. Specific IgE and skin reactivity significantly decreased at T2. There was no difference in those parameters between the groups.

Conclusion: After achieving desensitisation to cow milk with oral immunotherapy, a maintenance regimen with milk given twice weekly is as effective as the daily maintenance.

42

A weekly maintenance dosing regime for peanut immunotherapy: clinical and immunological parameters

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Background: Peanut allergy is severe and rarely resolves. We implemented a maintenance oral immunotherapy protocol in 19 peanut allergic subjects who had been successfully desensitised to peanut. Subjects were re-evaluated during a period of 30 months of follow-up.

Methods: Nineteen peanut-allergic children who had completed oral immunotherapy and tolerated a daily dose of 800 mg peanut protein were studied. Daily maintenance (800 mg protein) was followed after a median of 25 months by weekly maintenance doses (800 mg protein).

Peanut-specific serum IgE, skin prick test, allergen-specific intra and extracellular cytokines were measured pre-immunotherapy and at intervals up to 30 months of maintenance treatment.

Results: Weekly maintenance dosing was well tolerated by our subjects who no longer avoided peanuts in their diet, and reported no reactions to incidental peanut ingestion. There was an increase in peanut-specific intracellular IFN-γ (median pre:0.8 v median 12 m:1.9 v median 30 m:4.3, $P = 0.03$* v $P = 0.02$) and a decrease in IL-4 (median pre:56.8 v median 12 m:37.4 v median 30 m:42.8, $P = 0.05$ v $P = 0.13$) during 30 months of maintenance immunotherapy. Extracellular peanut-stimulated IL-5 and IL-13 decreased and extracellular IL-10 increased over the same period. Peanut specific IgG4 (median pre:0.58 v median 12 m:8.14 v median

30 m:15.6 mg/l, $P = 0.0001$*** v $P = 0.02$*) increased and peanut specific IgE (median pre:29.7 v median 12 m:14.2 v median 30 m:7.6 ku/l, $P = 0.03$* v $P = 0.0005$***). and skin prick test wheal diameter (median pre:8.5 v median 12 m:5 v median 30 m:5 mm, $P = 0.0008$*** v $P = 0.01$*) decreased.

Conclusion: Changing to a weekly maintenance dose after 2 years of daily maintenance immunotherapy was well tolerated, subjects enjoyed a more relaxed diet and protection from incidental peanut ingestion was maintained. Our laboratory findings show a shift from an allergen-specific Th2- to Th-1 response, which may indicate the development of immunological tolerance.

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Oral Abstract Session 8

Mechanisms of allergen specific immunotherapy

43

Clinical efficacy of allergen-specific sublingual immunotherapy correlates with the induction of tolerogenic dendritic cell, but not CD4+ regulatory T cell, markers

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Background: Sublingual allergen specific immunotherapy (SLIT) is a powerful approach to elicit tolerance. The exact immune mechanisms involved are not yet fully understood, but the induction of regulatory T cells is considered to be critical.
Method: Herein, we investigated changes at the level of peripheral CD4+ T cells and dendritic cells (DCs) in relationship to clinical benefit in 82 grass pollen allergic patients included in a double-blind, placebo-controlled SLIT study performed in a challenge chamber. Blood samples were collected before and after 2 and 4 months of treatment with a sublingual tablet containing either a five-grass pollen extract or a placebo. To assess allergen-specific CD4+ T cell responses, surface phenotype, proliferative responses, cytokine production and gene expression were analysed in PBMCs after *in vitro* allergen stimulation, or among MHC class II/peptide-tetramer positive cells. In parallel, markers for both regulatory and effector DCs identified by proteomic approaches were quantified by real-time PCR in PBMCs.
Results: When monitoring Th1, Th2 and Treg markers, only minor changes were observed in the phenotype of CD4+ PBMCs in both active and placebo groups. In patients receiving active tablets, IL-4 and IL-10 gene expression, IL-10 secretion and percentages of potential 'pro-allergic' Th2A cells were decreased, but without any correlation with clinical benefit. pMHCII-tetramer analysis of allergen-specific CD4+ T cells confirmed that SLIT clinical efficacy is not associated with dramatic changes in the polarisation of circulating CD4+ T cell. No significant changes in expression of effector DC markers were detected during treatment. In contrast, a clearcut upregulation of tolerogenic DC markers such as C1Q and Stabilin-1 was observed in active patients exhibiting clinical responses during treatment.
Conclusion: This study emphasizes the interest of monitoring molecular changes at the level of peripheral blood DCs to detect early polarisation of adaptive immune responses during immunotherapy.In this regard, a molecular signature specific for tolerogenic DCs, but not regulatory T cells, is associated with early SLIT efficacy.

44

Evaluation of ultra-rush immunotherapy for Virginia live oak in a pollen challenge chamber identifies cytokine and transcriptional correlates of protection

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Background: Pollen challenge chambers (PCC) have not been utilised to study the effectiveness of ultra-rush, high-dose subcutaneous immunotherapy (URSCIT) to any aeroallergen.
Methods: Thirty-four Virginia Live Oak (VLO) sensitive participants underwent two pre-treatment PCC exposures (visit 1) to VLO pollen to establish baseline symptomatic responses. Participants were then treated with URSCIT to VLO pollen extract in a double-blinded, placebo-controlled, parallel fashion with 17 participants in each group. Participants were subsequently exposed to VLO pollen in the PCC at 10 and 11 (visit 2) and at 30 and 31 (visit 3) days post-URSCIT. Total symptom scores (TSS) were recorded. Whole blood was collected and subjected to genome-wide transciptomic analyses (i.e, RNA-Seq; $n = 64$ from pre- and post-visits 1 and 3). Nasal secretions ($n = 44$; visits 2 and 3) were collected and examined for 13 cytokine/chemokines. Linear generalised estimating equation (GEE) models were used to evaluate changes in TSS and RNA-seq data.
Results: Mean TSS decreased over the three PCC exposures, but the magnitude of the decline was greater in those receiving USCIT compared with those in the placebo arm (decline in TSS of 1.34 vs 0.30), reaching a trend for statistical significance ($P = 0.198$). IL-10 levels in nasal secretions were lower in the URSCIT compared to the placebo arm, both before PCC visit 2 ($\log_2$ difference $= -1.79$, $P = 0.030$] and after PCC visit 3 ($\log_2$ difference $= -1.73$, $P = 0.034$). There was a trend for lower IL-6 levels in the URSCIT arm after visits 2 and 3 compared with the placebo arm ($\log_2$ differences of -1.09, $P = 0.074$ and -1.67, $P = 0.127$, respectively). Comparison of the fold changes in the gene expression levels by RNA-Seq at post-visit 1 vs post-visit 3 demonstrated that a greater number of genes had lower expression levels in the URSCIT compared to the placebo arm. Among the genes showing the most significant changes over time was Fibronectin 1, with lower levels in the placebo arm compared to the URSCIT arm.
Conclusion: Following challenge with VLO, compared with placebo, URSCIT associates with a trend in reduced symptoms and two correlates of protection: lower IL-10 levels in nasal secretions and resistance to a decline in Fibronectin 1 levels, a correlate of a Th_1 responses. These findings also highlight the utility of PCC in clinical trials as well as pathogenesis studies for allergic rhinoconjunctivitis.

45

Grass pollen allergic patients treated with subcutaneous immunotherapy have elevated Src-homology-2 domain-containing inositol-5′phosphatase 1 and 2 (SHIP-1 and SHIP-2) protein levels

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Background: Reduced basophil histamine release is observed in patients treated with subcutaneous immunotherapy (SCIT). We examined the effect of SCIT on intracellular protein concentrations of spleen

tyrosine kinase (Syk), SHIP-1 and SHIP-2, three key regulators of basophil releasability in the FcεRI-pathway.

Method: We recruited patients with seasonal rhino-conjunctivitis (16 on SCIT for 2 years, six untreated allergic controls). Blood basophils were purified both during offseason (spring) and the grass pollen season (summer). Purity of basophils (96.7 ± 0.17%, mean ± SEM) was assessed by flow cytometry (CD45+, CD203c+, FcεRIa+). Cell lysates were evaluated for Syk and SHIP1 expression by quantitative Western blotting. Basophil activation test (BAT) was employed to assess allergen-specific releasability [log (grass pollen) required for half-maximal activation or LC50].

Results: Basophil releasability (BAT) increased in untreated patients during pollen season (LC50 −0.547 to −0.771, $P = 0.04$) while SCIT-treated patients maintained off-season releasability (−0.371 to −0.410, $P = 0.46$). There were no significant differences in offseason SHIP-1 levels in treated compared to untreated patients ($P = 0.22$). A fall in SHIP-1 levels was observed from offseason to the pollen season for untreated patients (31.176 ± 1.554–11.171 ± 2.251, $P = 0.01$) and treated patients showed a similar trend (45.249 ± 17.054–22.817 ± 4.819, $P = 0.08$). During the pollen season treated patients had elevated SHIP-1 levels compared to untreated patients (22.817 ± 4.819 vs 11.171 ± 2.251, $P = 0.03$). We observed higher SHIP-2 levels offseason in treated patients (177.170 ± 41.058–34.479 ± 16.067, $P = 0.004$), but no significant differences in season (164.306 ± 38.257–113.005 ± 3.585, $P = 0.10$) We observed no significant changes in Syk expression.

Conclusion: Natural allergen exposure in sensitised patients decreases basophil SHIP-1 predisposing them to enhanced releasability. Reduced basophil releasability and positive response to SCIT is significantly correlated with increased expression of SHIP-1, suggesting a new therapeutic approach.

46
A novel allergen-adjuvant conjugate suitable for allergen-specific Th2 redirection

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Background: In a previous paper we described a substituted 8-hydroxyadenine named SA-2 as able to *in vitro* revert the T_H2 phenotype of human allergen specific T cells by triggering TLR7.

The conjugation of the adjuvant to the allergen is a novel and efficacious strategy of allergen immunotherapy because it enhances the immunogenicity and reduces the allergenicity of the vaccine. In this work we synthetized another adenine derivative named SA-26E, which was chemically conjugated to the purified natural allergen Derp2 (nDer p2-Conj) from *Dermatophagoides pteronyssinus* and we assessed this conjugate for its immunomodulatory activity on human cells.

Method: nDer p2-Conj was characterised by MALDI-TOF analysis and evaluated for induction of NF-κB in CD14+ cells, TLR-triggering in transfected HEK293 cell lines, cytokine production in BDCA4+ and CD14+ cells, Th-skewing effects in allergen-specific human T cell lines and T cell clones and allergenicity in BASOTEST assays.

Results: nDer p2-Conj induced nuclear translocation of p50 and trigger TLR7, induced innate cells to produce IFN-α and IL-12 at similar levels than dispersible TLR ligand R-848. As a consequence, the conjugate reverted T_H2-prone allergen T cell lines into IFN-γ-producing cells (T_H1/T_H0 phenotype) as assessed by cytokine production in the supernatants, intracellular cytokine expression and T_H-related transcription factor expression (GATA-3 and T-bet). nDer p2-Conj was also able to revert the phenotype of T_H2 established cells, as demonstrated by culturing CRTH2 cells with this conjugate. We further demonstrated that the T_H1 differentiation induced by allergen conjugate can be reverted by the addition of neutralising antibodies against T_H1 citokines. In addition, Derp2 specific T cell clones derived from the T cell lines cultured in the presence of Derp2 conjugate showed a T_H1/T_H0 profiling. Finally, the nDer p2-Conj reduced basophils activation in comparison with uncoupled allergen protein.

Conclusion: The system of chemical conjugation to relevant proteins as allergens confers the ability to induce the production of regulatory cytokines in innate immunity cells by TLR7 triggering and to down-regulate T_H2 allergen-specific T cells, suggesting a relevant role in the development of novel immunotherapeutic strategies in allergic disorders.

47
Sustained effect of grass pollen subcutaneous immunotherapy on desensitisation of allergen specific basophil response

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Background: The long-term effect of grass pollen SCIT on basophil allergen specific response is not completely understood. We investigated the involvement of this cellular factor with the induction of tolerance after discontinuation of pollen SCIT.

Method: Basophil CD63 response and humoral markers were follow-up in 20 subjects just before starting and after build-up phase of grass pollen SCIT, before first pollen season and then 1–2 years after the period of 3–5 years of SCIT. The basophil response was also monitor after removal of IgG antibodies. Clinical outcomes included seasonal symptoms and use of rescue medication.

Results: The desensitization of basophil allergen specific response and clinical tolerance was maintained after 1–2 years of grass pollen SCIT discontinuation. Although SCIT-induced grass pollen-specific IgG4 levels decreased to near pre-SCIT levels after discontinuation, the removal of IgG antibodies, at that point, revealed the increase of basophil response to pretreatment level.

Conclusions: Grass pollen immunotherapy induces allergen-specific basophil desensitisation that persists after treatment discontinuation and that could account for long-term clinical tolerance.

48
Local nasal antiinflammatory IgG4 responses are induced following grass pollen sublingual and subcutaneous immunotherapy

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Rationale: Grass pollen sublingual (SLIT) and subcutaneous (SCIT) immunotherapy are associated with induction of serum IgG4-associated blocking antibodies that

prevent IgE-facilitated allergen binding to B cells (IgE-FAB). We hypothesised that local nasal IgG4 antibodies are induced and inhibit IgE-FAB after SLIT and SCIT.
Methods: Nasal fluid and serum were obtained from SLIT-treated ($n = 9$), SCIT-treated ($n = 8$), untreated allergics ($n = 10$) and non-atopic controls ($n = 10$). Specific IgE and IgG4 to *Phleum pratense* (Phl p) components were measured by the ImmunoCap Immuno Solid-phase Allergen Chip (ISAC) micro-array system using the biochip technology. Inhibitory activity of nasal fluid and serum were measured using the IgE-Facilitated Allergen Binding (FAB) assay.
Results: Untreated allergics had elevated levels of rPhl p 1 ($P = 0.002$) and rPhl p 5-specific IgE ($P = 0.001$) in nasal fluid compared to non-atopic controls. Specific IgE to rPhl p 1 and rPhl p 5 in nasal fluid in SLIT- and SCIT-treated patients was unchanged compared to untreated allergics. Recombinant Phl p 5-specific IgG4 levels were increased in SLIT- and SCIT-treated patients compared to untreated allergics ($P = 0.001$; $P = 0.002$) and non-atopic controls ($P = 0.001$; $P = 0.003$). Interestingly, inhibitory activity in nasal fluid for co-operative binding of allergen-IgE complexes to B cells in SLIT- and SCIT-treated patients was significantly increased compared to untreated allergics ($P = 0.002$, $P = 0.004$). These increases in inhibitory activity in nasal fluid paralleled those in serum after both SLIT ($P = 0.001$) and SCIT ($P = 0.002$). The magnitude of this suppression was numerically greater with local antibodies (SLIT: 48%; SCIT: 40%) when compared to peripheral antibodies (SLIT: 35%; SCIT: 32%).
Conclusions: IgG4 antibodies with inhibitory activity for IgE-FAB are induced in nasal fluid following SLIT and SCIT and have potential as biomarkers for monitoring immunotherapy.

Oral Abstract Session 9

Allergen-specific T and B cell responses

49

IL-27 supresses allergen-driven TH2 responses and IL-27+ dendritic cells are significantly reduced in grass pollen allergic individuals compared to non-atopic controls

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Rationale: Interleukin (IL)-27 belongs to the IL-12 superfamily and consists of EBI3 and IL-12p28 subunits. IL-27 is produced by dendritic cells (DCs) and has immunomodulatory effects on T cells. We hypothesised that IL-27 suppresses *ex-vivo* allergen-induced T helper 2 responses. Furthermore, IL-27 has differential expression on DCs in grass pollen-allergics and non-atopic controls. IL-27-primed DCs inhibit grass pollen-induced T cell proliferative.
Methods: PBMCs obtained from grass pollen-allergics ($n = 10$) and non-atopic controls ($n = 9$) were stimulated with LPS (100 ng/ml) for 24 h. IL-27-producing DCs were quantified by flow cytometry. The effect of IL-27 on grass pollen-driven PBMC proliferation was measured by ^{3}H-thymidine incorporation. Transcription factors and cytokines mRNA expression were measured by RT-PCR. Proliferative responses of CD4+ T cells co-cultured with IL-27-treated DC in the presence of allergen was assessed.
Results: Proportions of IL-27-producing DCs were decreased in allergic patients compared to non-atopic controls ($P = 0.003$). IL-27 significantly supressed PBMC proliferation following *ex-vivo* grass pollen stimulation (100 ng/ml, $P = 0.0078$). This suppression was observed in allergen dose-dependent manner. mRNA expression in PBMC culture for T-bet and c-Maf were up-regulated ($P = 0.02$; $P = 0.02$). GATA-3 was down-regulated ($P = 0.02$). IL-27 significantly down-regulated IL-4 ($P = 0.02$) and IL-5 ($P = 0.03$) and up-regulated of IL-10 ($P = 0.02$) and IFN-g mRNA expression ($P = 0.03$). IL-27 inhibited IL-4 production from Th2 clones ($P = 0.01$) when stimulated with anti-CD3/CD28. Moreover, T effector cells proliferation was supressed when allergen stimulated IL-27-primed DC were cultured with T effector cells ($P = 0.03$).
Conclusions: Our findings suggest dysregulation of IL-27 production in DCs from allergics compared to non-atopic controls. IL-27 down regulates Th2-driven allergic responses. Whether this is a potential immunomodulatory mechanism during allergen immunotherapy remains to be determined.

50

Heterogeneity of specific CD4+ T cell responses to peanut allergic components: prospects for suitable immunotherapeutic strategies

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Background: Peanut proteins comprised of at least 11 allergic components. The contribution to specific immune responses by each component in each individual is not known. Details characterisation of immune response to allergic components of peanut is required to develop suitable immunotherapeutic strategies.
Method: pMHCII-tetramer and CD154-based assay were used in an *ex vivo* approach to assess at a single cell level the specific CD4+ T cell responses to Ara h 1, 2, 3, 6, 8 and 9 in children and adults with and without peanut allergy. The frequency, surface marker phenotype and cytokine profile of these cells were directly analyzed by flow cytometry. A correlation between specific immune response to each peanut allergic component and contribution to clinical symptoms was also evaluated.
Results: Peanut allergen-specific CD4+ T cells were detected in all of the subjects with and without peanut allergy tested. We observed heterogeneity of phenotype within the allergen-specific CD4+ T cells that depends on the epitope for which the cells are specific. T cell epitopes associated with production of IL-10 or IFN-g are recognised at low frequencies in both allergic and healthy individuals. In contrast, allergy-associated epitopes are only recognised in allergic individuals by high frequency, terminally differentiated allergen-specific CD4+ T cells specifically associated with CCR4 and CRTH2 expression and TH2 cytokine production. Interestingly, we also observed inter-individual variations of the specific immune response to peanut allergic components. This differential ability of the allergic individual to respond to peanut allergens appears to be influenced by HLA class II allele polymorphisms.
Conclusion: Ability to identify immunogenicity and type of response elicited by each peanut allergic component appears to be critical to future success in vaccine development against peanut allergy. Understanding the type of cellular response and the role of genetic restriction may allow to target immune response to critical peanut allergen and to uncover the optimal type of cellular immune response necessary for protection.

51

Epitope recognition patterns of monomeric peanut-specific IgA in humans diverge significantly from dimeric peanut-specific IgA in human colostrum and human and mouse serum

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Background: Human IgA is unique among immunoglobulin isotypes regarding its normal existence in monomeric (m-IgA) (≈80% in serum) and dimeric (d-IgA) (in secretions and ≈20% in serum) forms. Plasma IgA is derived mostly from the bone marrow while secretory IgA is assembled locally in mucosal tissues and glands. However, it is unknown if the germline and maturation path, and therefore specificity, of the food-antigen-specific IgA plasma-cells in mucosal surfaces is similar to the bone morrow and splenic plasma-cells.
Objective: We sought to identify the m-IgA and d-IgA binding epitopes of the major peanut allergens Ara h 1, 2 and 3 in human colostrum and human and mouse

serum and compare them with known peanut specific IgE binding epitopes εεεεε.

Methods: Serum samples from six non-allergic non-sensitised individuals, six highly allergic and sensitised patients, six naïve mice and six peanut sensitised mice were used in addition to commercial colostral IgA from at least 10 human donors. Fast protein liquid chromatography was utilised to manually isolate m-IgA and d-IgA from a by-product of intravenous immunoglobulin manufacture derived from more than 3000 healthy plasma donors. Study samples were tested by means of peptide microarrays for IgA binding with synthetic overlapping peptides spanning the sequences of Ara h 1, 2 and 3 molecules.

Result: Human serum m-IgA recognised only 31.1% of Ara peptides recognised by serum d-IgA, which recognised the same peptides as d-IgA in colostrum. The recognition patterns in naïve and sensitised mice resembled that of human d-IgA. Peanut-specific IgE-binding epitope patterns are similar to peanut-specific d-IgA.

Conclusion: There is large diversity in epitope recognition profiles between m-IgA and d-IgA, suggesting a different regulation and maturation pathway and potentially a different germline origin. The neutralisation properties of allergen-specific d-IgA antibodies suggest a potential role for these molecules in passive immunisation against known allergens.

52
Possible regulatory role of galectin-9 on *Ascaris summ*-induced eosinophilic lung inflammation in mice

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Background: Galectin-9 (Gal-9) is a member of the galectin family of lectins that exhibit binding affinity for β-galactosides. We found a T cell line-derived Gal-9 with novel eosinophil chemoattractant activity, but its role in eosinophilic inflammation of the lung is unknown. We evaluated the role of Gal-9 in *Ascaris suum*-induced eosinophilic lung inflammation in mice.

Method: To evaluate the role of Gal-9 in *Ascaris suum*-induced eosinophilic lung inflammation, we developed a mouse model of eosinophilic pneumonia induced by the *Ascaris suum* antigen, and analyzed eosinophilic inflammation in Gal-9 deficient mice. The therapeutic effects of recombinant Gal-9 on lung inflammation were also examined in this mouse model. To evaluate lung inflammation, numbers of inflammatory cells and cytokine levels in the bronchoalveolar lavage fluid (BALF) were estimated by flow cytometry and enzyme-linked immunosorbent assay, respectively.

Results: The BALF of this mouse model of eosinophilic pneumonia induced by the *Ascaris suum* antigen contained increased numbers of inflammatory cells and elevated Gal-9 levels. Compared with wild-type mice, the BALF of Gal-9 deficient mice contained higher numbers of both eosinophils and T helper type 2 (Th2) cells. Th2 cytokines and eotaxin levels were also higher, and levels of CD4+CD25+Foxp3+ regulatory T cells were lower in Gal-9 deficient mice than in wild-type mice. Intranasal administration of recombinant Gal-9 prevented eosinophilic inflammation of the lung and upregulated the release of endogenous Gal-9.

Conclusion: Our findings suggest that Gal-9 negatively regulates Th2-mediated eosinophilic inflammation of the lung and Foxp3+ regulatory T cells might be involved in suppressing allergic lung inflammation.

53
Distinct antibody reactivity patterns to Bet v 1 and its bacterial homologues from *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Background: Structural homologues of the major birch pollen allergen, Bet v 1, can be found in all three evolutionary domains. Bacterial sources of Bet v 1 homologues are, among others, *Staphylococcus aureus,* which is frequently found in atopic dermatitis patients, and *Pseudomonas aeruginosa*, which is associated with pneumonia and urinary tract infections. Contact of the immune system with these bacterial Bet v 1 homologues is highly likely. Therefore, we aimed to investigate the connection between birch pollen allergy and humoral immune resonses to bacterial Bet v 1 homologues.

Method: Bet v 1.0101, SA2116 from *S. aureus* and PA1206 from *P. aeruginosa* were expressed in *Escherichia coli* and purified via standard chromatographic methods. Secondary structures were checked by circular dichroism spectroscopy. The IgE and IgG responses to Bet v 1.0101, SA2116 and PA1206 were determined for 12 sera from Bet v 1-sensitised birch pollen allergic, four grass pollen allergic and two house dust mite allergic patients and 10 sera from non-allergic individuals.

Results: The three recombinant proteins showed similar structures in CD-spectroscopy. All sera from birch pollen allergic patients showed IgE binding to Bet v 1.0101, whereas none of the bacterial Bet v 1 homologues bound IgE. In contrast, all sera contained IgG specific for all tested proteins. Sera of birch pollen allergic patients possessed higher IgG titers to Bet v 1.0101 than to SA2116 and PA1206. In contrast, sera of grass pollen and house dust mite allergic patients as well as non-allergic individuals' sera displayed higher IgG titers to the bacterial Bet v 1 homologues than to Bet v 1.0101.

Conclusion: In our study, we showed that IgG reactivity patterns to Bet v 1 and its bacterial homologues differed between birch pollen allergic patients and the other groups studied. Thus, antibacterial immune responses might be linked to the development of birch pollen allergy.

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54
Phenotypic and functional characterisation of human allergen-specific memory B cells

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

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

(PLA), we measured the frequency of PLA-specific memory B cells and generated PLA-specific B cell clones.

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

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

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Oral Abstract Session 10

From mice to men: novel mechanisms and treatments for asthma

56
Allergic airway inflammation induces dendritic cell phenotypes in airway sensory ganglia

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Background: Dendritic cells (DC) play a decisive role as antigen-presenting cells in the allergic airway inflammation. It has been shown that neuropeptides of sensory neurons like calcitonin gene-related peptide (CGRP) can attract and modulate immune cells like DC during the allergic airway inflammation. The colocalisation of DC in specific airway sensory ganglia has not been explored yet.

The aim of the present study is to evaluate possible interactions of DC in sensory ganglia concerning calcitonin gene-related-peptides (CGRP)-expression during allergic airway inflammation.

Method: Using the mouse model of a chronic allergic airway inflammation, BALB/c mice were treated with intranasal house *dust mite* (*HDM*) *extract* (25 μg/50 μl) 5 days a week for 7 weeks. The sample material was harvested 24 h after final allergen challenge. Immunohistochemistry was performed to detect the colocalisation of DCs by MHC-II and CD11c, satellite glia cells (SGC) by glutamine synthetase and neurons by the neuronal marker PGP 9.5.

Results: The immune cells, which have DC-phenotype characteristics but are not Satellite glia cells, were found in the vagal sensory airway ganglia of the mouse under physiological conditions and they were significantly increased during an allergic airway inflammation (DCs/neurons: control 23.48 ± 7.613% vs HDM 49.75 ± 4.194%, $P = 0.0003$). Additionally, an increased number of CGRP positive neurons in vagal sensory airway ganglia during allergic airway inflammation was found (CGRP positive neurons/total neurons: HDM 52.07 ± 3.040% vs control 21.63 ± 3.799%, $P = 0.0001$).

Conclusion: The finding of the presence of DC in the airway jugular-nodose ganglion indicates a role of the DC in these ganglia under physiological conditions. The increased numbers of DC and CGRP-positive neurons in these ganglia suggest the involvement of these cells the pathogenesis of allergic airway inflammation. However, the exact functions of DC and CGRP in allergic airway inflammation remain to be explored in future studies.

57
The α-melanocyte stimulating hormone-melanocortin receptor axis in the pathogenesis of allergic bronchial asthma

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Background: Allergic bronchial asthma develops on the basis of a chronic inflammation of the airways. According to the hygiene hypothesis a dominant T helper 2 (TH2) type immune response is critically important for the initiation of this inflammatory response. However, the mechanisms leading chronification of the allergic immune-response in asthmatic airways remain elusive, but suggest incessant secretion of pro-inflammatory mediators triggered by allergen inhalation. This in turn overwhelms inherent regulatory feed-back loops, which virtually control inflammatory reactions by the production of antiinflammatory factors. One of these mediators could be the α-melanocyte-stimulating hormone (α-MSH), which has been described to reduce inflammation in several dermatologic and neurologic disorders. This study aimed at investigating the role of α-MSH in asthma pathogenesis.

Method and results: In mouse models of acute and chronic experimental allergic asthma we found that α-MSH is produced in inflamed airways and that the amount of α-MSH released into the broncho-alveolar lumen increases with intensity and duration of allergic airway inflammation. Thereby, α-MSH is mainly produced by the airway epithelium and to a much lower amount by alveolar macrophages. From the five melanocortin receptors (MC-R) that have been identified so far, we found expression of only MC5-R in healthy animals, mainly by alveolar macrophages and airway epithelial cells. In contrast, animals with experimental asthma revealed expression of MC5-R not only in the airway epithelium and alveolar macrophages, but also in infiltrating eosinophils. Additionally, we detected expression of MC1-R, which is not expressed in healthy animals, by infiltrating neutrophils and fibroblasts in the tunica adventitia of inflamed vessels.

Conclusions: We found that α-MSH is mainly produced by the airway epithelium depending on the strength and duration of the inflammatory response in the airways together with an altered expression pattern of the melanocortin receptors in animals with experimental allergic asthma. These data suggest an important role of the airway epithelium in regulating the allergic immune response in asthmatic airways by releasing α-MSH.

58
Adoptive transfer of Th clones confer late-phase asthmatic response in mice

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Background: Helper T (Th) cells have been implicated in asthma. To investigate a role of Th cells in airflow limitation, T cell-transfer model was analyzed for possible immediate and late phase asthmatic responses after antigen challenge.

Method: Ovalbumin (OVA) specific Th clones were established from either the regional lymphnodes of Balb/c mice immunised with OVA/CFA or splenocytes of DO11.10 transgenic mice expressing T cell receptor specific for $OVA_{323–339}$/H-2^d. Th clones were adoptively transferred into unprimed mice. Upon challenge with either OVA or $OVA_{323–339}$, Penh was continuously analyzed by unrestrained whole body plethysmography (BUXCO). Airway resistance was also monitored by resistance/compliance analyzer under anesthetized condition. Supernatants of stimulated Th clones were analyzed for contractile activity using collagen gels embedded with murine primary bronchial smooth muscle cells.
Results: Penh values were significantly increased 6 h after OVA challenge, when mice were transferred with Th clones, T5-1, T6-2, T6-4, and T6-7, but not by other Th clones, BF7, T6-1, or T6-10. Airflow limitation was confirmed by a direct measurement of airway resistance under anesthetized, restrained, and intubated conditions. The airflow limitation was also efficiently induced by $OVA_{323–339}$, a T cell epitope peptide. Contractile activity was detectable in the supernatants of T6-2 stimulated with immobilised anti-CD3.
Conclusion: Activation of Th cells resulted in an airflow limitation besides eosinophilic inflammation, AHR, and mucous hyperplasia. T cell-derived bronchoconstrictor might be a target for treatment-resistant asthma.

59
Microbiome composition affects airway immunity against respiratory syncytial virus infection in mice

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Background and objective: Early bacterial colonization is necessary for the development and maturation of neonatal immunity. Inadequate balance between the microbiome composition and innate or adaptive immune system of the neonate might contribute to respiratory syncytial virus (RSV) induced disease severity. To investigate if intestinal microbial composition affects host RSV induced immune responses, we altered the gut microbiome in a mouse model for primary RSV infection and in a FI-RSV induced vaccination model for enhanced disease.
Materials and methods: Microbiome composition was altered in C57BL/6 mice using either a 7 week dietary intervention with specific oligosaccharides (scGOS/lcFOS/pAOS) or a 4 week broad spectrum antibiotic treatment during the FI-RSV vaccination. Fecal taxonomic composition and lung RSV specific immune responses were determined.
Results: During primary RSV infection, specific oligosaccharide intervention increased the number of IFN-gamma producing CD4+ T cells 8 days post infection compared to control diet. Moreover, in the FI-RSV model, dietary intervention decreased lung total cell influx, eosinophilia and the number of IL-4, -5 and -13 producing CD4+ T cells. Lower microbial diversity induced by broad spectrum antibiotics during FI-RSV vaccination correlated with decreased numbers of IFN-gamma producing CD4+ and CD8+ T cells 6 days after viral challenge.
Conclusions: Specific modulation of the microbial composition and diversity correlate with different host immunity response against RSV and suggests that optimising the early microbial implementation may have an impact on the susceptibility to RSV induced disease.

60
Efficacy of a mixture of release-active rabbit polyclonal antibodies to histamine, bradykinin and morphin in the treatment of mouse model of allergic asthma

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Background: Allergic asthma as a complex disease characterised by airway obstruction, airway inflammation and airway hyperresponsiveness to a variety of stimuli requires the discovery of new therapeutic strategies. The aim of this study was to investigate the efficacy of Rengalin (Ren) (a mixture of release-active rabbit polyclonal antibodies to histamine, bradykinin and morphin) in the treatment of mouse model of allergic asthma (MMA).
Method: MMA was developed as previously described. Mice in study group 1 were intragastric (i.g.) administered with 0.25 ml of Ren, beginning 5 days before and then during sensitisation period; mice in group 2 were treated with i.p. injections of Dexamethasone (DM) 0.25 ml in dose 3 mg/kg before INA; mice in group 3 were treated during sensitisation period with Desloratadine (DL) by i.g. administration of 0.25 ml in dose 50 μg/kg; mice with MMA (group 4) did not receive any treatment. Twenty-four hours after the last INA airway hyperreactivity (AHR) to methacholine (Mch) was measured by whole-body plethysmography. Forty-eight hours after the last INA blood was taken for cell count, bronchoalveolar lavage fluid (BALF) was collected for estimation of inflammatory cells and lungs were removed for histological examination. Serum anti-OVA IgE, IgG, IgG1 and IgG2a were measured by ELISA at the end of sensitisation and after INA.
Results: Treatment of mice with Ren, DM, and DL significantly reduced AHR to Mch compared to untreated group 4 mice with MMA. The levels of anti-OVA IgG2a in groups treated with Ren, DM, and DL were decreased after INA in mice treated with DM. Anti-OVA IgG1 and total IgG antibody responses were decreased in all treated groups especially after INA in mice treated with Ren and DM. Treatment of mice with Ren significantly reduced neutrophil number and partly increased lymphocyte number in BALF. Treatment with DM reduced eosinophil count in BALF while treatment with DL led to decrease alveolar macrophages count and increase neutrophil number in comparison to untreated MMA group. In peripheral blood substantial decrease in eosinophil count was observed in all treated groups while the number of neutrophils was increased. Treatment of mice with Ren, DM and DL led to the reduction of allergic inflammation.
Conclusion: This study provides a new therapeutic approach in the treatment of asthma suggesting that Rengalin given during the time of allergen exposure might alter disease progression in patients with respiratory allergy.

Oral Abstract Session 11

New aspects in the diagnosis of drug allergy

61

Usefulness of skin tests for the diagnosis of immediate type hypersensitivity reactions to proton pump inhibitors

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Background: Limited data are available about the value of skin tests in the diagnosis of PPI-induced hypersensitivity reactions and the cross-reactivity among PPIs. The aim of this study was to assess the role of skin testing in the diagnosis of PPI-related immediate hypersensitivity reactions and the cross-reactivity patterns among PPIs in patients with typical features of PPI-related hypersensitivity reactions.

Method: The study was designed in a prospective, national, multicentre nature. Sixty-five patients (mean age: 44.08 years, F/M:53/12) with a suggestive history of a PPI-induced immediate hypersensitivity reaction and 30 control subjects (mean age: 34.83 years, F/M:17/13) were included. The severities of the reactions were assessed with the Ring-Messmer scale. Standardised skin prick tests with omeprazole, lansoprazole, pantoprazole, rabeprazole, esomeprazole and intradermal tests with omeprazole, pantoprazole, esomeprazole were performed. Oral provocation tests (OPTs) with the PPIs other than the culprit PPI that displayed negative results in skin tests were performed in 61 patients and diagnostic OPTs with the suspected PPI were performed in 12 patients.

Results: Lansoprazole was the most frequently involved drug with 52 patients (80.0%), followed by esomeprazole in 11 (16.9%), pantoprazole in 9 (13.8%), rabeprazole in 2 (3.1%) and omeprazole in one patient (1.5%). The severity of the reaction was grade1 in 32 (49.2%), grade2 in 22 (33.8%), grade3 in 11 patients (16.9%). Diagnostic skin tests with the culprit PPIs were positive in 20 patients (20/60, 33.3%). All skin tests in 30 control subjects were negative. The sensitivity, specificity, negative and positive predictive value of the skin tests with PPIs according to clinical history was 33.3%, 100%, 42.9%, 100%, respectively. A group of patients with positive skin test with lansoprazole were also positive on skin test with pantoprazole (3/17), esomeprazole (3/17) or omeprazole (1/17). Eight of the 52 patients who had a history of a hypersensitivity reaction to lansoprazole had a positive OPT result with at least one of the alternative PPIs (3/52 omeprazole, 3/52 rabeprazole, 2/52 pantoprazole, 1/52 esomeprazole).

Conclusion: Considering the high specificity, skin testing seems to be a useful method for the diagnosis of immediate type hypersensitivity reactions to PPIs and for the evaluation of cross-reactivity among PPIs. However, OPT should be performed in case of negativity on skin tests.

62

Diagnosis of chlorhexidine allergy in the Danish Anaesthesia Allergy Centre

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Background: Allergic reactions to the widely used disinfectant chlorhexidine are rare, but often severe and easily overlooked. Patients with suspected perioperative allergic reactions are especially at risk and testing for chlorhexidine is routinely carried out when investigating these patients in the Danish Anaesthesia Allergy Centre (DAAC).

The aim of this study was to evaluate the different diagnostic tests used in the diagnosis of chlorhexidine allergy.

Method: All patients investigated in DAAC in the period July 2004-July 2012 were included ($n = 343$ patients). Specific IgE for chlorhexidine (Immunocap®, Phadia AB, Sweden) was analysed in 334 patients and histamine release test (HR) (RefLab ApS, Denmark) in 257 patients. Skin testing in duplicate was carried out using skin prick test (SPT) with chlorhexidine digluconate 5 mg/ml in 339 patients and intradermal test (IDT) with chlorhexidine digluconate 0.002 mg/ml in 333 patients. Criteria for positivity were: SPT wheal ≥3 mm of the negative control; IDT diameter of wheal greater than or equal to twice the diameter of negative control; specific IgE >0.35 kUA/l; HR class ≥1.

Chlorhexidine allergy was assumed if two or more tests were positive.

Results: In total, 39 patients had one or more positive tests. Of these, 25 patients fulfilled the criteria for allergy to chlorhexidine; 24/25 were SPT-positive, 16/25 were IDT-positive, 25/25 were IgE-positive and 13/23 were HR-positive. Of the 14 patients with only one positive test 4/14 had a positive SPT, 2/14 had a positive IDT, 6/14 were specific IgE-positive and 2/11 were HR-positive. In our population the rate of false positive tests was low for all tests. The rate of false negative tests was especially low for SPT and specific IgE.

Conclusion: Twenty-five of 343 patients fulfilled the criteria for allergy to chlorhexidine on the basis of two or more positive tests. Fourteen had only one positive test. The remaining 304 were negative in all tests. The high concordance between tests in this study suggests that basing the diagnosis of chlorhexidine allergy on two or more positive tests improves diagnostic accuracy. Obviously, a positive provocation would be needed to confirm this, but a validated provocation model is not yet available.

Surprisingly, using our criteria for positivity for IDT, only 16 patients out of the 25 patients with allergy had a positive test. There is no international consensus on the criteria for a positive IDT and this should be the subject of further studies.

63

IgE-mediated anaphylaxis and allergic reactions to idursulfase in patients with Hunter syndrome

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Background: Enzyme replacement therapy (ERT) with recombinant human idursulf-

ase is effective for the treatment of mucopolysaccharidosis (MPS) type II. However, various adverse events can occur in relation to infusion of idursulfase. The purpose was to evaluate the occurrence of infusion-related allergic reactions, including anaphylaxis, to idursulfase in patients with MPS II receiving ERT and to elucidate its possible mechanism.

Method: A total of 34 patients with MPS II were enrolled to receive ERT with Elaprase® at a dose of 0.5 mg/kg intravenously once a week. Information regarding the symptoms, frequency and timing of anaphylaxis during treatment was analyzed. Formation of anti-idursulfase IgE antibody was assessed by skin prick test (SPT) and enzyme-linked immunosorbent assay (ELISA). Western blotting was performed to confirm the reaction between idursulfase and specific IgE.

Results: Three patients (8.8%) showed anaphylaxis by infusion of idursulfase. No deaths occurred during the study. Anti-idursulfase IgE antibody was detected by SPT and ELISA. Immunoblotting with patients' sera and Elaprase® showed a single band of specific IgE binding to the protein around 70 kD, and idursulfase did not display amino acid sequence homology to known allergens. SPT with idursulfase demonstrated positive results in all patients with anaphylaxis. However, we failed to reveal any risk factors for the development of infusion-related immediate-type allergic reactions.

Conclusion: Anaphylaxis related to infusion of idursulfase is mediated by anti-idursulfase IgE antibody, which might be produced by de novo synthesis. SPT might be useful in predicting the occurrence of anti-idursulfase IgE-mediated anaphylaxis during infusion.

64

Drug provocation test duration in the diagnosis of non-immediate reactions to amoxicillin in children

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Background: Drug Provocation Test (DPT) can be considered of paramount importance in those patients with suspected drug hypersensitivity who have tested negative for the *in vivo* and/or *in vitro* test, who have no risk factors and for whom diagnosis is mandatory. The general guidelines for performing a DPT are a single blind placebo controlled test under strict hospital surveillance with emergency room facilities. While in case of immediate reactions the DPT procedure has reached a good level of standardisation, in case of non-immediate reactions many questions remain open. It is still a matter of debate whether a full therapeutic dose should be given for a number of days similar to a therapeutic regimen or just for 1 day.

Method: We reviewed charts of patients referred to the Allergy Unit of Anna Meyer Children's Hospital for suspected drug allergy from 2008–2011. All patients with history of Amoxicillin reactions underwent Skin Prick Tests (SPTs) and Intradermal Tests (IDTs) with the culprict drug according to EAACI guidelines. Moreover, most of those children resulted negative to the skin tests were challenged with Amoxicillin. We performed a three step protocol (10–20–70% of the therapeutic dose) every 30' on the first day.

The day after Amoxicillin was administered in one single dose and the challenge was continued at home for a total of 5 days.

Results: The most common suspected agents were beta-lactams antibiotics (55.7%). Amoxicillin predominated with maculopapular exathemas (45.9%). Two hundred patients underwent SPTs/IDTs and nine out of 200 resulted positive. One hundred and fifty-two out of 200 children had history of non-immediate reactions. One hundred and seventy-seven DPT were performed with Amoxicillin for a total of 5 days each child. Diagnosis of Amoxicillin allergy was confirmed by DPT in 17 patients (9.6%). Of those 14 had history of non-immediated reactions. The DPT reactions occurred in four patients (26.6%) on day 5.

Conclusion: According to our results, the cumulative dose can be exclusively reached after a number of days of drug administration in case of non-immediate reactions. In order to avoid missing diagnosis of drug hypersensitivity in a significant number of cases a long term DPT protocol should be recommended in those patients with history of adverse drug reactions occurred during the course of a therapeutic regimen.

65

Human leukocyte antigen genotype in antiepileptics hypersensitivity reactions: a pilot study in Brazilian patients

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Background: A strong pharmacogenetic association has been reported in Chinese patients between human leukocyte antigen (HLA)-B*1502 and carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS), while HLA-A*3101 was associated with CBZ-induced hypersensitivity reactions among subjects of Northern European ancestry. Our aim was to analyze the association between antiepileptics hypersensitivity reactions and polymorphisms of HLA-B*1502 and HLA-A*3101 in a population of São Paulo, Brazil.

Method: Case-control study in which we genotyped the HLA-B alleles of samples obtained from 54 subjects with varying severities antiepileptics hypersensitivity reactions (AHR), 83 tolerants, and 45 control subjects, all from São Paulo, Brazil. The phenotype evaluation was based on standardised scoring systems using an adapted ENDA (*European Network of Drug Allergy*) questionnaire, medical records and on the clinical follow-up in our Allergy Clinic. The patch test with the culprit drug was performed according to the ENDA recommendations.

Results: We studied 185 subjects, 65% were female and mean age was 43.6 years. Eigthy percent had mixed ethinicity. Fifty-four cases were validated as AHR, 31 Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS), 12 Stevens-Johnson Syndrome (SJS) and 11 maculo-papular exanthema. Of all 41 drug patch tests, 28 (68%) were positive, in both SJS and DRESS. The HLA variants observed in our case and control groups had homogeneous distribution. None of our study groups presented positive association with HLA-B*1502 and HLA-A*3101 polymorphisms.

Conclusion: There was no association between HLA-B*1502 and HLA-A*3101 polymorphisms genotype and AHR. It is unlikely these polymorphisms are going to be a satisfactory genetic marker of AHR in our population. Due to the high degree of linkage disequilibrium in the MHC, the identification of an association does not necessarily indicate that this locus is the causal variant. Given the rarity of such reactions, this is only going to be possible through multicenter international collaborations.

66

Use of nasal inspiratory flow rates in the measurement of ASA induced respiratory reactions

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Background: Nasal ketorolac challenge with modified oral aspirin challenge is a

safe and effective alternative for desensitising patients with aspirin exacerbated respiratory disease. In addition to clinical judgement, objective tests assessing nasal flow may help in diagnosing nasal reactions. To evaluate the feasibility of peak nasal inspiratory flow (PNIF) as an objective measurement in the assessment of a reaction to nasal ketorolac or oral aspirin, and to determine changes in PNIF that have adequate sensitivity and specificity.

Method: One hundred and fifty-one patients referred to the Scripps Clinic (San Diego, California) for aspirin challenges and desensitisation, from March 1, 2009 to July 31, 2012 and 14 healthy controls participated in the study. The percent decreases in PNIF measurements of nasal reactors were compared with the nonreactors' measurements. The ROC curve was constructed to asses the diagnostic performance of PNIF measurement for clinically positive nasal challenge.

Results: A total of 165 subjects participated in the study. One hundred and fourteen patients (69.1%) had a positive reaction to the nasal ketoroloc challenge. There was no statistical difference between nasal reactors and nonreactors regarding gender, baseline FEV1, and use of systemic steroid before challenge. The mean percent decrease in PNIF was significantly higher in the reactor group (-30 ± 29 vs -7 ± 16, $P < 0.001$). A cut-off value of 25% decrease in PNIF had the maximum sensitivity and specificity (56.1% and 94.1%) as well as maximum PPV and NPV (95.5% and 49.0%) (AUC = 0.755, SE = 0.037, 95%CI = 0.682–0.818, $P < 0.001$).

Conclusion: The high specificity and PPV of a 25% drop in PNIF found in the ROC analysis indicate that PNIF measurements can be used for assessing nasal reactions during nasal ketorolac challenges in the diagnosis of aspirin exacerbated respiratory disease.

Oral Abstract Session 12

Epidemiology of allergic rhinitis

67
The diversity of adolescent rhinitis: a cluster analysis

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Background: Rhinitis affects many adolescents and often shows comorbidity with asthma. We hypothesised that like asthma, adolescent rhinitis may exhibit clinical heterogeneity that could be characterised by cluster analysis.

Methods: The Isle of Wight birth cohort ($n = 1456$) was established in 1989 to study the natural history of allergic diseases. Participants were followed up at 1, 2, 4, 10 and 18 years. Cluster analysis was performed on the population that had rhinitis at age 18 ($n = 468$) using 12 variables selected from potentially relevant 'associated' factors at 18-years; sex, wheeze at 18, eczema at 18, ocular symptoms at 18, family history of rhinitis, age that rhinitis appeared, atopic status at 18, total IgE, bronchial reversibility, BHR, mean FeNO (exhaled nitric oxide), FEV_1, FVC, FEV_1/FVC, $FEF_{25–75}$, and Body Mass Index. Clusters were further characterised by allergic comorbidity throughout the first 18-years of life, environmental exposures at 18-years and rhinitis morbidity indices.

Results: Five clusters were identified:

Cluster 1 (50/468; 10.7%): Female (86%) atopic (77%) rhinitis with high comorbid wheeze (80%) and asthma (66%). This group had high FeNO, high BHR and greatest airflow limitation. Rhinitis had had high impact on sleep and daily activities. This group showed high need for rhinitis treatment.

Cluster 2 (98/468; 20.9%): Male (99%) atopic (81%) rhinitis with little comorbid wheeze (14%) and diagnosed asthma (9%). This group showed low BHR and least airflow obstruction. Rhinitis morbidity was intermediate with high treatment need.

Cluster 3 (170/468; 36.3%): Female (79%), strong family history of rhinitis (39%) with little comorbid atopy (58%), wheeze (28%) and asthma (20%). This group exhibit low FeNO and lowest BHR. Rhinitis morbidity and treatment needs were moderate.

Cluster 4 (54/468; 11.5%): Female (65%) overweight (44%) non-atopic (49%) rhinitis with intermediate comorbid wheeze (43%) asthma (43%). They showed lowest FeNO but high airflow limitation. They had low rhinitis treatment needs.

Cluster 5 (96/468; 20.5%): Male (68%) atopic (93%) early onset (6.7 years) rhinitis with high comorbid wheeze (67%) and asthma (59%). They showed high FeNO and normal lung functions. They had persistent symptoms with high treatment needs.

Conclusion: Clinically distinctive adolescent rhinitis clusters are apparent with varying gender, asthma, atopy and obesity associations plus different symptom severity and treatment needs.

68
Rhinitis in a community elderly population: its associations with atopy, asthma and comorbidity

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Background: Rhinitis is one of the most frequent medical conditions. However, there is still scarce of epidemiologic evidence for rhinitis in the elderly population. The present study aimed to investigate the prevalence of elderly rhinitis and the relationships with asthma and other comorbidity.

Method: A cross-sectional analysis was performed using the baseline dataset of the Korean Longitudinal Study on Health and Aging (KLoSHA), a community-based elderly population cohort in Korea (aged ≥65 years). Structured questionnaires were utilised to define rhinitis, asthma and comorbidity, and allergen skin prick tests were used to define atopy. Health-related quality of life was assessed by short-form 36 (SF-36) questionnaires.

Results: A total of 982 elderly subjects (98.2%) were included in the present study. The prevalence of rhinitis was 25.6%, and the age-related decrease was evidently observed after the ages of 90 years. The prevalence of atopy was 17.2% (18.8% among rhinitis subjects), and atopy did not have significant association with rhinitis. Asthma-rhinitis relationships were assessed in multivariate logistic regression analyses, and they were found to be significant, independently of atopy. Among comorbid conditions, irritable bowel syndrome (IBS) showed marginal associations with rhinitis, particularly for rhinitis without atopy. In SF-36 questionnaire analyses, rhinitis was related to the reduction in physical aspects of quality of life.

Conclusion: The present study demonstrated that elderly rhinitis had high prevalence as a non-allergic form, and also was a significant disorder in relation to asthma association and its impact on quality-of-life. Potential relationship between non-allergic rhinitis and IBS warrants further elucidation.

69
Natural history of local allergic rhinitis

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Background: Local allergic rhinitis (LAR) is a common respiratory disease with a prevalence of 25.7% in rhinitis population. However, whether LAR is a first step in the development of classical allergic rhinitis (AR) with systemic atopy or an independent entity remains unknown. The objectives of this study were to evaluate the natural history of LAR and possible conversion to AR over time.

Method: A 10-years-follow-up study was designed to evaluate 194 adult LAR patients and 97 healthy controls. LAR patients had positive response to nasal allergen provocation test (NAPT) with at least one aeroallergen. Demographic and clinical questionnaire, spirometry, SPT, and serum specific IgE antibodies to common

aeroallergens were yearly evaluated. NAPT were performed at baseline and after 5 and 10 years of evolution. The study was approved by the local ethics committees. All participants were informed and signed the corresponding informed consent.

Results: These data represent the results of the first 5 years of the follow-up. Most LAR patients were non-smoker women with moderate/severe persistent perennial rhinitis. At initial evaluation conjunctivitis (52.3%) and asthma (18.8%) were the most frequent comorbidities, and *D. pteronyssinus* (51.1%) the main specific aeroallergen detected by NAPT. After 5 years a worsening of rhinitis with increase of persistence and severity symptoms (26.2%), and new association to conjunctivitis (7.9%) and asthma (5.6%) were detected. Systemic atopy was detected by SPT and/or serum specific IgE in LAR (6.81%) and control group (4.5%), without significant differences.

Conclusion: These results show that a similar proportion of LAR patients and healthy controls developed systemic atopy, suggesting LAR and classical AR can be two independent entities. These findings need to be confirmed in the 10-year-follow-up study actually in progress.

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70
Epidemiology of rhinitis in elderly: a nationwide survey

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Background: Epidemiologic data on rhinitis in the elderly is scarce, despite the increase in the senior population over the last decades, together with a concern for better quality of life (QoL). This study aimed to estimate the prevalence of rhinitis in the population aged above 65 years old in mainland Portugal and to characterise and classify rhinitis in this age group.

Method: A cross-sectional, nationwide, population-based survey was accomplished through questionnaire application to responders aged above 65 years old, living in mainland Portugal, who gave their informed consent. Data was collected by face-to-face interview as previously described (Todo-Bom A, et al. Allergy 2008). Current rhinitis (CR) was defined as the presence of at least two out of three rhinitis symptoms (repeated sneezing and/or itchy nose, blocked nose for more than 1 h or runny nose without having a cold or flu), either usually or in the last 12 months. Rhinitis severity was assessed using a visual analog scale (VAS), as previously described (Bousquet J, et al. Allergy 2007). Overall rhinitis impact on QoL was evaluated using a 0–100 VAS.

Results: Data was obtained from 3678 responders; 58.5% were female; mean (standard deviation) age 74.1 (7.0) years old. The prevalence of CR was 29.8% (95% confidence interval 28.4–31.3); no differences were found for gender, age or urban/rural living area. Of seniors with CR, 49.1% had mild intermittent, 7.0% mild persistent, 27.5% moderate-severe intermittent and 16.4% moderate-severe persistent rhinitis. Most subjects (58.6%) referred that rhinitis symptoms had started before the age of 40. Seniors reported a mean decrease of 40% in their usual QoL due to nasal symptoms. Only 38.6% of the patients with CR had been physician-diagnosed and 38.7% were under treatment for this disease in the previous year. Allergic conjunctivitis symptoms were referred by 68.6% of subjects with CR (population prevalence 20.5% (95% confidence interval 19.2–21.8).

Conclusion: Rhinitis and rhinoconjunctivitis are common diseases in the population aged more than 65 years-old and are frequently underdiagnosed and undertreated. This was the first nationwide epidemiological survey classifying rhinitis according to ARIA guidelines in this age group. More than 40% of seniors presented moderate-severe disease.

71
First data of the European registry of severe allergic reactions

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Background: Anaphylaxis is a severe allergic reaction that can be life-threatening. The registration of anaphylaxis through a standardised system enables the comparison and analysis of data throughout Europe.

Method: The participating countries of the European anaphylaxis registry are Austria, Bulgaria, France (Allergy Vigilance Network), Germany, Greece, Poland, Spain and Switzerland. The data are obtained using a password-protected online questionnaire.

Results: From 2011-05-31 until 2013-01-14, 2079 anaphylactic cases were registered by the following countries: Germany (n = 1297), France (n = 383), Switzerland (n = 201), Austria (n = 104), Spain (n = 45), Poland (n = 36), Greece (n = 8), Bulgaria (n = 5).

The main causes of anaphylaxis were venom (42%) followed by food (29%) and drugs (12%). In Austria, Bulgaria, Germany and Switzerland venom was the leading cause of anaphylaxis. In Greece, Poland, Spain and France food allergens were most commonly named. The most commonly affected organ was the skin (84%), followed by the respiratory tract (63%), the cardiovascular system (60%) and the gastrointestinal tract (37%).

In most patients (48%) symptoms occurred in <10 min after allergen exposure. Symptoms started after <10 min in 61% of the reactions caused by venom, in 42% caused by drugs and 41% caused by food. Patients reacted most commonly at home (25%) followed by garden or park (20%). Treatment was performed mostly frequently by a professional (70%), in 10% of cases by lay and in 6% first by lay followed by a professional. The professionals were mainly emergency physicians and the lay persons family members of the patient.

Conclusion: The European data reveal that venom and food allergens dominate among the elicitors of severe allergic reactions in Europe and that an immediate treatment in <10 min after the onset of symptoms is very important. As the main location of the reaction is at home, patients and family members need to be encouraged to use their emergency drugs.

72
Allergic sensitisation in elderly patients with rhinitis

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Background: It is widely believed that allergen sensitisation declines with age. As a result, physicians often disregard the allergic component in the pathogenesis of respiratory conditions in the elderly. Atopy

is rarely considered in the clinical assessment of the geriatric rhinitis patients, and these patients are infrequently referred for allergy evaluation.

Method: The study included patients older than 65 years with rhinitis and an age- and gender-matched control group. Skin prick tests (SPT) with inhalant allergens (house dust mites, Alternaria, trees pollen, grass pollen, ragweed and mugwort pollen, animal dander) were performed on all the subjects. Detailed medical history was obtained and a questionnaire inquiring about the severity of symptoms, medication, family history of atopy was administered.

Results: A total of 71 patients with rhinits/rhinoconjuctivitis (mean age 69.8 years) were recruited, 11 of them also had asthma. Thirty-one patients (43.6%) had at least one positive SPT result, compared to only 10 subjects (14%) in the control group. The most common allergic sensitisation was found to be to house dust mites in both groups. In the rhinitis group, 13 patients were found to be polysensitised. Symptom scores revealed that 44 patients (61.9%) assessed their nasal symptoms as severe.

Conclusion: Despite the immune modifications occurring in the old age, the prevalence of allergic sensitisation in geriatric patients with rhinitis is substantial. If properly evaluated, these patients, who often present with severe symptoms, can benefit from preventive measures such as allergen avoidance and even specific immunotherapy.

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Oral Abstract Session 13

New horizons in molecular allergology

73
Mapping of the immunodominant regions of shrimp tropomyosin Pan b 1 by basophil activation test and skin prick test

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Background: Shellfish allergy is one of the most common food allergies and a main cause of life threatening allergic reactions to food. The major allergen in shellfish is the heat-stable muscle protein tropomyosin. Epitope mapping of shrimp tropomyosin has been done by IgE-binding assays with short synthetic peptides, but this provides little information about which domains that are responsible for IgE-receptor crosslinking on effector cells. Our aim was to map the immunodominant regions of shrimp tropomyosin by IgE-crosslinking studies.
Method: Five overlapping peptides (P1-P5) spanning the entire sequence of *Pandalus borealis* tropomyosin (Pan b 1) were cloned and expressed in *Escherichia coli,* each including a 22 amino acid long N-terminal His-tag. The five peptides were identified by MS analysis, and IgE-receptor crosslinking was investigated by basophil activation test (BAT) and skin prick test (SPT) with Norwegian shrimp allergic adults. A mixture of the five peptides in equal molar ratios was also included in the studies.
Results: The five Pan b 1 peptides were 81–100 amino acids long and had a calculated molecular weight of 9.5–11.7 kDa. A 15–68% amino acid recognition was obtained. In BAT, peptides induced activation at high concentrations and showed individual variations at lower concentrations. The peptide mixture induced a comparable activation of basophils as whole tropomyosin, also at lower concentrations. SPT studies showed positive responses to P1, P3 and P5 for all patients and increased responses to the peptide mixture compared to whole tropomyosin.
Conclusion: For the first time the immunodominant regions of shrimp tropomyosin were mapped by IgE-receptor crosslinking studies with peptides long enough to display both conformational and sequential epitopes. No specific part of tropomyosin was found to be immunodominant for all patients tested; the patients were either predominantly sensitised to the middle peptide or to the N- and C-terminus peptides of the protein. These results correlated well with previous microarray data with short synthetic peptides. Dividing shrimp tropomyosin into five peptides did not reduce the allergenicity of the protein.

75
Allergenicity of short peptides from the major peanut allergen Ara h 2

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Background: The 2S-albumins Ara h 2 and Ara h 6 are the most potent allergens for peanut-allergic patients. Protein unfolding has been reported to significantly decrease the allergenic activity of 2S-albumins. Chemically reduced and alkylated (r/a), *i.e.* irreversibly denatured, Ara h 2 and Ara h 6 have thus been suggested to provide safer alternative than native allergens for immunotherapeutic treatment. In this regard, we investigated the residual allergenic potency of r/a Ara h 2.
Method: IgE reactivity of 21 peanut-allergic patients toward native and r/a Ara h 2 was evaluated by performing IgE-binding inhibition assays. Synthetic peptides containing linear B-cell epitopes of Ara h 2 were also tested. The IgE-binding capacity of full-length allergens and short peptides were then correlated with their ability to trigger the degranulation of rat basophil leukemia (RBL) SX38 passively sensitised with immunopurified IgE antibodies from peanut-allergic patients.
Results: A significant IgE-reactivity toward r/a Ara h 2 was evidenced in most patients and was found to be equivalent to that of native Ara h 2 in two patients. Two synthetic peptides, of 15 and 27 amino acids residues in length, displayed IgE-binding capacities comparable to that of full-length r/a Ara h 2. The 27 residues long peptide also exhibited a capacity to trigger basophil degranulation similar to that of r/a Ara h 2. Moreover, this peptide was as potent as the native Ara h 2 when RBL cells were sensitised with IgE antibodies from the patient exhibiting the highest IgE-reactivity toward denatured Ara h 2.
Conclusion: Although reduction and alkylation generally diminishes the allergenic activity of Ara h 2, some peanut-allergic patients may remain highly reactive to short peptides of Ara h 2. Other modifications of Ara h 2 should be thus considered in order to develop hypoallergenic molecules for specific immunotherapy.

76
Identification of salivary lipocalins as new allergens on body-fur of male Syrian hamster

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Background: Different species of hamsters, e.g. Syrian (*Mesocricetus auratus*) and Siberian (*Phodopus sungorus*), are increasingly popular as domestic pets. Several cases of asthma upon contact with hamsters and anaphylaxis following hamster bites have been described, but the allergen (s) responsible are either unidentified or poorly characterised. Several human allergens of animal origin have been found to be lipocalins present on body-surface of animals. In Syrian hamster, MSP proteins comprising of 24 and 20.5 kDa major forms (differing only in the presence and absence of N-glycosylation) are expressed in submandibular glands (SMG) of males and detectable in their saliva. MSP are well characterised proteins and cDNA sequencing had revealed MSP to be a lipocalin.
Objectives: To determine whether

1 MSP lipocalins are detectable in body-fur of Syrian hamsters,
2 whether MSP lipocalins are allergens, and
3 whether similar lipocalins/allergens are present in SMG or body-fur of the other popular pet, Siberian hamster.

Method: Extracts of SMG, fur and saliva from male and female Syrian hamsters were run in SDS-PAGE. Gels were stained or blotted and probed with rabbit antisera

against natural MSP. Recombinant MSP was expressed in *E. coli*. Fur extracts of male Syrian hamsters were investigated in IgE immunoblots using sera of five patients allergic to hamster. Sera were also assayed for IgE reactivity with recombinant MSP in ELISA. Western blots using MSP antisera were performed on SMG and fur extracts of Siberian hamsters.

Results: Protein bands of MSP were detectable at 24 and 20.5 kDa in SMG extracts, saliva and fur extracts of male Syrian hamsters, but not females. Three out of five patient's sera reacted with 24 and 20.5 kDa MSP lipocalins of male Syrian hamster fur. The same three patients had IgE antibodies to rMSP in the range of 1.6–30.6 kU_A/l. Rabbit antibodies against MSP did not show any cross-reaction with SMG or fur extracts of Siberian hamsters.

Conclusion: Two forms of a salivary lipocalin (MSP) were detected male-specifically in body-fur of Syrian hamster and identified as allergens. Immunologically similar lipocalin allergens were not detectable in Siberian hamster. MSP lipocalin allergens are unique in that their presence in body-fur is sex-dependent. MSP may provide a new tool for diagnosis of allergy to Syrian hamster.

77
Dog saliva – an important source of dog allergens

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Background: Allergy to dog (*Canis familiaris*) is a worldwide common cause of asthma and allergic rhinitis. However, dog dander extract in routine diagnostics is not an optimal predictor of IgE-mediated dog allergy. Our objective was to evaluate dog saliva as an allergen source for improved diagnostics of allergy to dog.

Methods: IgE binding proteins in dog saliva and dander extract were analysed by immunoblot and mass spectrometry (LC-MS/MS) using pooled or individual sera from dog allergic patients ($n = 13$). Sera from 59 patients IgE positive to dander and 55 patients IgE negative to dander but with symptoms to dog were analysed for IgE against saliva and dander by ELISA. Basophil stimulation with dog saliva and dander extract was measured by flow cytometry among three dog allergic patients. Additionally, IgE binding protein profiles of saliva from different breeds were investigated by immunoblot.

Results: Greater number and diversity of IgE binding proteins was found in saliva compared to dander extract and varied among dog breeds. In saliva Can f 1, 2, 3, 6 and the saliva allergen candidates; BPIFA2, Mucin-5B, ANGPTL5 and dog IgA were identified. The majority of the 59 dog dander positive sera ($n = 44$) were IgE positive to dog saliva. Among patients IgE negative to dander, but with symptoms to dog, 20% were IgE positive to saliva. The biological activity of saliva was confirmed by basophil degranulation. Dog saliva gave rise to higher basophil activation than dog dander in all patients.

Conclusions: Dog saliva is an allergen source for improved component-resolved diagnostics of and vaccine against dog allergy. The IgE binding protein profile of saliva from different dogs varies.

78
Enzymatic and allergenic analyses of house dust mite extracts from *Dermatophagoides siboney, Dermatophagoides pteronyssinus* and *Blomia tropicalis*

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Background: Molecular characterisation of several house dust mite allergens has elucidated their enzymatic proteolytic properties. At least 19 allergen groups have been described, four of them are proteolytic enzymes, including cysteine, trypsine, chemotrypsine and colagenolytic proteases. In addition, other works indicate that enzymatic activity can be linked to allergenicity. On the other hand, the presence of active proteases in biopharmaceutical products could lead to protein degradation affecting product stability. The objective of this work was to study the gelatinolytic activity and its relationship with the IgE-binding activity of different components of allergenic extracts from *Dermatophagoides siboney* (*Ds*), *D. pteronyssinus* (Dp) and *Blomia tropicalis* (Bt) and to study the effect of temperature and oxidation agents on the proteolytic activity.

Method: Freeze-dried allergen Extracts: VALERGEN-DS, VALERGEN-DP and VALERGEN-BT (BIOCEN, Cuba). IgE Western Blotting, using human serum pool from selected allergic patients. The enzymatic analyses were made by gelatinolytic zymography. SDS-PAGE-Gelatine 1%. The gel was incubated during 75 min in a reaction buffer (Glicine 0.1 M pH 8.5) at 37°C.

Results: The gelatinolytic profile of *Bt* consisted of eight separate bands. Only the 30 kDa band was resistant to incubation in denaturing conditions at 100°C, during 5 min. In the *Dp* extract, four gelatinolytic bands were detected, two of them are thermoresistant, including the most intense, which corresponds to the major allergen Der p 1. In spite of the taxonomic and allergenic similarity between *Dp* and *Ds*, in the last specie, it was detected up to six enzymatically active bands, whereas only Der s 1 was thermoresistant. Oxidation of *Ds* extracts using metal ions (Zn^{1+}, Cu^{2+}) showed that the high molecular weight protease is inhibited proportionally to the ion concentration. Another middle weight band notably increased the activity suggesting metal dependence. The overall comparison of zymograms with the allergenic profiles, as measured by Western Blotting, suggested that most allergenic components have enzymatic activity in the case of *Bt*, in contrast to *Dp* or *Ds*. An important finding is that incubation at high temperatures can destroy most of the enzymatic bands, retaining IgE activity.

Conclusion: The finding could be advantageously applied to the development of allergen vaccines with reduced enzymatic activity.

Oral Abstract Session 14

New developments in the treatment of allergic rhinitis

79

A new tool to assess treatment benefits in French patients with allergic rhinitis: the French version of the Patient Benefit Index (PBI)

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Background: Allergic rhinitis (AR) deteriorates the quality of the life of the patients and the treatment objective is to restore it. To appreciate the satisfaction of the patients with respect to their treatment is important but few tools are available. The Patient Benefit Index (PBI) assesses treatment benefit in patients with AR. It includes two self-administered questionnaires with 25 questions each. The Patient Needs Questionnaire (PNQ) explores the patient's expectations regarding the treatment, and is used at the start of treatment. The Patient Benefit Questionnaire (PBQ) is administered during treatment, to evaluate the treatment benefit, regarding the same points than PNQ. For each question, partial PBI are calculated as the product of (expectation/PNQ)*benefit. The PBI ranges from 0 (no benefit) to 4 (maximal benefit) by summing partial PBI. Our aim was to validate the French version of the PBI (F-PBI).
Method: The English PBI was translated in French by two independent translators following good practice. The questionnaires (PNQ and PBQ) were self-administered by patients in the BENEFICA survey (a prospective, observational study involving patients with allergic rhinitis who start a treatment with a H1-antihistamine). The properties of the F-PBI were studied in a sample of patients drawn up from the BENEFICA population. Symptoms (mini-RQLQ, discomfort) were evaluated before treatment (W0) and 2 weeks later, during treatment (W2). PNQ was self-administered at W0 and PBQ at W2.
Results: A sample of 385 patients was drawn up from BENEFICA: mean age 39 ± 14 years, women 53%, duration of rhinitis 14 ± 11 years, 82% of patients with moderate to severe persistent rhinitis (ARIA). Expectations of patients could be summarized in three dimensions (factorial analysis with Varimax rotation explaining 71% of the global variance): symptoms relief, restoration of social life, decrease of psychological impact. PBI score was 2.75 ± 0.79 and was superior to 1 (threshold for clinically relevant benefit) for 97% of patients. Internal coherence was excellent (Cronbach alpha 0.88). PBI was moderately correlated to change in miniRQLQ ($r = -0.49$, $P < 0.0001$) and change in discomfort ($r = -0.39$, $P < 0.0001$), due to its richer conceptual content. PBI was greater in patients willing to continue the treatment (2.78 ± 0.76 vs 1.32 ± 0.86, $P < 0.0001$).
Conclusion: The F-PBI is validated. It has the same conceptual content than the English PBI. Its properties allow the PBI to be used by clinicians.

80

A new allergic rhinitis therapy (MP29-02) is more effective than current first line monotherapies in providing allergic rhinitis patients relief from their nasal and ocular symptoms (rTNSS, rT5SS, rT7SS)

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Background: New AR treatments should provide relief from all symptoms associated with the disease. Three efficacy endpoints are presented here to match patient symptom patterns:

1 Reflective total nasal symptom score (rTNSS; nasal congestion, nasal itching, rhinorrhoea and sneezing; AM + PM).
2 Reflective total of five symptom scores (rT5SS; rTNSS plus ocular itching).
3 Reflective total of seven symptom scores (rT7SS) comprising the rTNSS plus the ocular symptoms of itching, watering and redness.

The aim was to assess the efficacy of MP29-02 (a novel intranasal formulation of azelastine hydrochloride [AZE] and fluticasone propionate [FP]) in providing relief for SAR patients presenting with common symptom patterns compared to AZE or FP nasal sprays and placebo.
Method: Six hundred and ten moderate-to-severe SAR patients (≥12 years old) were randomised into this double-blind, placebo-controlled, 14-day, parallel-group trial to MP29-02, commercially-available AZE or FP nasal sprays, and placebo (all one spray/nostril bid [total daily doses: AZE = 548 μg; FP = 200 μg]). The primary efficacy variable was change from baseline in rTNSS (AM + PM; max = 24), over 14-days. Reflective ocular symptom score (rTOSS) was an important secondary endpoint. Change from baseline in rT5SS (max = 30) and rT7SS (max = 42) were assessed post-hoc.
Results: MP29-02 patients had a significantly greater reduction in rTNSS (−5.31) vs FP (−3.84; $P = 0.0031$), AZE (−3.25; $P < 0.0001$) and placebo (−2.02; $P < 0.0001$), with a relative difference of 47% to FP and 66% to AZE. MP29-02 reduced rT5SS significantly more (−6.72) than either FP (−4.81; $P = 0.0020$), AZE (−4.23; $P < 0.0001$) or placebo (−2.83; $P < 0.001$) (49% to FP; 64% to AZE). Similarly, MP29-02 most effectively treated the entire rhinitis symptom complex, reducing the rT7SS from baseline (−8.74) significantly more than FP (−6.05; $P = 0.0013$), AZE (−5.83; $P = 0.0004$) and placebo (−3.55; $P < 0.0001$). Here the relative difference was 52% and 56% to FP and AZE, respectively. These benefits were observed during the first day of treatment and sustained over the entire course of treatment.
Conclusion: Compared to currently available first-line therapy for AR, MP29-02 most effectively treated patients presenting with common AR symptom patterns. Such a treatment option might preclude the need for concomitant therapy. Given these findings, MP29-02 could be considered as first-line treatment for both nasal and ocular symptom relief in AR management.

81
A new therapy (MP29-02) is effective for the treatment of chronic rhinitis: results from a randomised long-term trial

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Background: MP29-02, a novel intranasal formulation of azelastine hydrochloride (AZE) and fluticasone propionate (FP), provides significantly superior relief from the symptoms of SAR than current first-line therapy, measured over 14 days. However, almost half of all AR sufferers in Europe and the US have persistent AR, so there are many patients who need symptomatic relief for >14 days. The aim was to evaluate the long-term efficacy of MP29-02 vs FP in patients with chronic rhinitis.
Method: Six hundred and twelve chronic rhinitis patients (i.e. perennial allergic rhinitis [PAR] or non-allergic rhinitis; ≥12 years) were enrolled into this randomised, open-label, active-controlled, parallel-group study to MP29-02 (one spray/nostril bid) or commercially available FP nasal spray (two sprays/nostril qd) for 52 weeks. Safety was assessed as the primary outcome. Efficacy was assessed secondarily by change from baseline in PM reflective total nasal symptom score (rTNSS, range 0–12). Time to achieve 100% reduction from baseline in PM rTNSS and% symptom-free days were assessed post-hoc in all patients and in the PAR sub-population.
Results: MP29-02 reduced patients' PM rTNSS from baseline significantly more than FP, from Day 1 up to and including week 28 (−2.88 vs −2.55; Diff: −0.35; 95% CI: −0.59, −0.11; $P = 0.0048$), with treatment difference maintained for 52 weeks. By Day 1 almost twice as many MP29-02 patients achieved 100% rTNSS reduction than FP patients. Within the first month, 70% of MP29-02-patients experienced 100% reduction in PM rTNSS vs 59% of FP patients, and did so 8 days (median) faster than FP ($P = 0.0024$). Similarly, in the PAR sub-population, more patients treated with MP29-02 achieved 100% response, and did so 8 days (median) faster than FP within Month 1 ($P = 0.063$). MP29-02 patients experienced 172.8 symptom free days in the total population [8.4% more than FP patients ($P = 0.0005$)] and 173.8 symptom free days in the PAR population [7.3% more than FP patients ($P = 0.0122$)].
Conclusion: These results confirm MP29-02's large therapeutic spectrum (i.e. both SAR and PAR) and its consistent superiority over an intranasal steroid. Seven out of 10 patients experience 100% rTNSS reduction, with twice as many patients achieving this level of response at day 1 compared to FP. Given these findings MP29-02 can be considered the drug of choice for the treatment of SAR and chronic rhinitis.

82
Nasal provocation, skin reactivity and allergen-specific serum IgE levels in allergic rhinitis

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Background: The diagnosis of allergic rhinitis (AR) is normally based on the measurement of allergen-specific serum IgE levels, skin prick testing and/or provocation testing. In a couple of studies with grass or birch pollen allergic patients, the relation between these diagnostic parameters has been explored. The results were contradictory and no clear relationship between these parameters could be demonstrated. We examined a possible relationship between these parameters in birch or house dust mite (HDM) allergic patients.
Method: Patients with suspected birch or HDM-induced AR were screened prior to enrollment into two separate multicentre studies with birch or HDM immunotherapy. Patients with a positive medical history, a positive skin prick test (SPT) to birch pollen or HDM, and a specific serum IgE (sIgE) level to birch pollen or HDM of >0.7 U/ml underwent a standardised titrated nasal provocation test (TNPT) using 100, 1000 and 10 000 AU/ml of a birch pollen or HDM allergen extract. The concentrations eliciting a positive response (i.e. provocative dose) were compared with the sIgE levels and the outcomes of the SPT.
Results: Three hundred and seventeen patients were screened for the birch pollen immunotherapy study and 425 for the HDM study. All three diagnostic parameters were positive for 244 patients (119M/125F, mean age 36 year) in the birch study and for 287 patients (142M/145F, mean age 31 years) in the HDM study. For the birch pollen allergic patients the SPT and sIgE results were comparable between the three provocation concentrations (see Table). The results in HDM allergic patients, on the other hand, showed a decrease in sIgE level at the provocative dose of 10 000 AU/ml. For the SPT outcome in HDM allergic patients no difference was observed.
Conclusion: No apparent relationship could be demonstrated between the three diagnostic parameters for both birch pollen- and HDM allergic patients. From our results it appears that the outcome in neither the SPT nor the sIgE levels is predictive for the provocative dose in the TNPT.

83
Efficacy and safety of the probiotic *Lactobacillus paracasei* LP-33® in allergic rhinitis – a double-blind, randomised, placebo-controlled trial

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Background: Allergic rhinitis results from IgE-mediated immune responses. An imbalance between Th1 and Th2 cells is involved in IgE-allergic inflammation which may be improved by probiotics.
Objectives: To test the efficacy of the probiotic *Lactobacillus paracasei* LP-33®, a

Table 1. Results from the three diagnostic parameters.

	Birch pollen ($n = 244$)			HDM ($n = 287$)		
Provocative dose (AU/ml)	100	1000	10 000	100	1000	10 000
sIgE (U/ml), median (range)	28.4 (1.0–99.6)	23.0 (0.9–99.3)	22.8 (1.2–96.5)	29.1 (0.8–280)	32.5 (0.8–532)	17.6 (0.7–504)
SPT diameter (mm), median (range)	8.0 (3–20)	8.0 (3–20)	8.0 (3–20)	8.0 (3–18)	7.0 (3–18)	7.0 (3–21)

double-blind, placebo-controlled, randomised trial was carried out in patients with demonstrated AR to grass pollen and presenting altered quality of life during Loratadine treatment.
Methods: Subjects with persistent allergic rhinitis, symptomatic during the grass pollen season, and a positive skin test or specific IgE to grass pollens were included by GPs. All received Loratadine for 5 weeks. The primary endpoint was the improvement in Rhinitis Quality of Life (RQLQ) global score at the fifth week of LP-33® consumption compared with placebo. Secondary endpoints included nasal and ocular symptoms (individual and total symptom scores), visual analogue scale (VAS) and time of first exacerbation of the symptoms when Loratadine was stopped.
Results: Four hundred and twenty-five subjects were[m1] included in the study. Using ITT analysis, the RQLQ global score decreased significantly more in the active group than in the placebo group ($P = 0.0255$, difference = −0.286 [95%CI: −0.536; −0.035]). No significant differences were noted for the change of RTSS-5 global score between groups ($P = 0.1288$, difference = −0.452 [95%CI: −1.036; 0.132]). Significant differences in ocular symptoms (RQLQ) were observed between groups ($P = 0.0029$, difference = −0.4087 [−0.6768; −0.1407]).
Conclusions: This study performed by GPs shows that LP-33® improves the QOL of subjects with PER who are currently being treated with an oral H_1-antihistamine (Loratadine 10 mg daily). Whereas nasal symptoms had not changed, ocular symptoms had consistently improved.

84
Nasal allergen provocation test in nasal polyposis with and without allergy

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Background: CRSwNP is characterised by eosinophilic inflammation and local IgE production. The amount of local tissue IgE in CRSwNP is independent of the atopic status and serum IgE of the patient. Moreover patients with CRSwNP and pollen allergy do not show prominent symptoms during season.
Method: Four groups of patients ($n = 48$) underwent nasal allergen provocation test for grass pollen. We included 12 patients with allergic rhinitis based on grass allergy, 12 patients with CRSwNP without grass allergy, 12 patients with CRSwNP with grass allergy, and 12 control patients. The diagnosis of grass allergy was based on skin prick test and RAST. The test was positive based on change in nasal airflow measured by active anterior rhinomanometry and symptoms. In annex, VAS scores were performed before and after NAPT.
Results: The nasal allergen provocation test was positive in 19% of the patients with CRSwNP without allergy and in 54% of the patients with CRSwNP with allergy. In contrast 100% of the patients with allergic rhinitis developed a positive provocation test, whereas in the control group 8% of the patients developed a positive provocation test. CRSwNP without allergy did not show a significant increase in VAS scores of complaints. In contrast, allergic rhinitis patients and CRSwNP patients with grass allergy developed a significant increase in nasal obstruction and nasal drip. However, in allergic CRSwNP patient the symptoms after provocation were significantly lower compared to allergic rhinitis patients.
Conclusion: This suggests that local IgE present in these patients are functional after allergen provocation with grass pollen. However there is a reduced reactivity after grass pollen stimulation in CRSwNP compared to allergic rhinitis. This reduced reactivity is most likely due to the polyclonality of local IgE or IgG4 blocking activity in CRSwNP.

Oral Abstract Session 15

Asthma control: newest findings

85

The incidence of malignancy is similar among asthma patients treated with or without omalizumab: results from the long-term EXCELS study

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Background: The long-term safety of omalizumab was evaluated in a real-world phase IV study (EXCELS), conducted as part of an FDA postmarketing commitment. This was in response to earlier reports in clinical studies of adolescents and adults with asthma and other allergic disorders, that malignant neoplasms were observed in 20 of 4127 (0.5%) omalizumab-treated patients compared with five of 2236 (0.2%) control patients.

Method: In this prospective, observational study, patients (≥12 years of age) with moderate-to-severe allergic asthma were enrolled in two cohorts which differed according to whether they were receiving omalizumab (OMA cohort) or had never received omalizumab (non-OMA cohort) at baseline. Patients in the OMA cohort who started treatment within 7 days before and up to 30 days after enrollment were defined as 'new starts'. Asthma therapy changes were permitted during the study. The primary outcome measure was all confirmed, study-emergent primary malignancies, including non-melanoma skin cancer, adjudicated by an external independent oncologist.

Results: The study population included 5007 in the OMA cohort (18 426 person-years), containing 587 OMA new starts (1967 person-years), and 2829 patients in the non-OMA cohort (10 844 person-years). Median follow-up was approximately 5 years and 56% of patients completed 5 years of follow-up. There were more patients with severe asthma in the OMA cohort vs the non-OMA cohort (50% vs 23%) but other baseline characteristics were generally similar across the cohorts. Rates of confirmed, study-emergent primary malignancies were similar in the overall OMA cohort, OMA new starts and the non-OMA cohort (Table).

Conclusion: In this prospective, observational study with >29 000 total person-years of follow-up (up to 5 years per patient), the incidence of malignancy was similar among asthma patients treated with or without omalizumab.

CI, confidence interval.

[a]Allows for multiple events per patient.
[b]All rates and their differences are expressed as per 1000 patient-years.

86

Bronchial thermoplasty – persistence of benefits out to 5 years in patients with severe asthma

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Background: Long-term 5-year benefits and safety of Bronchial Thermoplasty (BT), a therapeutic bronchoscopic procedure for the treatment of severe persistent asthma, are presented from the AIR2 Trial.

Methods: Patients with severe asthma (uncontrolled despite high doses of ICS [>1000 μg BDP equivalent/day] and LABA) who underwent BT in the AIR2 Trial were followed to 5 years. Adverse events (AEs), severe exacerbations (requiring oral corticosteroids), and healthcare utilisation events including emergency room [ER] visits were assessed during quarterly phone calls and annual in-office evaluations. Durability of treatment effect was assessed using a non-inferiority test for proportion of subjects experiencing severe exacerbations annually compared to Year 1 post-BT (52 weeks beyond 6 weeks after last BT). The upper 95% confidence limit of the difference in proportions in each year minus Year 1 had to be <20% to establish non-inferiority.

Table 1.

	OMA cohort			Rate differences: OMA vs non- OMA cohort		Rate ratios: OMA vs non-OMA cohort	
	All (n = 5007)	New starts (n = 587)	Non-OMA cohort (n = 2829)	All	New starts	All	New starts
Malignancy events[a]	295	32	190				
Malignancy rate (95% CI)[b]	16.0 (14.2, 17.9)	16.3 (11.1, 23.0)	19.1 (16.5, 22.0)	−3.06 (−9.19, 2.03)	−2.80 (−11.02, 5.90)	0.84 (0.62, 1.13)	0.85 (0.49, 1.36)

Results: 162/190 (85.2%) subjects enrolled in the BT group completed the 5 years of follow-up. Proportion of subjects experiencing severe exacerbations in each of Years 1–5 were (point estimate [95% CI]): 30.9% [24.2, 37.7], 23.0% [16.6, 29.5], 35.0% [27.6, 42.4], 38.0% [30.4, 45.5], and 21.6% [15.3, 27.9] respectively. Compared to the 12 months prior to study entry (51.6%), the average reduction over 5 years post-BT was 42.4%. Non-inferiority criterion was met. Severe exacerbation rates (events/subject/year) were 0.88 for the 12 months prior to study entry, and 0.49, 0.41, 0.58, 0.60, and 0.31 respectively for Years 1–5 post-BT. Compared to the 12 months prior to study entry the average reduction in proportion of subjects with ER visits for respiratory symptoms over the 5 year post-BT period was 77.4%. Over the 5 year period, no serious procedure-related complications or unanticipated AEs were observed, there was no increase in the proportion of subjects reporting respiratory AEs, and pre- and post-bronchodilator FEV_1 remained stable (no deterioration).

Conclusion: BT provides persistent long-term benefits out to at least 5 years in adult patients with severe uncontrolled asthma. The sustained reduction in asthma exacerbation rate and ER visits 5 years after treatment demonstrates the safety and durable effectiveness of this disease-modifying therapy.

87

Safety profile and pharmacokinetics of SB010, an inhaled GATA-3-specific DNAzyme, in phase I clinical trials in healthy and asthmatic subjects

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Background: T helper 2 (Th2) cells and their master transcription factor GATA-3 were shown to play a central role in the pathology of allergic bronchial asthma. SB010 (a nebulisation solution of the human GATA-3-specific DNAzyme hgd40) has been developed as a treatment for Th2-driven asthma. DNAzymes are single-stranded catalytic DNA molecules that specifically bind and cleave target mRNA sequences. Aim of the clinical studies was to investigate safety, tolerability and pharmacokinetics of orally inhaled SB010 in healthy subjects and patients with allergic bronchial asthma.

Method: We performed three subsequent Phase I trials: single dose application in healthy subjects (study 1, $n = 24$), multiple dose application in healthy subjects (study 2, $n = 48$) and multiple dose application in patients with allergic asthma with airway hyperresponsiveness (AHR) (study 3, $n = 24$). All studies were performed as randomised, double-blind, placebo-controlled, parallel group (per dose level) dose-escalation studies in male Caucasian subjects. SB010 was applied as nebulised solution via a controlled breathing system in six dose levels ranging from 0.4 to 40 mg (study 1) and in three dose levels ranging from 5 to 20 mg (studies 2 and 3). Adverse events, vital signs, clinical chemistry, hematology, urinalysis, ECG, pulmonary function testing and overall tolerability were assessed in 108 subjects totally. hgd40 plasma concentrations were analyzed using a highly specific and sensitive hybridization ELISA system.

Results: In all three studies, all doses were well tolerated, no serious or severe adverse events and no dose limiting effects were observed. Occasional adverse events (such as headache or cough) were of minor clinical relevance and were fully reversible during the respective study periods. Maximum plasma concentrations of hgd40 were always detected within the respective highest dose group: at 1 h after single administration in healthy subjects (29.2 ± 20.6 ng/ml) at 1 h after the last of multiple applications in healthy subjects (10.0 ± 14.5 ng/ml) and 0.75 h after single application in asthmatic patients (14.0 ± 16.6 ng/ml). In all subjects hgd40 was no longer detectable at 12 h after administration.

Conclusions: Overall, inhaled SB010 turned out to be well tolerated after single or multiple inhalative exposure in healthy male subjects and single inhalative exposure in patients with allergic asthma with AHR. Based on these results the therapeutic efficacy of SB010 will now be evaluated in Phase IIa clinical trials.

88

Once-daily tiotropium improves lung function and reduces risk of asthma exacerbation/worsening in patients with symptomatic asthma, regardless of allergic status

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Background: The once-daily long-acting anticholinergic bronchodilator tiotropium improved lung function and reduced exacerbation risk in patients with symptomatic asthma despite treatment with inhaled corticosteroids (ICS) plus long-acting β_2-agonists (LABAs). Pre-planned subgroup analyses were performed to evaluate whether the allergic status of these patients influenced the way they responded to tiotropium.

Methods: In two replicate, randomised, 48-week, parallel-group Phase III studies (NCT00772538, NCT00776984), patients with severe asthma were randomised to receive once-daily 5 µg tiotropium (via Respimat® Soft Mist™ Inhaler) or placebo as add-on therapy to high-dose ICS + LABAs. Antihistamines, omalizumab and oral corticosteroids were permitted during the run-in, treatment and follow-up periods. Patients with potentially allergic asthma were identified in a pre-planned subgroup analysis using the following criteria: total serum IgE (>430 µg/l) or clinical judgement ('yes').

Table 1.

	Time to first severe asthma exacerbation			Time to first asthma worsening		
Clinical judgement at randomisation	Events tiotropium/placebo	Risk reduction,%	HR (95% CI)	Events tiotropium/placebo	Risk reduction,%	HR (95% CI)
Yes	89/109	18	0.82 (0.62–1.08)	149/191	30	0.70 (0.56–0.86)
No	33/40	25	0.75 (0.47–1.19)	77/96	32	0.68 (0.51–0.92)

Results: Of the 912 randomised patients, 398 and 559 were identified with potentially allergic asthma, according to IgE serum levels or clinical judgement, respectively. A similar proportion of tiotropium and placebo patients received concomitant systemic antihistamines or anti-allergic agents during the treatment period. The co-primary end points of peak and predose (trough) forced expiratory volume in 1 s improved with tiotropium compared with placebo in both trials 1 and 2, irrespective of allergic status. The response to tiotropium was also independent of the blood eosinophilia. Pooled data analysis demonstrated an increase in time to first severe asthma exacerbation and time to first asthma worsening following tiotropium vs placebo, regardless of allergic status (see Table). Safety profile was similar across treatment groups.

Conclusions: Once-daily 5 μg tiotropium as add-on therapy to ICS + LABAs and allergic background medication improves lung function and asthma exacerbations in patients with symptomatic asthma, irrespective of patient allergic status.

89

Tiotropium added to inhaled corticosteroids and long-acting β_2-agonists reduces episodes of asthma worsening, irrespective of selected patient baseline characteristics

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Background: Two replicate double-blind, parallel-group trials (NCT00772538, NCT00776984) compared once-daily long-acting anticholinergic bronchodilator tiotropium (5 μg) vs placebo (via Respimat® Soft Mist™ Inhaler) as add-on to ICS + LABAs for 48 weeks in asthmatic patients. Tiotropium improved lung function and severe exacerbations in patients with symptomatic asthma despite high-dose ICS, LABAs and additional treatments based on guidelines. Tiotropium also significantly reduced the risk of asthma-worsening episodes (defined as progressive increase in symptoms and/or ≥30% decline in best morning PEF for ≥2 consecutive days). Subgroup analyses were performed to determine if improvements in asthma worsening were associated with differences in patient baseline characteristics.

Methods: At study entry patients had post-bronchodilator $FEV_1 \leq 80\%$ predicted, ACQ score ≥1.5, and at least one asthma exacerbation in previous year. Prespecified subgroup analyses included disease duration, FEV_1 level and reversibility, and mean ACQ score at randomisation. Pre-planned *post hoc* subgroup analyses included age and smoking status.

Results: Of 912 patients randomised, 456 received tiotropium and 456 received placebo. Overall, tiotropium increased time to first asthma worsening (risk reduction 31%; HR 0.69; $P < 0.001$) vs placebo; 226 patients on tiotropium and 287 patients on placebo experienced ≥1 asthma worsening. Tiotropium reduced the risk of asthma worsening in all subgroups (see Table).

Conclusions: In patients with symptomatic asthma despite the use of ICS + LABAs, the addition of once-daily tiotropium increased time to first asthma worsening vs placebo, irrespective of selected baseline characteristics.

90

Safety, tolerability, and efficacy of brodalumab (AMG 827) in subjects with moderate to severe asthma with high bronchodilator reversibility: subgroup analysis of a randomised, double-blind, placebo-controlled, multiple-dose study

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Background: As part of a prespecified subgroup analysis of a phase 2 study, we evaluated the efficacy of brodalumab, a human anti-IL-17 receptor A monoclonal antibody, in a high airflow reversibility subpopulation of asthma patients.

Method: Adult (age 18–65 years) subjects with inadequately controlled asthma were randomised to receive brodalumab (140, 210, or 280 mg) or placebo every 2 weeks. High reversibility (post-bronchodilator FEV_1 improvement ≥20%) was one of nine prespecified subgroup analyses. Analyses used last-observation-carried-forward imputation and included all randomised subjects receiving ≥1 dose of investigational product. Endpoints included ACQ, lung function (FEV_1 and PEF), and asthma symptoms; all *P* values were nominal.

Results: The high reversibility subgroup comprised 37% (112/302) of the full study population and was composed of more females (66% vs 55%) and atopic subjects (87% vs 81%) than the low reversibility subgroup ($n = 190$). As expected, ACQ scores, rescue SABA use, and FeNO were higher and pre-bronchodilator FEV_1 and PEF were lower in the high reversibility than in the low reversibility population.

Table 1. Risk reduction of tiotropium in time to first asthma worsening by subgroup analyses.

Parameter at randomisation	Events tiotropium/placebo	Risk reduction, %	HR (95% CI)
Age, years: <40; 40–60; ≥60	38/46; 129/148; 59/93	29; 27; 38	0.71 (0.46–1.09); 0.73 (0.57–0.92); 0.62 (0.45–0.86)
Smoking status: ex-smoker (>2–10 pack-years); never smoked/ex-smoker (≤2 pack-years)	45/56; 181/231	49; 26	0.51 (0.34–0.76); 0.74 (0.61–0.90)
Asthma duration, years: 5–<20; ≥20	50/56; 176/231	23; 33	0.77 (0.53–1.14); 0.67 (0.55–0.82)
FEV_1,% of predicted normal pre-bronchodilation: <60%; ≥60%	96/120; 130/167	27; 34	0.73 (0.56–0.95); 0.66 (0.53–0.84)
FEV_1 reversibility (FEV_1 increase of ≥12% and ≥200 ml from baseline): yes; no	107/154; 119/133	34; 26	0.66 (0.52–0.85); 0.74 (0.57–0.94)
ACQ score: <2.6; ≥2.6	112/149; 114/138	37; 25	0.63 (0.49–0.81); 0.75 (0.59–0.97)

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Although treatment effect on ACQ scores at week 12 for the full study population was not statistically significant ($P = 0.37$; linear trend test), the minimal important difference (MID) for ACQ (0.5) was met with an estimated treatment difference of −0.53 ($P = 0.02$) in the 210 mg group of the high reversibility subpopulation; efficacy was not increased with a higher 280 mg dose (−0.38 treatment difference in ACQ change; $P = 0.06$). Least squares mean differences (210 mg) from placebo in FEV_1 and PEF were 0.14 and 16.8 l/min ($P > 0.05$). There were numerical trends for reduced daily symptom scores (linear trend $P = 0.08$) and increased symptom free days (linear trend $P = 0.03$). Treatment effects for the low reversibility subpopulation were not statistically significant ($P > 0.05$), except for a small reduction in improvement in night time symptoms and symptom free days. Overall, the most common AEs were worsening of asthma, upper respiratory tract infection, and injection site reaction. While more frequent in brodalumab-treated groups AEs were generally balanced among treatment groups.

Conclusion: While not effective in the overall population, brodalumab treatment was associated with a clinically meaningful response in a subpopulation of asthma with high airflow reversibility, suggesting a role of IL-17 in the pathophysiology in this patient subgroup.

Oral Abstract Session 16

Understanding urticaria

91

Angioedema and angioedema management from ASTERIA II: a phase III, randomised, double-blind, placebo-controlled study to evaluate the efficacy and safety of omalizumab in patients with chronic idiopathic/spontaneous urticaria (CIU/CSU) who remain symptomatic despite H1 antihistamine treatment

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Background: Angioedema is a common symptom of CIU/CSU and has a negative impact on patients' health-related quality of life. We describe the presence of angioedema and how patients managed it during the 12-week treatment period in ASTERIA II.

Method: Subjects ($n = 323$) were randomised 1:1:1:1 to receive placebo or omaliziumab 75, 150 or 300 mg at Baseline and Weeks 4 and 8. Angioedema and angioedema management were assessed via the Urticaria Patient Daily Diary. We summarized the percent of patients with angioedema, the mean number of days with angioedema, and how patients managed angioedema (response categories: did nothing; took medication; contacted health care provider; visited health care provider; went to the hospital emergency room; was hospitalised).

Results: During the week prior to Baseline, angioedema was a prevalent symptom with 37.8% to 46.3% of patients reporting it; for those with angioedema, they reported having it for approximately half of the days during that week (3.4–3.7 days). During the last week of the 12-week treatment period, fewer patients reported angioedema across all treatment arms (6.8–28.4%) and had it for fewer days during that week (1.8–2.5 days); however, the reduction in patient-reported angioedema was higher in omalizumab treated patients. Angioedema management at Baseline and throughout the course of the treatment period generally consisted of low intensity interventions, if any: most patients reported doing nothing or taking medication, and few patients reported having called or visited their health care provider; none reported visiting the hospital emergency room nor being hospitalised.

Conclusion: Omalizumab was efficacious in reducing patient-reported angioedema in CIU/CSU patients who were symptomatic despite H1 antihistamine treatment.

92

Detection of serum specific IgE to bacterial derived extracellular vesicles in chronic urticaria

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Background: Chronic urticaria (CU) is a common immunologic disease in skin, but its pathogenic mechanisms are not fully understood. Recent publications suggested bacteria derived extracellular vesicles (EVs) could induce immune dysfunctions in various allergic diseases. Considering bacteria is colonizing on skin to induce immune responses in skin allergy disease, we detected serum specific IgE and IgG1levels to bacterial derived EVs in CU patients.

Method: Ninety-five CU patients and 49 healthy controls were enrolled from Ajou University Hospital, Suwon, Korea. EV extracts from three different bacteria including *Acinetobacter baumannii, Enterobacter, Staphylococcus aureus* were isolated. Serum specific IgE and G1 antibodies to EVs were detected using an ELISA technique. Positive cut off value was determined from the mean + SD of absorbance values of healthy controls.

Results: The serum specific IgE and IgG1 levels to *Acinetobacter baumanii* derived EV were significantly higher than those of controls with a positive significant correlation between specific IgE and IgG1 levels ($r = 0.47$, $P < 0.0001$). No significant differences were found in specific IgE and IgG1 levels to other two bacteria derived EVs. The patients with high specific IgE to *Acinetobacter baumanii* was significant younger, while no significant differences were found in gender, history of allergic diseases, urticaria activity score, atopy and total IgE level between positive and negative groups.

Conclusion: We confirmed that a subpopulation of CU patients possesses serum specific IgE and IgG1 antibodies to *Acinetobacter baumanii* derived EVs. Further investigations will be needed to investigate their pathogenic role in CU.

Table 1.

	Baseline				Week 12			
	Placebo ($n = 79$)	Omalizumab 75 mg ($n = 82$)	Omalizumab 150 mg ($n = 82$)	Omalizumab 300 mg ($n = 79$)	Placebo ($n = 79$)	Omalizumab 75 mg ($n = 82$)	Omalizumab 150 mg ($n = 82$)	Omalizumab 300 mg ($n = 79$)
Angioedema present during week (%)	38.0	37.8	46.3	40.5	28.4	20.3	15.5	6.8
Mean number days angioedema present	3.4	3.7	3.5	3.6	2.4	2.5	2.5	1.8

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93
D-dimer is a biomarker of lack of responsiveness to antihistamines in chronic spontaneous urticaria

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Background: A marker of disease activity and of responsiveness to therapy is still missing in chronic urticaria (CU). Recent studies found that the disease is characterised by the activation of the coagulation cascade leading to thrombin generation and, in severe cases, to fibrinolysis. The present study investigated the usefulness of plasma D-dimer as a predictor of response to antihistamines in spontaneous CU.
Method: Disease severity and response to cetirizine were assessed by standardised procedures in 86 adults with spontaneous CU. Plasma D-dimer, ESR, CRP, and thyroid autoantibodies were measured, and autoreactivity was assessed by autologous serum and/or plasma skin tests.
Results: The response to cetirizine was satisfactory in 60% of patients and insufficient in 40%. D-Dimer plasma levels were elevated in 27/86 (31%) patients. Plasma D-dimer levels were inversely related to histamine response and, within subgroups showing similar disease severity, subjects showing signs of fibrinolysis were much more frequently cetirizine-resistant ($P < 0.001$) than those showing normal D-dimer levels. D-dimer levels did not correlate with thyroid autoimmunity, nor with autoreactivity, but were elevated in 6/7 (86%) of patients showing an elevated CPR, and in 3/5 (60%) subjects showing an elevated ESR. In two patients D-dimer follow-up data showed that levels within normal limits coincided with periods of effectiveness of antihistamine treatment, whereas elevated D-dimer levels were associated with unresponsiveness to cetirizine even at high doses.
Conclusion: Elevated D-dimer levels represent a biomarker of chronic urticaria patients that are less responsive to antihistamine treatment. This observation suggests the existence of complentary pathogenic pathways that parallel histamine release from mast cells in a subset of patients.

94
Circulating level of CD4+CD25+ Foxp3+ T cells in patients with chronic urticaria; is there any alteration?

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Background: CD4+CD25+ regulatory T (Treg) cells play critical roles in maintaining peripheral tolerance and preventing autoimmunity, but the characteristics of Treg cells have not yet been clarified in CU. We determined the frequencies of circulating CD4+CD25+ FOXP3+ T cells, and serum level of IL-10, TGFβ, and IL-17 in CU patients compared with healthy control subjects, to identify whether level of CD4+CD25+ Foxp3+ T cells differ in CU patients.
Method: Peripheral blood mononuclear cells (PBMCs) were obtained from CU patients and healthy controls. The frequency of CD4+CD25+ T cells in PBMCs and expression levels of FOXP3 was detected by flow cytometery. The serum level of IL-10, TGFβ and IL-17 were measured by ELISA.
Results: A decreased percentage of Treg cells was detected in CU patients compared to control subjects. It was also detected in CAU as well as CIU patients compared to their respective control. No significant difference between the serum levels of IL-10, TGFβ, and IL-17 in CU patients and control subjects.
Conclusion: Our data demonstrates that the frequency of CD4+CD25+ FOXP3+ T cells in PBMCs was decreased in CU patients. Further studies are needed to clarify the exact role of Treg cells in the pathogenesis of CU and factors regulating their function.
Keywords: Treg, chronic urticaria, IL-10, IL-17, TGFβ.

95
Serum autoantibodies to thyroid antigens in patients with chronic urticaria

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Background: Chronic urticaria (CU) is a common skim allergic disease in which autoimmune mechanisms have been involved. Recent studies demonstrated that highly cytokinergic IgE antibodies bound on mast cell surface could recognise thyroid autoantigens. We hypothesised that serum specific IgE to thyroid autoantigens may be involved in CU pathogenesis.
Method: We enrolled 195 patients with CU and 76 normal healthy controls (NC). Serum specific IgE to two thyroid autoantigens, thyroid peroxidase (TPO) and thyroid globulin (TG) were measured by ELISA, while specific IgG antibodies to TPO and TG were measured by radioimmunoassay. Positive cut off value for ELISA results was determined from mean + 3SD of absorbance values of NCs. Binding specificity was confirmed by ELISA inhibition test. Basophil activation test (BAT) with TPO was performed using basophils from the patients with high specific IgE to TPO.
Results: High serum specific IgE to TPO was detected in 5% of CU patients compared to NC (1%). High specific IgE to TG was detected only in 1% of CU and NC groups. When clinical parameters were compared between positive and negative groups, total IgE level was significantly higher in positive group ($P < 0.05$) with a significant positive correlation between total IgE and specific IgE to TPO levels ($P < 0.05$). No significant differences were found in atopy, urticaria activity score and other CU related clinical parameters between the two groups. No associations were found in prevalence between IgE to TPO and IgG to TG or TPO. BAT showed a significant upregulation of CD63 or CD203 expression with additions of TPO.
Conclusion: We confirmed circulating serum specific IgE to TPO in a sub-population of CU patients, which could activate basophil in CU, suggesting a possible involvement of autoimmune mechanism in CU pathogenesis.

96
Basophil activation test to decipher the heterogeneous nature of chronic spontaneous urticaria

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Background: The pathomechanism of chronic urticaria (CU) is still incompletely understood. In half of the patients autoantibodies against IgE or its receptor (FceRI) were identified, their relevance remains unclear. Other autoreactive serum components like coagulation factors, complement products or cytokines may also play a role. Recently, we optimised the basophil activation test for CU (CU-BAT), as an *in vitro* alternative to the autologous serum skin test. Our aim is to use this method to distinguish antibody and non-antibody mediated CU subtypes.

Method: Sera of well-defined CU-BAT positive patients were separated according to the molecular weight (MW) into two fractions using different filtration methods. The low MW fraction (<100 kDa) was analyzed by a multiplex platform (MSD, Mesoscale Disc.) to confirm the absence of antibodies. Inhibition experiments were performed with different phosphoinositol-3-phosphate kinase inhibitors (PI3KI) for the delta and gamma isoform, IC-87114 and AS-605240 respectively (Selleck Inc.). The ability of the different serum fractions to induce activation of donor basophils was defined as CD63 up-regulation using flow cytometry.

Results: Activating serum factors could be found in both, the low and high MW serum fraction. The delta PI3KI isoform efficiently inhibited FceRI signaling in a dose-dependent manner, whereas the gamma isoform did not. Therefore, we could either prove or exclude FceRI involvement in the serum-induced activation of donor basophils.

Conclusion: In concordance with other studies, we could prove autoantibody involvement in CU only in certain cases. We could also prove that activating factors can be found in the antibody-free, low MW serum fraction. Their exact nature remains to be defined. Our optimised CU-BAT assay is stable and reliable and may allow us to distinguish different CU forms. This may help to decipher the heterogeneous nature of CU and pave the way to an optimised, pathomechanism related therapy of CU.

Oral Abstract Session 17

Innate immunity

97

Ontogeny of toll-like and NOD-like receptor-mediated innate immune responses in high and low risk infants: the immune tolerance in early childhood (ITEC) study

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Background: Altered response of cord blood mononuclear cells (CBMCs) to Toll-like receptor (TLR) and Nucleotide-binding oligomerization domain (NOD)-like responses have previously been observed in infants who subsequently develop allergy. There is no information on responses to other pattern recognition receptors or comparing infants from a family who are at either high risk or low risk for developing allergic disease.

Objectives: To measure cytokine production of cord blood mononuclear cells to agonists of Toll-like and NOD-like receptors and inflammasome agonists in high risk and low risk groups.

Methods: We conducted a prospective cohort study on two study sites: Southampton and Isle of Wight. Recruitment occurred antenatally. Inclusion criteria for high risk ($N = 35$) was at least two first-degree relatives with atopy and for low risk ($N = 10$) neither parent with atopy. Cord blood was collected at birth and CBMCs cells cryopreserved. CBMCs were stimulated with TLR2 (PGN-SA), TLR4 (LPS-EK), TLR2/6 (Pam2CSK4), TLR7/8 (R848) ligands, as well as NOD1 (iE-DAP) and NOD2 (L18-MDP) and inflammasome (LPS + NRLP3) ligands for 24 h and the production of Th1 (IFN-γ), Th0 cytokines (IL-2, IL-12, TNF-α, TNF-β, IL-1β,-4,-6,-8,-12p70) and Th2 cytokine (such as IL-4, IL-9, IL-10, IL-13, IL-22) measured using a Flow cytomix multiplex assay.

Results: Significant differences between high risk and low risk groups in cytokine production in response to a number of PRR ligands was observed. Low risk infants produce higher levels of IL-6 (compared to high risk infants in response to TLR4, TLR7/8, NLRP3/LPS ligand; IL-10 (see Table 1) and IL-22 production was also different between groups in response to a number of PRR ligands. (TLR2, TLR7/8, NOD1, NLRP3/LPS) between the groups. No significant differences were found for other cytokines.

Conclusions: We report for the first time that cord blood mononuclear cell innate immune responses to a range of PRR agonists are different between infants at high or low risk for allergy. We would like to acknowledge the SCBR, WTCRF and Isle of Wight staff who supported this project.

Table 1.

	Control	TLR4	TLR 2/6	TLR 7/8	NOD1	NOD2	NLRP3	NLRP3/ LPS
High risk								
Median	0	27.99	6.18	29.78	0	0	0	0
25th, 75th centiles	0, 0	11.1, 94.5	0, 12.6	11.1, 54.5	0, 0	0, 0	0, 0	0, 3.7
Number	35	35	30	34	28	28	34	32
Low risk								
Median	0	27.54	14.67	19.04	9.055	0	4.715	12.59
25th, 75th centiles	0, 9.4	16.6, 51.6	9.2, 21.5	12.8, 84.1	0, 9.6	0, 10.6	0, 12.1	0, 16.4
Number	10	10	9	10	8	9	10	9
Comparison *P values	0.002	0.559	0.378	0.511	<0.001	0.048	0.002	<0.001

*Two-sample Wilcoxon rank-sum test comparison.

98

Stimulation of human dendritic cells with allergy-protective cowshed bacteria leads to synergistic activation through multiple innate recognition systems

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Background: An increasing number of epidemiological investigations show that an early-life contact with cowsheds, farm animals and/or raw milk can protect from the development of allergic diseases later in life. Several bacterial species have been isolated from cowsheds – namely *Acinetobacter lwoffii* F78, *Lactococcus lactis* G121 and *Staphylococcus sciuri* W620 – and showed allergy-protective effects in various mouse models and can potently activate human dendritic cells. In addition, it is also known that this activation is mediated by different innate recognition systems. Recently, Ege *et al.* could show that the protective effect of the farm environment correlates with its microbial diversity.

Method: Different combinations and concentrations of *A. lwoffii* F78, *L. lactis* G121 and *S. sciuri* W620 were investigated regarding their activation capacity on human monocyte-derived dendritic cells. ELISA was used for analyzing the upregulation of cytokines, especially with respect to their T_{Helper} cell polarisation capacity. By means of FACS analysis the upregulation of the costimulatory molecules CD40, CD80 and CD86 was studied.

Results: All three combinations of cowshed bacteria induced an increase in the release of different cytokines. Synergistic effects could be detected for all tested cytokines, but the extent of those effects was dependent on the cytokine and the concentration of bacteria used for stimulation. FACS data showed no synergistic upregulation of the costimulatory molecules after stimulation with *L. lactis* G121, *A. lwoffii* F78 and *S. sciuri* W620.

Conclusion: These data suggest that a combination of bacteria leads to synergistic activation of human dendritic cells which could be one reason for the correlation of enhanced allergy-protection and microbial

diversity in the farming environment (supported by DFG SFB/TR22, project A02).

99
MicroRNA signatures associated with effector or regulatory dendritic cells

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Background: Dendritic cells (DCs) act as master regulators of the adaptive immune response controlling the early step in the balance between tolerance and immunity. These different functional programs can be controlled by microRNAs (miRNAs). The later are small non-coding RNAs capable to regulate the translation of one or several genes. In this study, our goal was to characterise miRNAs involved in the control of DC polarisation towards a suppressive/regulatory or effector/inflammatory functional program.
Method: In a first set of experiments, we developed culture conditions to generate human monocyte-derived DCs (MoDCs) supporting the differentiation of T helper type 1, T helper type 2 or regulatory T cells (subsequently termed DC1, DC2 and DCreg respectively). The expression of 989 miRNAs was assessed in modulated DCs from five different donors using a microarray approach. The modulation of miRNA expression in polarised DCs was confirmed by real-time PCR.
Results: Culture conditions allowing the polarisation of MoDCs into DC1 or DC2 were successfully established using a cocktail of cytokines and/or TLR agonists. DCreg were obtained after treatment with the glucocorticoid dexamethasone. These DC subsets were confirmed to drive the induction of the following cytokines by resting CD4+ T cells: IFN-γ (for DC1), IL-4, IL-5 and IL-13 (for DC2) and IL-10 (for DCreg) and to express genes specific from each polarisation. Relative comparison of miRNA expression profiles between untreated DCs, DC1, DC2 and DCreg revealed that 13 miRNAs were specifically modulated in these cells. Interestingly, we also establish that three miRNAs are antagonistically regulated between effector DCs (DC1 and DC2) and DCreg and are thus potentially acting as critical molecular switch to orient early immune responses towards tolerance or inflammation.
Conclusion: Polarised DCs, including DC1, DC2 and DCreg, have distinct miRNA expression patterns. The biological role of miRNAs specifically modulated in DC subsets is being addressed in *in vitro* cultures by selective blockade of the appropriate miRNA. The relevance of miRNAs as prognostic or follow-up markers of allergen immunotherapy efficacy will be discussed.

100
Biological profile and regulatory capacity of human IL-10 (over)expressing B cells

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Background: Regulatory cells are essential for the maintenance of immunological tolerance and homeostasis. Immunoregulatory role of B cells was first described in experimental models of normal immune response and autoimmunity. In humans, B cell depleting therapy was found to be associated with the exacerbation of ulcerative colitis and psoriasis induction suggesting B cell regulatory capacity. Distinct B cell subsets with regulatory properties, mediated mainly through provision of IL-10, have been described in both mice and humans. Recently, our group provided clear evidence linking IL-10 secretion and antiinflammatory IgG4 production in subpopulation of human B cells, revealing it as important regulators of immune responses mediated through multiple suppressive mechanisms.
Method: In order to address the suppressive capacity of human IL-10 producing B cells in different compartments of immune response they were first transfected to overexpress IL-10 and then co-cultured with distinct autologous immune cells *in vitro*. Their effect was compared to the one of the control transfected cells in the corresponding co-cultures samples.
Results: In comparison with control transfected cells, B cells overexpressing IL-10 produce less proinflammatory TNF-α and IL-8 and in the same time secrete more antiinflammatory IL-1Ra and VEGF. In addition, they express more CD25, GARP and costimulatory surface molecules than control transfected B cells. When compared with control vector transfected B cell co-cultures, IL-10 overexpressing B cells mediated:

1 Strong inhibition of TLR-2L and TLR-4L elicited cytokine release from PBMC;
2 Shift of MDDCs toward more immature state upon LPS stimulation, with more CD14 and less CD80 and CD86 expressed;
3 Suppression of antigen triggered specific proliferation of PBMC.

Conclusion: These results bring strong evidence for immuneregulatory properties of IL-10 overexpressing B cells directly on magnitude of proinflammatory cytokine production, maturation state of MDDC and antigen specific proliferation, comprising the capacity to regulate both innate and adoptive arms of immune response.

101
Exposure to pollen substances activates the inflammasome machinery in human keratinocytes

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Background: Following pollen exposure, cytokines and chemokines play an important role in the immunological cascade leading to allergic sensitisation and elicitation. Former studies showed that pollen impact the physical barrier of the skin by influencing the expression of adhesion molecules. In this study we expand this data showing an influence of pollen derived mediators on the immunological barrier of human skin by activation of innate components of the immune system – the inflammasome. Recent studies point to an involvement of the NLRP3 inflammasome both in allergic sensitisation by shaping a Th2 response and in skin barrier function influencing skin homeostasis.
Method: To expand knowledge in this field we investigated the impact of pollen derived mediators on the inflammasome of human primary keratinocytes (KC). KC were stimulated with aqueous pollen extracts (APE) of birch and ragweed for different time periods. Cell-free supernatants as well as cell lysates were analyzed for Interleukin (IL)-18, IL-1 beta and IL-1 alpha release. Protein level of active Caspase-1 was determined by Western Blot.
Results: Results revealed that treatment of KC with both birch and ragweed pollen leads to a rapid release of both IL-1 beta and IL-1 alpha. Notably, ragweed pollen exhibit a higher potency for the induction of extracellular secretion of IL-1 in human keratinocytes compared to birch pollen. An even more distinctive effect could be achieved with both pollen extracts under inflammatory conditions provoked by prestimulation of the keratinocytes with TNF-alpha and IFN-gamma. Furthermore, IL-18 was enhanced in cell lysates of APE stimulated KC. Pollen also lead to the pro-

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duction of active Caspase-1 pointing to a Caspase-1 dependent processing and secretion of IL-1beta and thus activation of NLRP3 response after pollen exposure.

Conclusion: In summary, our results support the hypothesis that pollen influence the immunological barrier of the skin via the NLRP3 inflammasome of human keratinocytes. Thus, pollen themselves provide the danger signal necessary not only for sensitisation but also for elicitation of inflammatory allergic skin reactions.

102
Recombinant human pulmonary surfactant protein-D (rhSP-D) modulates Th2 responses and suppresses IgE-facilitated allergen binding to B cells

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Background: Recombinant fragment of human surfactant protein-D (rhSP-D) has been shown to suppress house dust mite and *Aspergillus fumigatus* (Afu) induced allergic inflammation in murine models. We hypothesised that rhSP-D suppresses *ex-vivo* grass pollen induced PBMC proliferation and suppresses CD23-mediated IgE-facilitated allergen binding (FAB) by B cells to Th2 cells.

Method: PBMCs and sera were obtained from six grass pollen allergic individuals ($n = 6$). *Ex-vivo Phleum pratense* (5 μg/ml)-driven PBMC proliferative response was measured by 3H-*thymidine* incorporation *assay*. Binding of rhSP-D to *Phleum pratense* extract was examined by indirect ELISA and Western blotting. Allergen-IgE complexes binding to CD23-enriched B cells pre-treated with rhSP-D was assessed by IgE-FAB assay.

Results: Grass pollen-driven PBMC proliferative response was significantly increased following *ex-vivo* allergen-stimulation (5 μg/ml, $P = 0.0260$). This proliferative response was suppressed in the presence of 5 μg/ml rhSP-D ($P = 0.0022$). Interestingly, rhSP-D was shown to bind *Phleum pratense* in a dose-dependent manner at 0 μg/ml (optical density @ 415 nm, $n = 4$; mean = 0.20), 1 μg/ml (0.95), 5 μg/ml (1.00), 10 μg/ml (0.75) and 20 μg/ml (0.35). This binding was calcium-dependent and was inhibited in the presence of 5 mM EDTA ($P = 0.0225$). Western blot analysis indicated three binding sites of rhSP-D to Phleum pratense extract, observed around 50, 40 and 38 kd respectively. The ability of rhSP-D to bind to various cell types in PBMC's was confirmed by confocal microscopy and flow cytometry. The binding of allergen-IgE complexes to B cells was reduced by 44%, when CD23-enriched B cells were pre-treated with rhSP-D ($n = 6$, actual P value = 0.0001). This decrease in allergen-IgE binding to B cells was associated with reduction in CD23 expression of B cells ($P < 0.001$, actual P value = 0.0002)

Conclusion: rhSP-D supresses *ex-vivo* allergen induced proliferative response and interferes with the co-operative binding of allergen-IgE complex to B cells. Its modulatory effect in supressing Th2 cytokine responses remains to be determined.

Oral Abstract Session 18

Markers of chronic rhinosinusitis

103

Correlation of symptom severity in patients with chronic rhinosinusitis and aspirin exacerbated respiratory disease and *in vitro* results of functional eicosanoid testing

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Background: The syndrome of aspirin exacerbated respiratory disease (AERD) is characterised by an imbalance within the eicosanoid metabolism. The incidence of this imbalance is often underestimated in the population of chronic rhinosinusitis and nasal polyp patients. Functional eicosanoid testing is increasingly discussed as a helpful method for detecting eicosanoid imbalance *in vitro*.
Method: Chronic rhinosinusitis (CRS) symptoms (nasal obstruction, rhinorrhea, asthma and number of previous sinunasal operations (NPSO) were documented from 21 patients (age 27–77 years). All patients had undergone at least one surgical intervention for CRS/nasal polyps. In addition, samples of peripheral blood were collected and processed by functional eicosanoid testing (FET).
Results: The severity of symptoms and the degree of eicosanoid imbalance determined by FET (0.9–2.6) revealed to be positively correlated. Furthermore, the frequencies of previous sinus surgical interventions and asthma closely correlated with the severity of the respective FET-scores.
Conclusion: Our results show a positive correlation between typical symptoms of CRS and eicosanoid imbalance in patients with recurrent CRS/nasal polyps. We therefore conclude that the FET offers a valuable diagnostic tool for detecting AERD even in the absence of Samter's triad. Further studies involving larger patient cohorts will have to be conducted to support these data.

104

Chronic rhinosinusitis with nasal polyps: a pilot study on immunologic dysregulation and the role of sensitisation to aeroallergens

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Introduction: The ethiopathogenesis of chronic rhinosinusitis with nasal polyps (CRSwNP) is still unknown, although a reliable hypothesis consists in an imbalanced immunologic response in respiratory tract, that involves both innate and acquired immunity.
Aims: To fully characterise a group of CRSwNP patients candidate for Functional Endoscopic Sinus Surgery (FESS) and to find immunological markers predicting of relapsing forms after surgery.
Methods: Thirty patients (18 men, 12 female) with CRSwNP eligible for FESS were evaluated before and after surgery. Preoperative investigation included: allergologic assessment, spirometry, methacholine challenge, blood eosinophilia, fractional exhaled nitric oxide (FeNO) determination, histolopathological and immunological study on removed polyps. Post-surgical follow-up (at 3 and 27 months) included: nasal fibroendoscopy, FeNO determination and spirometry.
Results: 18/30 subjects were atopic, 16/30 were asthmatics and 10/30 had NSAIDs hypersensitivity. At the long-term post-surgical follow-up (27 ± 7 months), 21/30 (70%) were relapsers, 15/18 (83%) among atopics, 6/12 (50%) among non atopics ($P = 0.06$). Among atopics, 15/18 were relapsers (83%), 12/18 (66%) were polysensitised. Total IgE median level was 1615 UI/ml in relapsers, 79 UI/ml in non-relapsers (ns). FeNO decreases after FESS (43.1–26.6 ppb, ns). The decrease was however lost at the long-term follow-up (FeNO = 37.7 ppb). FeNO was higher in atopics, in particular in relapsers and asthmatics. Tiffeneau Index was lower in asthmatic relapsers vs non relapsers (94.7 vs 106.4, $P = 0.02$). Histopathologic analysis showed that 18/29 (62%) had eosinophilia (≥11) in NPs. 13/29 (45%) presented a thickening of basement membrane, marked (grade 2) in eight of them (61.5%), 7/8 were relapsers. Regarding immunopathology, in relapsers, FeNO negatively correlated with intracellular Foxp3 expression ($P = 0.04$), especially in atopic relapsers (r: −0.89, P: 0.00006). In atopic relapsers, there was also a significant correlation between FeNO and intracellular GATA-3 expression (r: 0.7, P: 0.007). Finally we found a characteristic perivascular distribution of pentraxin-3 (PTX3) in 11/26 patients (42%), nine of them relapsers (82%).
Conclusions: Allergy, severe asthma, bronchial inflammation, NSAIDs hypersensitivity, high level of total IgE and low intracellular expression of Foxp3 are possible useful prognostic factors for NPs relapse after FESS.

105

The association of T cell polarisation and allergic inflammation markers in patients with nasal polyposis

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Introduction: Nasal polyposis (NP) is a chronic inflammatory disease of unknown etiology with elevated levels of eosinophils in nasal and paranasal mucosa. Bacterial, fungal and viral infections, allergic and environmental factors are known causes. The aim of this study was to measure and compare some parameters on T cell polarisation and the allergic inflammatory mediators in nasal fluid and serum between atopic and non-atopic patients with nasal polyposis.
Method: Fourteen patients (eight non-atopic, six atopic) with similar age, gender and body mass index were enrolled in this study. Nasal lavage fluid and serum were obtained from patients. Atopy was determined by skin prick test. While major basic protein (MBP), eosinophil cationic protein (ECP), macrophage inflammatory protein-1 alpha (MIP-1α), interleukin (IL)-17A, monocyte chemoattractant protein (MCP)-1, neopterin, IL-12p70, IL-10 levels were measured by ELISA; IL-4, IL-17 and

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interferon gamma were analyzed by flow cytometry in nasal lavage fluid and serum samples. Quality of life was assessed with the *Sino-Nasal Outcome Test-20 questionnaire and Nasal Obstruction Symptom Evaluation scale*.

Results: MBP, ECP and MİP-1α levels in nasal lavage fluid were significantly higher in non-atopic patients with NP (respectively, $P = 0.039$, $P = 0.003$). The other measurements did not show statistically significant difference among atopic and non-atopic patiens with NP.

Conclusion: Significantly elevated MBP, ECP and MİP-1 levels found in the nasal lavage fluid of non-atopic patients with NP suggest that local factors play an important role in the development of nasal polyps without relationship with atopy.

Keywords: nasal polyposis, major basic protein, eosinophil cationic protein.

106
Augmentation of CXCL10 expression in nasal fibroblasts derived from patients with recalcitrant chronic rhinosinusitis associated with bronchial asthma

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Background: The prevalence of chronic rhinosinusitis (CRS) that is refractory to traditional therapy appears to be increasing, and CRS that is refractory to traditional therapy tends to be associated with bronchial asthma (BA), especially, aspirin-intolerant asthma (AIA). After viral infections, patients with CRS associated with BA usually experience exacerbations of their CRS symptoms, including nasal polyposis, in comparison with CRS patients without BA. Alternatively fibroblasts as an important component of the epithelial mesenchymal trophic unit play a key role in maintaining tissue homeostasis and may also have the potential to contribute to disease pathogenesis through their contribution to inflammatory responses. On the basis of these findings, we hypothesised that CRS patients with BA are more susceptible to inflammation of the nasal and paranasal mucosa depending on the antiviral response of nasal fibroblasts.

Method: Tissue specimens were obtained from the nasal polyps of three groups of CRS patients, a group that did not have BA (CRS-NA group), a group with aspirin-tolerant asthma (CRS-ATA group), and a group with AIA (CRS-AIA group). Nasal polyp fibroblasts (NPFs) were isolated from the specimens and stimulated with poly I:C. By using a DNA microarray and performing a hierarchical clustering analysis we were able to identify a cluster containing genes that were up-regulated after poly I:C stimulation. To confirm the results of the analysis data, we used quantitative real-time PCR (qRT-PCR) and an enzyme-linked immunoadsorbent assay (ELISA).

Results: Expression of *IFN-inducible protein 10 (IP-10)/CXCL10* transcript was higher in the NPFs of the CRS-AIA group and CRS-ATA group than in the CRS-NA group and control group. These findings were confirmed by qRT-PCR and ELISA.

Conclusion: The results of this study suggest that the increased poly I:C-induced CXCL10 expression in NPFs derived from the CRS patients with BA is involved in susceptible to T helper (Th)1-type immune response in the nasal and paranasal mucosa by viral infection compared with CRS patients without BA.

107
Substance P level in tears after specific and non-specific nasal challenge in patients with seasonal allergic rhinitis

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Background: Naso-ocular reflex after allergen or irritant application onto nasal mucosa is induced by the release of inflammatory mediators and neurokininins from nasal mucosa and conjunctiva, through stimulation of different populations of sensory receptors. The hypothesis of the study was that neurogenic inflammation is contributing to the increased reflex response. The purpose of the study was to compare naso-ocular reflex response in patients with seasonal allergic rhinitis after different methods of nasal provocation by measuring objective and subjective outcomes, including substance P (SP) and tryptase concentrations in nasal lavage and tears.

Method: The study involved 20 patients, with seasonal allergic rhinitis, sensitised either to grass pollen or ragweed. They were subjected to four different nasal provocation tests out of season. They included non-specific provocation with 10 ml of distilled water mist, nasal provocation 1000 IU of ragweed or grass pollen allergen, 60–100 μg of histamine and 2 ml of 2% hyperosmolar solution, as non-specific provocation affecting different populations of sensory receptors. Response was measured using VAS scale on major nasal and ocular symptoms, acoustic rhinometry and nasal lavage prior to and after the provocation tests. Tears collection after provocations was done using Schirmer test strips, which also measured lacrimation. Schirmer score (amount of tears) was measured for each eye separately, but SP concentration was determined for both eyes together. Substance P (SP) and tryptase were measured in nasal lavage and tears.

Results: There is a significant correlation between Schirmer scores between allergen, histamine and hypertonic provocation (Spearman rho 0.73, 0.75 and 0.77, respectively. SP levels in tears after allergen provocation correlated significantly with Schirmer scores after allergen (rho 0.66), histamine (0.62) and hypertonic (0.57) provocation. However, SP level after histamine provocation correlated signifacantčy only with Schirmer score after histamine provocation and SP level after hypertonic provocation did not correlate with Schirmer test. Tryptase was not detected in tears.

Conclusion: Significant correlation between the results of different provocation stimuli and SP levels in tears are suggesting that intensity of naso-ocular reflex is also related to neurogenic inflammation.

108
Characterisation of two epithelial cell air-liquid interface (ALI) culture models for human healthy nasal mucosa and nasal polyps

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Background: Submerged culture of primary human airway epithelial cells undergo a dedifferentiation with loss of many features of the *in vivo* airway epithelium. However, when cultured in an air-liquid interface (ALI), cells develop a well-differentiated, polarised, and pseudostratified epithelium.

Objective: To obtain and characterise human nasal mucosa and nasal polyp well-differentiated epithelia using an ALI culture system.

Methods: Nasal mucosa (NM, $N = 7$) and nasal polyps (NP, $N = 9$) were obtained from patients undergoing nasal corrective or endoscopic sinus surgery, respectively.

Epithelial cells were obtained from the explant method, and differentiated in ALI culture during 28 days. At different times (0, 7, 14, 21, and 28 days) a variety of analysis were performed: ultrastructure study by scanning (SEM) and transmission (TEM) electron microscopy; mucous (MUC5AC, MUC5B) and serous (lactoferrin) secretion by ELISA; cytokines and chemokines analysis by CBA (Cytometric Bead Array); and β-tubulin IV (cilia marker), MUC5AC (goblet cell marker) and p63 (basal cell marker) expression by immunocytochemistry.

Results: In both NM and NP ALI cultures, pseudostratified epithelium with ciliated, mucus-secreting, and basal cells were observed by SEM and TEM at days 14 and 28. Displaying epithelial cell re-differentation, β-tubulin IV and MUC5AC positive cells increased while p63 positive cells decreased overtime. In NP cultures MUC5AC secretion increased overtime compared to day 0 (100%), being significantly ($P < 0.05$) higher than in NM cultures at day 14 ($155 \pm 22\%$ vs $100 \pm 16\%$) and day 28 ($218 \pm 65\%$ vs $148 \pm 38\%$), whereas no differences were found overtime in MUC5B (increased) and lactoferrin secretion. GM-CSF, IL-8, and IL-6 secretion were significantly ($P < 0.05$) increased in NP compared to NM at day 14 (GM-CSF: $114 \pm 7\%$ vs $71 \pm 27\%$; IL-8: $66 \pm 23\%$ vs $29 \pm 7\%$; IL-6: $90 \pm 35\%$ vs $74 \pm 22\%$) and day 28 (GM-CSF: $153 \pm 48\%$ vs $74 \pm 21\%$; IL-8: $103 \pm 46\%$ vs $33 \pm 12\%$; IL-6: $280 \pm 138\%$ vs $126 \pm 28\%$) respectively (day 0 = 100%).

Conclusion: The ALI culture system provides a well-differentiated epithelium from human nasal mucosa and nasal polyp. These two *in vitro* models may be used to study a variety of inflammatory mechanisms and their regulation by proinflammatory agents and antiinflammatory drugs.

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Oral Abstract Session 19

Allergy: risk factors and prevention

109

Onset and persistence of respiratory/allergic symptoms in pre-school children: new phenotypes from the PARIS birth cohort

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Background: The natural course of childhood asthma and allergy is complex and not fully understood. The aim of this study was to identify phenotypes based upon the time course of respiratory/allergic symptoms throughout pre-school years.
Method: As part of the PARIS cohort, symptoms of wheezing, dry night cough, rhinitis and dermatitis were collected annually from birth to age 4 years. K-means clustering was used to group into phenotypes children sharing similar trajectories of symptoms over time. Associations of phenotypes with IgE sensitisation, allergic morbidity and risk factors were studied using chi-squared tests and multinomial regression.
Results: A group considered as reference consisting of children with low prevalence of symptoms (n = 1236 [49.0%]) and four respiratory/allergic phenotypes were identified. Transient rhinitis phenotype (n = 295 [11.7%]) showed no relation with allergic morbidity or IgE sensitisation, and was only associated with smoking exposure, which could irritate the airways. Transient wheeze phenotype (n = 399 [15.8%]) appeared as an infectious profile both in terms of morbidity and risk factors, without relation with IgE sensitisation. Lastly, two phenotypes associated with IgE sensitisation were identified, implicating the respiratory and skin systems respectively. Cough/rhinitis phenotype (n = 284 [11.3%]) included children with symptoms of dry night cough and rhinitis, half of the time associated with wheeze and itchy rash, whereas dermatitis phenotype (n = 308 [12.2%]) comprised children who essentially experienced skin disorders. Risk factors encompassed parental history of allergy, and potential exposure to stress and allergens.
Conclusion: This study provides evidence for the existence of different respiratory/allergic phenotypes before school age, potentially linked to irritation, infection or IgE-mediated sensitisation.

110

Health locus of control of children with asthma and allergic rhinitis

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Background: Locus of control (LOC) is the feature of personality that allows foreseeing human behaviour in social situations. In the area of medical rehabilitation a construct of LOC has been worked out Health Locus of Control (HLOC, HLC) and it is characterised by three dimensions: internal placement of the locus of control, external one- that depends on significant people, and external one that depends on favourable conditions or luck. HLOC is a variable that conditions the effectiveness of the treatment process in children. The aim of the research was to define the health locus of control for children with asthma and allergic rhinitis.
Method: The research included 124 children with allergies (40 with asthma, 36 with allergic rhinitis and 48 with coexisting asthma and allergic rhinitis) and 124 healthy ones. They aged 7–14. Methods used: analysis of documents and diagnostic survey together with Health Locus of Control Scales for Children G.S. Parcel, M.D. Meyer adapted by Z. Juczyński (Polish version).
Results: The analysis of the results of the research presents that children with allergies are characterised by more external LOC than healthy children (t = −2.084; P = 0.038), and the kinds of diseases do not differentiate the above variable. The comparison between the groups in the range of separate dimensions HLC presents statistically significant differences in the category Influence of others – it turned out that allergic children in a wider degree than healthy ones (t = 1.945; P = 0.05) think that their health is the result of other people's influence, especially of medical personnel. The external controllability in the aspect of healthy behaviours may be interpreted in a dual way: as a sign of patients' low activity in the process of treatment and as a sign of their trust in medical services. The factor that favours the internal LOC for both ill and healthy children is their older age (r = 0.509; P = 0.00).
Conclusion: Children with asthma and allergic rhinitis can get an internal health locus of control through more personal and interactive way of conducting the therapy and through integrated psycho-pedagogic activities.

111

Current rhinitis as a predictor of asthma severity in school-age children: population-based cohort study

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Background: There is a paucity of data on the effect that rhinitis has on the severity of asthma in childhood. Within the context of an unselected birth cohort study (Manchester Asthma and Allergy Study), we investigated the association between current rhinitis and asthma severity at age 8 years.
Methods: Children were followed prospectively from birth to age 8 years. A validated questionnaire was interviewer-administered to collect information on parentally-reported symptoms and medication use (n = 906). Current rhinitis (CR) was defined as sneezing, runny nose, or blocked nose in the absence of cold or flu within the last 12 months, and current wheeze as presence of wheezing or whistling in the chest in the last 12 months. Lung function was assessed by plethysmography (specific airway resistance-sRaw, n = 784) and spirometry (FEV_1, n = 693).
Results: The prevalence of CR was 28.7%, and of current wheeze 18%; the prevalence of current wheeze in those with CR was 33.6%. In a multivariate model, having at least one atopic parent (OR [95% CI], 2.16

[1.34–3.49], $P = 0.002$) and current wheeze (3.76 [2.58–5.47], $P < 0.001$) remained significant and independent associates of CR. Amongst children with current wheeze ($n = 162$), those with CR ($n = 87$) were significantly more likely to have frequent attacks of wheeze (four or more in the previous 12 months) compared to 75 children without CR (35/87 [40.2%] vs 16/75 [21.3%], $P = 0.01$, CR vs no CR respectively). In addition, children with CR were significantly more likely to visit their family doctor because of asthma (10/87 [11.5%] vs 1/74 [1.4%], $P = 0.01$). In the multivariate logistic regression analysis adjusted for the use of asthma medication, having CR remained a significant and independent risk factor for frequent attacks of wheezing (OR 3.74, 95% CI 1.64–8.50, $P = 0.002$). However, there was no difference in sRaw ($P = 0.107$) or FEV_1 ($P = 0.09$) between children with and without CR after adjustment for the presence of wheeze.

Conclusion: Amongst school-age children with wheezing, current rhinitis is a risk factor for more frequent/severe lower respiratory symptoms. However, in this age group, there is no association between the presence of rhinitis and reduced lung function.

112

Relationship between serum resistin and nasal obstruction as measured by active anterior rhinomanometry in children with persistent allergic rhinitis (PAR)

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Background: Nasal obstruction has been reported as a 'key symptom' of allergic rhinitis (AR) since it is deeply related with impaired quality of life and it reflects more directly the allergic inflammation in the nasal mucosa. This symptom can be objectified by active anterior rhinomanometry. Resistin is an adipokine which is known to be involved in inflammatory processes, exerting an important role in the regulation of cytokine production, even though its effective proinflammatory activity at nasal level has never been fully demonstrated.

Objective: To investigate the relationship between serum resistin levels and nasal obstruction assessed by an objective method such as active anterior rhinomanometry.

Methods: The study was performed at the Immunology and Allergology service of the Pediatric Department. Fifty-three children aged between 4 and 10 years of age affected by Persistent Allergic Rhinitis (PAR) and sensitised to house dust mites were enrolled. According to ARIA guidelines, patients were subdivided in two groups: mild PAR (22 children, 41.5%) and moderate-severe PAR (31, 58.5%). Serum resistin levels were detected in all children. The same day patients underwent active anterior rhinomanometry which was considered negative (no nasal obstruction) when the fraction of predicted values (p.v.) was ranged between 71% and 100% and positive when the fraction of p.v. was <70%.

Results: The serum resistin levels were significantly higher in children with moderate-severe PAR than in patients with mild PAR (5.2 vs 3.9 ng/ml, $P < 0.03$). Furthermore serum resistin levels were significantly higher in children with positive rhinomanometry compared to negatives (5.450 vs 3.6 ng/ml, $P < 0.03$). The fraction of predicted values of nasal flows in patients with nasal obstruction had a significant negative correlation with serum resistin levels ($r = -0.75$; $P < 0.001$).

Conclusion: These findings provide evidence that resistin levels are strongly related to nasal obstruction severity and clinical symptoms. Serum resistin is increased in children with severe nasal obstruction measured by an objective and quantitative approach. Further studies are needed to assess the role of resistin as a marker of the systemic inflammation underlying both the allergic trigger and nasal obstruction.

113

Eosinophil/basophil (Eo/B) progenitors in cord blood (CB) predict respiratory outcomes in early infancy

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Background: It has been shown that in infants at high risk of atopy cord blood hematopoietic progenitor cells predict the development of acute respiratory illnesses during the first 12 months. The aim of the present study was to investigate the predictive value of CB Eo/B progenitors regarding respiratory outcomes independently from the atopy risk. Furthermore, we analysed whether the Th1/Th2 balance at birth is of relevance for CB Eo/B progenitor cell recruitment.

Methods: In a sub cohort of 40 children of the LINA study (Lifestyle and environmental factors and their Influence on Newborns Allergy risk) frozen cord blood PBMCs were used for methylcellulose assays to assess Eo/B differentiation by colony formation (CFU) in the presence of IL-3, IL-5 or GM-CSF. Standardised questionnaires were recorded during 34th week of pregnancy and annually thereafter till the age of 2. *Ex vivo* stimulated CB cytokines were measured using the cytometric bead array (CBA).

Results: Enhanced CB IL-5- (but not IL-3- or GM-CSF-) responsive Eo/B CFU numbers predicted the occurrence of bronchitis and wheezing within the first 12 or 24 months ($P < 0.05$). For the CB Th2 cytokines IL-4 and IL-13 a positive correlation was seen with the number of IL-5 responsive Eo/B CFUs ($P < 0.05$).

Conclusions: Our data confirm the hypotheses that a modified Th2 milieu at birth may contribute to the recruitment and differentiation of Eo/B progenitors. We could further show that the predictive value of CB Eo/B progenitors in terms of respiratory illnesses is not restricted to high-risk children.

114

Prevalence and risk factors associated with allergic sensitisation, rhinitis and eczema among children: comparison of two birth cohorts from different cities in Brazil

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Background: We have evaluated the prevalence and risk factors for allergic sensitisation (AS), allergic rhinitis (AR) and allergic eczema (AE) among school children born and living in two Brazilian socio-economically different-cities: Ribeirão Preto (RP, southeast) and São Luís (SL, northeast).

Method: Children from the RP birth cohort were born in 1994 and participated in the present study at 10–11 years. Children from SL were born in 1997 and included at 7–8 years. Rhinitis and eczema were identified by information collected using the ISAAC questionnaire, applied to caregivers. AS was assessed by skin testing to inhalant allergens. Risk factors including sex, weight for gestational age, breastfeeding duration, family history of allergic diseases, maternal years of education, maternal smoking, presence of cat/dog at

home early in life, day care attendance, presence of another child in the room and hospitalisation for infection before 5 years of age were evaluated by logistic regression analysis and adjusted Odds ratios (OR) and 95% Confidence Intervals (CI) were calculated.

Results: Prevalence rates of AS, AR and AE in RP/SL were, respectively, 44.7/21.5% ($P < 0.01$), 29.4/9.5% ($P < 0.001$), and 10.1/7.9%. Children who were large for gestational age had higher risk for AE in both cities, with adjusted OR (CI) 3.3 (1.2–9.0; $P = 0.02$) and 2.8 (1.3–5.8; $P = 0.007$) in RP and SL, respectively. In RP, girls had lower risk of developing AS [OR = 0.7 (0.5–0.9, $P = 0.009$) and AR [OR = 0.6 (0.4–0.9, $P = 0.006$)]. RP children breastfed for 30 days or less showed a higher risk of AS [OR = 1.5 (1.0–2.1, $P = 0.038$)] and those who were never breastfed had a higher risk of developing AR [OR 1.9 (1.2–3.0, $P = 0.004$)]. In SL, hospitalisation for infection early in life was independently associated with AS, AR and AE [OR = 1.6 (1.4–2.2, $P = 0.006$); OR = 1.9 (1.1–3.2, $P = 0.014$) and OR = 1.9 (1.0–3.4, $P = 0.037$)], respectively. Presence of dogs at home in the first year of life was associated with AS [OR 1.5 (1.1–2.1, $P = 0.017$)] and AR [OR = 2.1 (1.2–3.5, $P = 0.008$)], and family history of allergic diseases was strongly associated with AR [OR = 2.9 (1.4–5.7, $P = 0.003$) in SL. Low maternal education (<9 years) was associated with protection [OR = 0.2 (0.1–0.9, $P = 0.03$) and children who did not go to day care centres were at higher risk [OR = 2.1 (1.0–4.2, $P = 0.037$)] for developing AE in SL.

Conclusion: Differences in socio-economic characteristics can have different impact on the association of risk factors and allergic diseases among Brazilian children.

Oral Abstract Session 20

Infection-allergy interactions: clinical implications

115

Exposure to hookworm prenatally and to worm infections in early childhood is inversely associated with eczema in childhood: results from a birth cohort in Uganda

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Background: The cause of the global increase in allergic conditions is not yet fully known but may be related to the decrease in infectious diseases. There is growing evidence that worms might be protective against allergy; it is not yet known whether this effect might be established prenatally or in early childhood.
Objectives: To investigate whether exposure to worms prenatally and in early childhood is associated with a reduced incidence of eczema in early childhood.
Methods: This birth cohort was part of a trial on anthelminthic treatment of worms during pregnancy that recruited pregnant women from Entebbe Hospital in Uganda. The children were followed up for 5 years, prospectively recording eczema. Worm status for the mothers was established at enrolment but for the children at each of the five annual visits. Associations between maternal or childhood worms and eczema were investigated using Cox regression.
Results: The study enrolled 2507 pregnant women. Hookworm was the most prevalent maternal worm at 45%. The prevalence of *T. trichiura* and hookworm, among the children, for the first 5 years was 21% and 6%, respectively. The incidence of eczema in the first 5 years was 4.68/100 pyrs. Maternal hookworm was inversely associated with eczema [aHR (95% CI), *P*-value; 0.71 (0.51–0.99), 0.04] and this effect increased with intensity of hookworm infection. Early childhood worms were also inversely associated with eczema; *T. trichiura* [0.35 (0.18–0.67), 0.002], hookworm [0.33 (0.11–1.02), 0.05]. Results on effect of treatment with albendazole and praziquantel during pregnancy on the incidence of childhood eczema have been presented at previous EAACI meetings.
Conclusions: This is the first study to demonstrate that exposure to hookworm prenatally and to *T. trichiura* and hookworm in early childhood may be protective against childhood eczema. This may be important for the primary prevention of eczema and possibly other allergic conditions, but first we need to understand the underlying mechanisms.

116

Inhibition of the airway epithelial antiviral response by pollen-derived non-allergenic substances

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Background: Asthmatics and allergic rhinitis patients are more susceptible to rhinovirus infections and exacerbations than healthy individuals. Aim of the study is to elucidate whether pollen exposure itself can compromise the innate antiviral response of nasal epithelium.
Method: Primary human nasal epithelial cells were stimulated with the viral mimic Poly IC in the absence or presence of low-molecular weight aqueous birch pollen extracts (APE). After 24 and 48 h, supernatants were sampled and analysed for chemokines and for type I (IFN-alpha, -beta) and type III (IFN-lambda 1 and −2) interferons. Additionally, supernatants were tested for their capacity to attract neutrophils and monocytes in transwell migration assays.
Results: Poly IC induced high levels of pro-inflammatory chemokines and type I and type III interferons. APE potently and dose-dependently inhibited this antiviral response. Poly IC-induced production of IL-8 was also inhibited. Concordantly, supernatants of Poly IC/APE-stimulated cells were less potent inducers of neutrophil chemotaxis compared to supernatants of Poly IC-stimulated cells. In contrast, monocyte chemotactic factors remained unchanged by APE (CCL3, CCL4) or were even induced (CCL2).
Conclusion: Low molecular weight compounds released from pollen inhibit antiviral type I and -III interferons and neutrophil chemotaxis. This might indicate a compromised response against respiratory viruses, e.g. rhinovirus, during episodes of pollen exposure.

117

Human rhinovirus 1B infection of human bronchial epithelial cells induces mature microRNA expression

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Background: Micro-RNAs (miRNAs) are a class of small non-coding RNA molecules that function though post transcriptional regulation of gene expression by a process termed RNA interference (RNAi). RNAi-mediated targeting of viral RNAs is recognised as an antiviral defense mechanism. The epigenetic effect of miRNAs can either be direct, by interfering with virus genome, or indirect, through downregulation of type I IFN genes. The aim of this study is to identify HRV-A1B spesific miRNAs in human broncheal cell line.
Method: *In silico* prediction of potential HRV-A1B specific human mature miRNAs was performed using two different prediction tools, namely miRBase and RNAhybrid. Human bronchial epithelial cells (BEAS-2B) were infected with HRV-A1B at a multiplicity of infection of one along with UV inactivated HRV1B (1 MOI) and zymosan, a TLR4 stimulator (100 μg/ml). RNA was isolated at different time points and the kinetics of eight miRNAs were evaluated. The expression of miRNAs was measured by miRNA specific RT-QPCR. The results were calculated according to the $2^{-\Delta\Delta C_T}$ method (FI). Statistical analysis was performed using Student's *t* test.
Results: Sixty two miRNAs were predicted to be able to bind to the HRV-A1B genome sequence. Eight miRNAs were selected according to their binding properties. We found replication dependent HRV-A1B specific induction in hs-miR-a (50 FI) and miR-b (24 FI) at 7 h after

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HRV1B infection and in hs-miR-c (4 FI) at 6 h after infection.

Conclusion: To our knowledge, this is the first study to demostrate replication dependent induction of HRV-A1B specific human miRNAs in human broncheal epithelial cell line. The expression levels of hs-miR-a, hs-miR-b and hs-miR-c were viral replication-dependent. Further experiments are needed in order to define the potential antiviral activity of the above miRNAs.

118
The role of M1 and M2 macrophages during rhinovirus-induced asthma exacerbation

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Background: Asthma is a heterogeneous inflammatory disorder of the airways characterised by chronic airway inflammation and airway hyperactivity and by symptoms of recurrent wheezing, cough and shortness of breath. The great majority of acute asthma exacerbations are associated with respiratory viral infections and, of viruses implicated, approximately 60% are human rhinoviruses. Macrophages (Mϕ) can be un-polarised (M0), or M1/M2 polarised by Th1/2 signals respectively, M2 Mϕ are believed to be important in asthma pathogenesis, and M1 in anti-infective immunity, however human data is insufficient. In this study we investigated characteristics of human Mϕ phenotypes *in vitro* and their role in rhinovirus-induced asthma exacerbation *in vivo*.

Method: A major 16 and a minor 1B serotypes of HRV were obtained from the Medical Research Council Common Cold Unit. Peripheral blood mononuclear cells (PBMCs) were isolated from the Component Donation Leucocyte cone of healthy donors[14] by Ficoll-Hypaque density gradient centrifugation. HRV16 experimental infections were induced in HRV16 seronegative moderate and mild atopic asthmatic and normal nonatopic age-matched subjects. PBMC and BAL cells from clinical samples and *in vitro* polarised MDM were used for flow cytometry analysis.

Results: We observed *in vitro*: (i) M1 but not M2 and M0 Mϕ are potent producers of type I and III IFNs and are more resistant to rhinovirus infection; (ii) M2 Mϕ produced higher levels of Th2-related chemokines (CCL22/MDC and CCL17/TARC) constitutively and of rhinovirus-induced IL-10; (iii) M1 Mϕ had up-regulated CD14, CD80, CD197 and CD54 surface markers.

Conclusion: In this study, we demonstrated that M0 and M2 Mϕ have increased susceptibility to HRV replication compared to M1 Mϕ. This is due to dramatic decreased ability of these subsets of Mϕ to produce type I and III IFN in response to viral infection. In contrast, M1 Mϕ were characterised by replication-, dose- dependent and receptor-independent up-regulation of IFN I and III gene expression and protein release. *In vivo* blood monocyte CD14 and CD80 and alveolar macrophage CD80 surface expression were significantly down-regulated during rhinovirus infection in asthmatic subjects and macrophage CD80 down-regulation was related to asthma severity and rhinovirus induced exacerbation severity.

119
Synthetic siRNAs decrease allergic inflammation, airway hyperresponsiveness and respiratory syncytial virusload in a murine model of respiratory syncytial virus-exacerbated allergic asthma

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Background: Respiratory syncytial virus (RSV) infection enhances allergic inflammation and airway hyper-reactivity (AHR) in patients with bronchial asthma (BA). High levels of IL-4 correlate with RSV-induced BA exacerbation frequency. There are no effective treatments of RSV-induced BA exacerbations. Drugs based on monoclonal antibodies against RSV or IL-4 are limited in applying because of high cost. RNA interference phenomenon allows to effectively silence gene expression of viruses and host genes. We designed two small interfering RNAs (siRNAs) against IL-4 (siIL4) and phosphoprotein P of RSV (siP) and tested them in a murine model of RSV-exacerbated BA.

Method: Female BALB/c mice were sensitised with three i.p. injections of 1 mg/kg ovalbumin (OVA) emulsified in 100 mg/kg of aluminum hydroxide on days 0, 14, 21 and then challenged with 10 mg/ml OVA by thee intranasal administrations (IA) on days 35, 36, 37. Mice were intranasaly (i.n.) treated with 1×10^7 $TCID_{50}$/mouse 24 h before first IA. siIL4 was i.n. delivered 48 and 5 h before first IA and 5 h before second IA in total dose of 5 mg/kg. siP was i.n. delivered 3 h before RSV treatment in dose of 3.5 mg/kg. Mice received the same doses of non-specific siRNAs were used as a negative control. AHR was assessed by pneumography 24 h after IA. Histological examination of lungs and cell composition of bronchoalveolar lavage (BAL) were evaluated by microscopy 48 h after IA. IL-4 gene expression and RSV load in lungs were measured by RT-PCR.

Results: Eosinophil infiltration in BAL of mice treated with siIL4 and siP was four times lower than that of control group that demonstrate reduction of allergic inflammation. Delivered siIL4 and siP showed twofold reduction of AHR compared to mice received non-specific siRNA. Histological alternations in lung tissue, presence of peribronchial infiltrates and eosinophil infiltration were notably decreased. Scores obtained from histological analysis of mice received siIL4 and siP were 1.8 times lower than that in negative control. mRNA expression of IL-4 detected by RT-PCR was threefold lower in mice received specific siRNAs. RSV genome copy number in lung tissue was 5.7-fold reduced as well.

Conclusion: We designed two siRNAs which effectively decreased of allergic inflammation, AHR and RSV load in a murine model of RSV-exacerbated BA that may be a promising approach in the treatment of RSV-induced BA exacerbations.

120
Altered immune proteome of *Staphylococcus aureus* under iron-restricted growth conditions

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Background: *Staphylococcus aureus* (*S. aureus*) is a Janus-faced microorganism: On the one hand it is a commensal in one third of the healthy population, on the other it causes a broad spectrum of infections. Additionally, *S. aureus* plays a role in allergic diseases such as atopic dermatitis, nasal polyposis and asthma. To elucidate the mechanism of *S. aureus* and allergy we are interested in the human antibody response to *S. aureus* proteins. In view of the iron deficiency *in vivo* we want to characterise the immune proteome of *S. aureus* under iron-restricted growth conditions.

Method: In this study we compared the extracellular proteome of a protein A deficient *S. aureus* strain (USA300) under iron-restricted and non-restricted *in vitro* growth conditions. For iron restriction 600 μM of the iron chelator bipyridyl were added to the TSB culture medium. After *S. aureus* had entered the stationary

growth phase the extracellular proteins were isolated from the culture supernatant, separated by 2D-PAGE (pH ranges 4–7 and 6–11) and identified by mass spectrometry. The protein identification was performed by mass spectrometry. For the analysis of the corresponding immune proteome the separated *S. aureus* proteins were transferred to PVDF-membranes, incubated with human serum and the binding of *S. aureus* specific antibodies was visualised (2D immunoblots).

Under iron-restriction the proteome of USA300 contained additional protein spots in the 2D gels, compared to the TSB control, and the expression of some proteins decreased. Antibody binding to the *S. aureus* proteome was much stronger, when the bacteria had grown under iron restriction: the number of spots and the spot intensities increased on the 2D immunoblots.

Results: Our data show that cultivation of *S. aureus* under iron-restricted conditions induces additional proteins. To some of these proteins antibody binding took place and consequently the proteins can be added to the group of immunogenic *S. aureus* proteins. Since we got more and stronger antibody signals than in TSB alone, we conclude that iron-restriction in TSB with bipyridyl is more closely related to the *in vivo* conditions under which *S. aureus* confronts the immune system.

Conclusion: Thus, iron-restriction during cultivation of *S. aureus* adds to our knowledge about the extracellular *S. aureus* proteome, the human antibody response directed against it, potential vaccine candidates and the *in vivo* situation of *S. aureus* infection or colonization.

Oral Abstract Session 21

The asthma burden: updated epidemiological data

121

Fruit and vegetable intake and its association with asthma in adults across Europe: evidence from the GA²LEN follow-up survey

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Background: Asthma is a chronic inflammatory disease with oxidative stress playing a major role in the expression of symptoms that characterise it. As part of the Global Allergy and Asthma European Network of Excellence (GA²LEN) we assessed dietary intake of an extensive range of fruits and vegetables which are naturally rich in antioxidant and antiinflammatory compounds to investigate its association with asthma in adults across Europe.
Method: Three thousand five hundred and four adults aged 15–77 years were recruited from 18 European centres participating in the GA²LEN Follow-up Survey. Participants answered a general questionnaire, and a common, validated, self-administered Food Frequency Questionnaire (FFQ) which assessed intake of 66 different fruits and vegetables. Asthma was defined as answering yes to ever having asthma, and to having at least one of the following symptoms at any time over the last 12 months:

1 Have you had wheezing or whistling in your chest?
2 Have you woken up with a feeling of tightness in your chest?
3 Have you been woken by an attack of shortness of breath? or
4 Have you been woken by an attack of coughing?

Effect of sub-groups of fruits and vegetables on asthma was investigated using univariate and multivariate logistic regression (adjusting for potential confounders). Regression models were fitted separately for each site, and results for each dietary exposure were pooled across sites using random effects meta-analysis on weighted samples. Heterogeneity was summarised using the I^2 statistic.
Results: Prevalence of asthma varied from 4.6% in Skopje to 40.8% in Southampton. Daily frequency of fruit intake was highest in Poland and Portugal. Daily frequency of total vegetable intake was highest in Poland, Portugal, and in the Nordic centres. The adjusted pooled effect estimate for total intake of fresh vegetables, showed a statistically significant negative association with asthma (adjusted Odds Ratio [OR]; 95% confidence interval [CI]) = 0.59 (0.41–0.86)). No significant association was observed between total fruits (OR (95% CI): 0.91 (0.67–1.24)) or any of the fruit/vegetable sub-groups and asthma.
Conclusion: In the GA²LEN Follow-up Survey, there was evidence supporting the notion that intake of a combination of vegetables may have a protective effect against asthma.

122

Plasma 25-hydroxyvitamin D associated with pulmonary function in Canadian adults with excess adiposity

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Background: Vitamin D deficiency is an important health issue particularly among people residing in northern countries. Both obesity and low levels of 25-hydroxyvitamin D (25(OH)D) have been linked with several health conditions including asthma.
Method: The analysis was based data from 3359 adults aged 18–79 years who participated in the Canadian Health Measures Survey in 2007–2009. Two-stage multiple linear regression analysis was conducted to determine the association between plasma 25(OH)D and pulmonary function and the effect modifications of sex and body mass index (BMI) in adults.
Results: Overall, 26% of the adults had plasma 25(OH)D level of <50 nM, which is considered as deficient (hypovitaminosis D). This deficiency was more prevalent among men in comparison to women (30% vs 23%). Regression analysis showed that deficient plasma 25(OH)D was associated with lower mean residual FVC and FEV_1 after adjustment for covariates. When stratified by sex and BMI, the associations were more marked in overweight and obese men. Vitamin D deficiency associated with pulmonary function was not statistically significant in normal weight men or in all women. Similar results were obtained when plasma 25(OH)D was examined as a continuous variable in the models.
Conclusion: Hypovitaminosis D may be a risk factor for lung dysfunction, especially for overweight and obese men. Further research is necessary to determine the mechanism of the interrelationship among vitamin D, adiposity and pulmonary function.

123

On the factors that contribute to the risk of asthma exacerbations in children and adolescents: the MAPCAH study

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Background: Studies of varying populations indicate that the risk of asthma exacerbations increases with increasing levels of ambient grass pollen. However, it is not known whether this effect is influenced by other factors such as the patient's gender, their sensitivity to other common allergens or the presence of other respiratory conditions such as a human rhinovirus (HRV) infection.
Objective: We estimated the effects of outdoor levels of pollen on asthma hospital admissions in children and adolescents, and whether the estimates differed between children with a respiratory viral infection and/or sensitised to allergens.
Method: We conducted the Melbourne Air Pollen Children and Adolescent Health (MAPCAH) case-crossover study of asthma incident hospital admissions in 644 children and adolescents in Melbourne, Australia between 2009 and 2011. All participants underwent skin prick tests for sensitivity to food and various pollen types shortly after admission for asthma. Nasal and throat swabs (NTS) were also

obtained to test for respiratory viral infections. We used regression for binary outcomes with logit transformation, possible correlation structure and controlling for the concentrations of pollutants. The primary exposure variable was daily concentrations of grass pollen (obtained using a Burkard spore trap) during the study period. Interaction terms were included in the regression models if there was evidence of effect modification from gender, presence of HRV and sensitisation.

Results: In adjusted models, exposure to grass pollen was associated with increased likelihood of admission with the strongest association in boys (Odds Ratio or OR = 1.08 per 20 grains/m^3, 95% CI 1.02–1.13). Among girls, grass pollen was only associated with increased likelihood of an admission in those sensitised to food (OR = 1.17, 95% CI 1.01–1.49). Overall, non-linear effects of grass pollen were observed up to 50 grains/m^3 ($P = 0.02$) in a semi-parametric regression model. There was little evidence of confounding or effect-modification due to HRV infection or sensitivity to pollen.

Conclusion: Boys exposed to high concentrations of grass pollen are at risk of an asthma exacerbation requiring hospitalisation, independent of HRV and allergen sensitisation. Further studies are required of causal mechanisms of grass pollen exposure in early life pathways to asthma.

124

Factors influencing the agreement between parental-reported usage and dispensed asthma drugs for adolescents – findings from the BAMSE-study

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Background: Asthma is one of the most common diagnoses in children with a prevalence around 10%. The knowledge about drug use among children is limited with uncertainties about the validity depending on data sources used.

Objectives: The aim of this study was to investigate how well parental reported use of asthma drugs in a questionnaire corresponded with register data on dispensed drugs. A secondary aim was to identify patient related factors that were associated with poor agreement.

Method: Data on parental reported use of asthma drugs from 3316 children (12–14 years old) in a population-based birth cohort in Stockholm, Sweden (the BAMSE-cohort) were compared with data on dispensed drugs the preceding 18 months from the Swedish prescribed drug register. The questionnaires were answered between the 3rd of April and the 7th of December 2008 and contained information on drug use during the preceding 12 months. Using parental-reported data as reference, sensitivity and specificity with 95% confidence intervals were computed for different antiasthmatic drugs, overall and for different patient related characteristics. Asthma was defined as fulfilling at least two of the following criteria: symptoms of wheeze in the last 12 months, have a doctor's diagnosis of asthma (ever) or using antiasthmatic drugs occasionally or regularly the last 12 months.

Results: The prevalence of asthma in the study population was 11%, and 91% of these children used asthma drugs the past 12 months according to the questionnaire. The sensitivity of the drug register was 0.76 (95% CI 0.71–0.81) and the specificity was 0.97 (CI 0.83–1.0). The sensitivity for boys was 0.82 (CI 0.76–0.87) and for girl 0.68 (CI 0.59–0.76). Including only children with current asthma the sensitivity increased to 0.79 (CI 0.72–0.85). Looking at children with at least 12 episodes of wheeze the last 12 months the sensitivity increased even more (0.86 CI 0.71–0.95). Factors associated with a decreased sensitivity were children with more than one home and children with siblings using asthma drugs (0.68 CI 0.57–0.78 and 0.69 CI 0.52–0.84 respectively).

Conclusion: One of four children with parental reported use of asthma drugs has not purchased antiasthmatic drugs within the preceding 18 months. The major determinants of poor agreement were female sex, living in more than one home and having siblings using antiasthmatic drugs.

125

Perinatal exposure to microbial agents in house dust in relation to respiratory health symptoms in children during the first 10 years. Results from the HITEA project

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Background: Exposure to microbial agents at home was observed to have protective effects on asthma and allergy symptoms mostly in cross-sectional studies among children from farming and rural surroundings.

Method: We aimed to assess whether the effects of measured early life microbial exposure (endotoxin, EPS and β(1-3)-glucans) could be also observed prospectively in children from urban areas in a longer time period up to 10 years of age. In subsets of three prospective German, Dutch, and Spanish birth cohorts from a predominantly urban environment (LISAplus, PIAMA and INMA), endotoxin, ß(1-3)-glucans, and Extracellular Polysaccharides (EPS) were measured in living room dust collected at 2–3 months of age. Respiratory symptoms and asthma during childhood were periodically reported by the parents through questionnaires up to 10 years of age. Logistic regression estimates were combined using random-effects meta-analyses.

Results: The total study population comprised 1429 children from three European countries. In preliminary analyses, early exposure to endotoxin, ß(1-3)-glucans, and EPS were not statistically significant associated with ever asthma between 1 and 10 years aOR: 0.94 (95% CI, 0.63–1.42, aOR: 0.96 (95% CI, 0.70–1.33), and aOR: 1.11 (95% CI, 0.80–1.54), respectively. We further observed no statistically significant

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association with wheeze symptoms and sensitisation to aero-allergens at any ages.

Conclusion: Our results are not consistent with the idea of life-long priming of the immune system by early life exposure to microbial agents in children from urban areas. The settled dust components from farm children and children from urban areas might significantly differ in concentration and composition.

126

Polymorphisms in region 20p13-p12 and their interaction with environmental tobacco smoke exposure in relation to asthma severity

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Background: *ADAM33* is the first asthma gene identified by positional cloning. Since the first report, there have been a number of replications studies with diverse results. Possible explanations for the heterogeneity between the studies include the effects of other genes in the vicinity, gene-gene and gene-environment interactions. We aimed to investigate the association between genes in the 20p13-p12 region (*ADAM33* and flanking genes *ATRN*, *GFRA4*, *SIGLEC1* and *HSPA12B*) and markers of asthma severity (lung function and severe exacerbations) amongst schoolchildren with asthma. The effect of early-life environmental tobacco smoke (ETS) exposure on asthma severity in the context of genetic variants was also evaluated.

Method: Four hundred and twenty-three children with asthma aged 5–18 years were recruited into the study if the following criteria were met: (i) physician-diagnosed asthma, (ii) asthma symptoms within the previous 12 months, and (iii) use of anti-asthma medication. We measured lung function (FEF_{50}) using spirometry and extracted data on hospitalisation for severe asthma exacerbation from medical records. Early-life environmental tobacco smoke (ETS) exposure was assessed by questionnaire. We genotyped 124 single nucleotide polymorphisms (SNPs) from five genes in the 20p13-p12 region.

Results: Twenty-nine SNPs were significantly associated with FEF_{50}, of which 12 (six *ARTN*, one *ADAM33*, four *SIGLEC1* and one *HSPA12B)* remained significant after false discovery rate (FDR) correction. Two SNPs (one in *ADAM33* and one in *SIGLEC1*) were associated with severe exacerbations. We observed a significant interaction between nine SNPs and early-life ETS exposure in relation to FEF_{50}, and one SNP in relation to severe exacerbations. For example, for rs512625 in *ADAM33* there was significant interaction with ETS exposure in relation to severe exacerbations ($P_{int} = 0.02$) and FEF_{50} ($P_{int} = 0.03$); G-allele homozygotes had higher risk of being hospitalised [aOR 9.15, 95% CI 2.28–36.89] and had significantly poorer lung function if exposed to ETS, with no effect of ETS exposure amongst A-allele carriers.

Conclusion: Genetic variants in 20p13-p12 region have impact on severity of disease among children with asthma and interact with early life ETS exposure in modifying asthma severity.

Oral Abstract Session 22

The diversity of cutaneous allergy

127

Hereditary angioedema due to C1 inhibitor deficiency: clinical descriptive study in an international cohort

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Background: Hereditary angioedema due to C1 inhibitor deficiency (HAE-C1-INH) is a rare disease with a high impact on health related quality of life (HRQoL). An international multicentre study for the development of a specific HRQoL questionnaire for HAE-C1-INH was carried out. We report the demographic and clinical data of the participating sample.

Material and methods: A self-administered clinical questionnaire was completed by HAE-C1-INH patients in 2008–2010. Data entered on symptoms and treatment refered to the prior 6 months. The results were analysed with statistical software SPSS v 9.0.

Results: Two hundred and ninety patients (200 female, 232 type I) recruited from 11 countries (Argentine, Austria, Brazil, Canada, Denmark, Germany, Hungary, Israel, Poland, Romania, Spain). Mean age was 41.5 years (18–84 years).

Mean age at onset of symptoms was 11.8 years and at diagnosis 26.4 years. Mean delay in diagnosis was 14.4 years. Eleven patients (3.8%) were diagnosed before onset of symptoms. Eight patients (2.8%) were asymptomatic.

Intubation/tracheotomy had ever been needed in 34 patients (once 67.9%; more than once 31.9%).

Attack triggers along life were identified by 89% of patients (stress, traumatism, infections, menstruation, other).

Patients reported at least 2591 episodes in the last 6 months: peripheral (39.5%), abdominal (35.4%), facial (6.5%), upper airway (5.4%), genital (9.2%) and other (3.8%). Treatment with plasma derived C1INH (pdC1INH) was administered in 911 episodes and fresh frozen plasma (FFP) in 45.

One hundred and thirty-nine patients were on long term prophylaxis: 74.1% attenuated androgens (AA), 14.4% antifibrinolytics (AF) and 11.5% pdC1INH. Mean accumulated doses per week were: danazol 1100.2 mg, oxandrolone 43.7 mg, stanozolol 9.6 mg, epsilonaminocaproic acid 21 000 mg, tranexamic acid 11 503 mg, pdC1INH 1653 IU.

Short term prophylaxis was performed 59 times for dental work, diagnostic procedures, surgery or others with pdC1INH (41), FFP (1), AA (18) and AF (11).

Side effects were reported by 1.4% of patients for pdC1INH, 2.1% for AF, 14.1% for AA and 1.7% for other treatments.

A total of 105 and 42 days had been missed from work and school respectively due to angioedema attacks in the last 6 months.

Conclusion: This study represents a large cohort of HAE-C1-INH, reflecting demographical and clinical characteristics of patients from 11 countries worldwide.

128

Assessment of the delay in diagnosis in patients with hereditary angioedema with C1 inhibitor deficiency: findings from an international registry

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Background: The clinical features of hereditary angioedema (HAE) due to C1 inhibitor (C1-INH) deficiency include recurrent episodes of oedema most commonly affecting skin, the gastrointestinal tract, and upper airways. In HAE type I, antigenic and functional C1-INH levels are reduced, while in type II only functional levels are reduced. Because of its rarity, the fact that its symptoms overlap with those of other forms of angioedema, and a lack of knowledge of HAE among physicians, the disease is frequently misdiagnosed. The objective of this analysis was to assess the delay in diagnosis in patients with type I or II HAE enrolled in the Icatibant Outcome Survey (IOS; NCT01034969) registry, an international, prospective, observational study of HAE patients.

Method: Data used in this analysis were collected between July 2009 and October 2012 from patients with laboratory-confirmed HAE type I or II. Demographic and diagnostic information was collected from each patient, including the presence of a family history of the disease. Patients who were diagnosed prior to their first attack because of a family history of HAE were excluded from this analysis.

Results: Data from 188 patients with HAE type I and II (female: $n = 115$; HAE type I: $n = 173$; family history: $n = 130$) were analyzed. Nine patients diagnosed prior to their first attack were excluded. Patients from Germany ($n = 46$), Spain ($n = 46$), Italy ($n = 32$), France ($n = 23$), Denmark ($n = 19$), the UK ($n = 17$), Israel ($n = 4$) and Sweden ($n = 1$) were included. The median (range) delay in diagnosis in HAE type I or II patients ($n = 169$) was 9.0 years (0.0–62.0). Diagnosis was delayed in both HAE type I ($n = 154$; 8.5 [0–62] years) and II ($n = 15$; 20.0 [0–42] years; $P = 0.102$). Patients with HAE type I ($n = 173$) were diagnosed at an earlier age compared with those with HAE type II (median: 21.9 vs 31.8 years; $P = 0.049$). When patients with a family history of HAE ($n = 115$) were compared to those without a family history ($n = 34$), there was no significant difference in the delay in diagnosis (9.0 years [0–57.0] vs 6.5 years [0–62.0]; $P = 0.598$).

Conclusion: Patients with HAE type I and II, even those with a family history, may face a significant delay from time of first symptoms to diagnosis. To decrease this delay, physician awareness of HAE needs

to be increased and symptomatic patients should be tested for C1-INH function and levels. In addition, family members of newly diagnosed patients, including those who are asymptomatic, should be offered screening for HAE.

129
Early treatment with icatibant significantly reduces duration and time to resolution of hereditary angioedema attacks

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Background: Early treatment of attacks is recommended by The Hereditary Angioedema (HAE) International Working Group (HAWK) consensus and the World Allergy Organization (WAO) Guideline for the Management of HAE. We investigated the impact of early treatment with icatibant on attacks using data collected in the Icatibant Outcome Survey (IOS; sponsored by Shire HGT) registry.
Method: The IOS registry (NCT01034969) is an international, prospective, observational study that includes adult patients at least 18 years of age with a confirmed diagnosis of HAE type I or II. Attacks were treated with subcutaneous icatibant (30 mg) administered by a healthcare professional or self-administered. This analysis was based on data collected between July 2009 and October 2012. Analyses of attack duration (time between attack onset and complete resolution of symptoms) and time to resolution (time between first injection of icatibant and complete resolution of symptoms) were performed. Five hundred eighty-two attacks in 160 patients with type I or II HAE were evaluated.
Results: The majority (59.2%) of self-treated attacks were treated within 2 h, compared with 45% of healthcare professional-treated attacks ($P = 0.029$). Mean attack duration was significantly shorter for attacks treated <1 h after onset compared with those treated ≥1 h after onset (6.7 vs 17.1 h; $P < 0.001$). Similar results were observed for mean time to attack resolution (6.3 vs 9.2 h; $P = 0.03$). In addition, reductions in mean attack duration were observed for attacks treated <2 vs ≥2 h after onset (7.7 vs 20.0 h; $P < 0.001$) and <5 vs ≥5 h after onset (8.5 vs 24.6 h; $P < 0.001$).
Conclusion: These observational data are the first to provide evidence that, in real-world use, early treatment with icatibant significantly improves HAE attack outcomes by shortening time to attack resolution and attack duration. Patients with HAE who self-administered treatment were likely to treat HAE attacks earlier than those who were treated by a healthcare professional.

130
Effect of hydroxychloroquine treatment in the patients with anti-histamine refractory chronic spontaneous urticaria, randomised controlled trail

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Background: To assess the efficacy of hydroxycholoroquine (HCQ), 400 mg daily, for anti-histamine refractory chronic spontaneous urticaria (CSU).
Method: Hydroxycholoroquine vs placebo treatment study was a 3 months randomised controlled trial involving 39 patients with anti-histamine refractory, non-response to fourfold tablets of H1-antihistamine, chronic spontaneous urticaria. Treatment started with hydroxycholoroquine 400 mg/day or placebo tablets for 3 months. The patients continued to receive four tablets of H1 antihistamine. The markers of urticaria control were assessed at baseline and after 12 weeks as followed: Urticaria Symptom Score (USS) and Dermatology Life Quality Index (DLQI).
Results: Thirty four subjects were female and five subjects were male. The mean age was 32.7 ± 11.87 years. Nineteen subjects in the HCQ-treated group and 20 subjects in the placebo group were randomised in the study. No significant differences were found between the two groups in terms of sex ratio, age, duration of CSU, ESR, thyroid autoantibodies positive, ANA positive, and autologous serum skin test positive results. The reduced hive symptoms measure was the mean difference between the average of USS pre- and post-treatment scores. The mean USS decreased in both groups. The difference between the HCQ-treated group (29.47 ± 19.70) and the placebo group (4.90 ± 6.29) was significant (−24.57 ± 4.63, $P < 0.001$). Also, the improvement in quality of life was assessed by difference of mean scores of DLQI pre- and post-treatment. The difference between the HCQ-treated (7.58 ± 6.64) and placebo groups (1.75 ± 4.08) was significant (−5.83 ± 1.77, $P = 0.003$). We found one subject in the HCQ-treated group had a problem with headache at the first week of taking HCQ but she received medication until the end of the study. Four subjects in the HCQ-treated group reported their skin was darker
Conclusion: We have concluded HCQ was a useful short term treatment for patient with CSU who were refractory with fourfold H1-antihistamine regimen. The benefits of HCQ include enhancing quality of life, diminishing urticaria symptoms and safe use.

131
Anti-pruritic and antiinflammatory effects of cholecystokinin in allergic skin disorders

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Background: Cholecystokinin (CCK) is a gastrointestinal peptide hormone, which induces the release of bile and other digestive enzymes to facilitate digestion of fat and protein. It is also known to possess immunomodulatory actions, and predominant antiinflammatory effect has been suggested in pancreatitis and arthritis models. We have previously shown that topical application of CCK8S exerts an anti-pruritic effect in mice, demonstrating the potential for dermatological therapeutic usage. Nevertheless, studies relating to the interaction of this unique peptide with skin are still very limited. We sought to investigate the function of CCK on skin, by primarily focusing on inflammatory skin disorders.
Method: To evaluate the role of CCK in atopic dermatitis (AD), serum CCK8 level of AD patients was evaluated by ELISA. Various factors influence serum CCK concentration, and food intake is known to raise the value by approximately 10-folds. Therefore, we selected patients with serum CCK8 concentration below 100 pg/ml for assessment. In addition, to investigate the role of CCK in skin inflammatory condition, we utilised the contact hypersensitivity model using mice. CCK8S was applied before sensitisation and/or elicitation, and ear thickness was measured for comparison. Additionally, in the *in vitro* assay, cultured human vascular endothelial cells (HuVEC) were prepared, and upon stimulation with TNF-a, marked expression of intercellular adhesion molecule-1 (ICAM-1) was observed using confocal

microscopy. CCK8S was added to this setting, to observe whether CCK8S alter the expression of ICAM-1. At the same time, RT-PCR analysis to detect the expression of ICAM-1 at mRNA level was also conducted.

Result: ELISA of serum from AD patients showed a negative correlation between serum CCK8 level and serum IgE concentration ($R^2 = 0.80$). In the contact hypersensitivity experiment, skin application of CCK8S significantly attenuated the ear swelling in CCK8S applied groups compared to CCK untreated mice ($P < 0.005$). Accordingly, in the *in vitro* experiment, the augmented expression of ICAM-1 on HuVEC was profoundly depressed by the addition of CCK8S, particularly on the cell surface, and the expression of mRNA for ICAM-1 was decreased by the addition of CCK8S.

Conclusion: The present study indicates that CCK is capable of exerting strong anti-inflammatory in the skin, and the association with allergic skin disorders such as AD is addressed.

132
Interleukin 10 secreting T cells regulate contact hypersensitivity

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Background: Contact hypersensitivity (CHS) is the classical murine model of allergic contact dermatitis. While the process of sensitisation and elicitation is well investigated, mechanisms that determine the resolution of the inflammatory response are less well understood. Recent studies suggested a regulatory role of mast-cells (MC) and particulary MC-derived IL-10 (Grimbaldeston et al 2007) in the control of CHS, while others reported MC to be required for optimal CHS responses (Dudeck et al 2011).

Method and results: Based on these controversies we analysed the cellular sources of IL-10 during the resolution phase of CHS in the DNFB model using IL-10 transcriptional reporter mice (Vert-X). Neither at base line nor after allergen challenge MCs of the affected ear skin or the draining lymph nodes displayed IL-10 expression. In contrast, a clear IL-10 signal was observed in the T cell compartment. Induction of IL-10 expression was observed especially in $CD4^+$ $CD25^+$ T cells, with a maximum during the resolution phase (i.e. 120 h after the challenge). In addition, a marked increase of IL-10 positive mainly hapten specific $CD8^+$ cells was detected in the ear skin and to a lesser degree in the regional LN. The functional role of $CD4^+$ T cells in regulating the CHS response was confirmed using MHC class $II^{-/-}$ mice that lack $CD4^+$ T cells and displayed an augmented CHS response. Similarly, selective depletion of $Foxp3^+$ T cells in DEREG mice prior to challenge resulted in an augmented CHS response, suggesting a dominant role of $CD4^+$ $Foxp3^+$ regulatory T cells in attenuating the CHS response to DNFB. Finally, the inhibitory role of T cell derived IL-10 was confirmed in mice with a T cell specific IL-10 deficiency (IL-10 $^{fl/fl}$ CD4-Cre^+). Corresponding to previous reports (Roers et al 2004) these mice displayed an enhanced ear swelling response and a delayed resolution of the CHS response as compared to Cre^- controls.

Conclusion: Our results do not support the assumption that mast cell derived IL-10 is involved in limiting the CHS response. Instead they confirm a crucial role of $CD4^+$ T cells and suggest that also IL-10 producing $CD8^+$ T cells may play an additional regulatory role in the resolution of CHS.

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Oral Abstract Session 23

Improved management of food allergy

133
Hypoallergenic formulas effect gastric emptying: a blinded randomised controlled crossover study

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Background: Cow's milk protein (CMP) allergy affects 1.9–4.9% of UK children. In the UK alone it is thought that over £25 million per annum is spent on the management of CMP allergy which include hypoallergenic formulas. These feeds comprise of casein-based Extensively Hydrolysed Formula (EHF), whey-based EHF and Amino Acid Formulas (AAF). They are nutritionally diverse and are used to manage CMP related esophago-gastric dysmotility. From our clinical observations, these formulas seem to worsen some symptoms ie vomiting, suggesting that the components that make up these formulas may directly influence gut motility, independent of their hypoallergenicity.
Aim: Proof of principle study to evaluate whether hypoallergenic formulas impact on gastric emptying (GE).
Methods: Eight healthy non-allergic adult subjects were included in a double blind randomised trial of four formulas: whole CMP formula with whey/casein ratio 60/40 (Aptamil 1, Milupa), EHF whey (Pepti Junior, Cow and Gate), EHF casein (Nutramigen Lipil 1, Mead Johnson) and an AAF (Neocate LCP, Nutricia). GE was assessed using a non-invasive 13C Octanoic acid breath test. After an overnight fast, volunteers ingested a formula meal (350 ml, ~250 kcal) labelled with 100 mg sodium ^{13}C-octanoate. Consequently, basal breath samples were collected in exetainers. Test lasted in total 6 h with breath samples taken every 5 min (first 2 h) and after that every 10 min (4 h). $^{13}CO_2$ excretion data were analysed by non-linear regression and calculation of gastric half-emptying time ($t_{½}$).
Results: In these eight subjects (3 M, mean age 27.5 years; 21–51 years), median GE ($t_{½}$) was 87 min [IQR 63–133] for whole CMP, 111 min [IQR 75–157] for casein EHF, 152 min [IQR 93–259] for whey EHF and 127 [IQR 74–224] min for AAF. GE of whey EHF was slower compared to resp. whole CMP ($P = 0.01$, Δ 65 min) and casein EHF ($P = 0.03$, Δ 41 min). There was a trend towards slower GE of a whey EHF compared to AAF ($P = 0.08$, Δ 25 min). Finally, a tendency for slower GE was observed in AAF and in casein EHF compared to whole CMP (resp $P = 0.06$, Δ 40 min; $P = 0.09$, Δ 24 min).
Conclusion: This pilot study indicates that GE rates in healthy adults differs substantially for different hypoallergenic formulas. These results indicate that, irrespective of the presence of allergy, these formulas may influence intrinsic GE properties which may contribute to their clinical effects. Further studies are required to elucidate the factors underlying this difference in GE.

134
International study of risk mitigating factors and in-flight allergic reactions to peanut and tree nut

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Background: Three studies have analyzed in-flight peanut/tree nut reactions, although exclusively among Americans.
Objective: To study the international in-flight experience and determine the efficacy of certain risk-mitigation strategies.
Methods: A 47 question on-line survey was distributed through the websites and social media outlets of the member organizations of the Food Allergy & Anaphylaxis Alliance. Both persons reporting an in-flight reaction and non-reactors were surveyed to assess details of air travel preparation and any reported reaction. Data were analyzed to determine the association between flying behaviors, reported reactions, and nationality.
Results: Three hundred and forty-nine reactions were reported among 3273 respondents from 11 countries. 13.3% received epinephrine as treatment. Flight crews were notified regarding 50.1% of reactions. Sxity-nine percent of all respondents reported making a pre-flight accommodation request, though just 55% of reactors did so vs 71.6% of non-reactors ($P < 0.001$). Adjusted odds of epinephrine use were increased with reported gastrointestinal or cardiovascular symptoms, or notifying the crew. Passengers requesting any accommodation, requesting a peanut/tree nut free meal, wiping their tray table, avoiding airline pillows or blankets, requesting a buffer zone, requesting other passengers not consume peanut/tree nut-containing products, or who reported not consuming airline-provided food had significantly lower adjusted odds of reporting a reaction.
Conclusions: In-flight peanut and tree nut reactions occur internationally. Epinephrine was sparsely used to treat reactions. We identified eight risk-mitigating behaviors associated with lower odds of a reported reaction. Future study is necessary to further validate the effectiveness of these passenger-initiated risk-mitigating behaviors.

135
Omalizumab as adjuvant treatment in oral induction of tolerance to cow's milk

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Background: Allergy to cow milk proteins affects 2–6% of children. Around half of children overcome their allergy to cow′s milk at the age of 1 year and 80–90% at 5 years of age however some remain allergic. Therapy with anti-IgE antibodies has been tried to accelerate tolerance with good results however up to this date there are no clear guidelines as to how it should be administered neither is there sufficient data on long term outcomes. We hope to describe our experience with oral induction of tolerance to cow′s milk focusing on patients who received Omalizumab.
Methods: We included children aged 5 years or older, diagnosed with allergy to cow′s milk, enrolled in our oral induction of tolerance protocol between October

2010 and December 2011. Levels of total IgE and IgE, IgG, IgG4 to casein, α-lactoalbumin, β-lactoglobulin and to cow's milk were measured prior to enrollment and after completing the protocol. Before starting the protocol a controlled oral challenge was performed except in patients who had a positive accidental exposure or anaphylaxis in the past 2 months. Only patients with a positive challenge were included in the protocol. We started with the administration of a total of 1 ml on the first day, raising the doses each week until reaching 200 ml, maintaining a daily dose. Symptoms were treated with antihistamines and patients that did not respond sufficiently to antihistamines therefore making it difficult to proceed with the protocol were treated with Omalizumab. Omalizumab was withdrawn 6 months after reaching its maximum doses.

Results: Forty patients had a positive oral challenge of which 38 were included. Thirty-seven patients completed the protocol and nine required therapy with Omalizumab. The most frequent symptoms during the oral provocation tests were cutaneous ones. Total IgE significantly increased throughout the protocol. Seventy-seven percent of patients who received treatment with Omalizumab were asthmatic.

Conclusions: Asthmatic patients are 6.5 times more likely to require Omalizumab to complete the protocol successfully and achieve tolerance. Furthermore patients who required Omalizumab had a higher initial IgE to casein compared to the rest of the subjects. In our experience, Omalizumab is effective even during an OIT protocol not just as pretreatment therapy prior to the protocol as seen in other studies. Furthermore its withdrawal did not provoke any adverse effects or relapse of allergy to cow's milk to date.

136

Consumer perceptions of precautionary labelling in families of children with food allergy and anaphylaxis and the level of risk to these consumers in the consumption of food products that bear precautionary labelling in Australia

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Background: Consumer perception regarding precautionary labelling for those most at risk of anaphylaxis is unknown. The level of contamination of food products that bear precautionary labelling is also unknown.

Objective: To understand consumer perception regarding precautionary labelling and assess the risk to consumers who consume food products with precautionary labelling.

Design/participants: We conducted a questionnaire-based study of 293 parents of children with an existing doctor diagnosis of food allergy to examine behaviour regarding precautionary labelling. Responses were compared between those with a history of anaphylaxis and those with a history of mild to moderate allergic reactions. The allergen content of high risk foods, namely chocolates, breakfast cereals, muesli bars, savoury biscuits, and sweet biscuits (128 samples in total) was analysed by Enzyme-Linked Immuno Sorbent Assay (ELISA) for peanut, hazelnut, milk, egg, soy and lupin protein.

Setting: The Department of Allergy at The Royal Children's Hospital, Melbourne, Australia between August-October 2011 (93% response rate).

Results: Avoidance of foods with precautionary labels differed depending on the wording of the precautionary statement, with 65% of participants ignoring the statement 'made in the same factory' compared with 22% for 'may be present'. There was no evidence of a difference in participants' behaviour depending on whether or not the child had a history of anaphylaxis.

Only nine products (7.0%) with precautionary labelling had detectable levels of peanut, with concentrations ranging from >2.5 to <50 ppm for whole peanut. Of all other samples that had precautionary labelling for hazelnut, milk, egg, soy or lupin, none were found to have a detectable level of those allergens.

Conclusions: Consumers are assuming that there is a gradient level of risk based on wording of precautionary statements and appear to be complacent with precautionary labelling, including those caring for children with a history of anaphylaxis. The majority of products with precautionary labelling assessed in this study contained no detectable levels of allergen suggesting that risk currently being taken by Australian allergic consumers is probably low except in the context of peanut allergy. Even when peanut contamination was present, it is unlikely that the dose of peanut detected would cause a reaction in the majority of the peanut allergic community.

137

Evidence-based efficacy of a cognitive behavioural therapeutic intervention in children with food allergy

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Background: To date, no controlled study has assessed the efficacy of any psychological intervention intended to moderate the psychological impact of food allergy. This study aimed to evaluate the effectiveness of a developmentally appropriate Cognitive Behavioural Therapy (CBT) intervention specifically developed for children and teens with IgE-mediated food allergy aiming to improve food allergy related quality of life (FAQL) in children and teens.

Method: Two experimental groups; the intervention group ($N = 24$) and a control group ($N = 45$) with children aged between 6 and 16 years. Both groups had previously undergone a food challenge. The control group received information and support at clinic, and the intervention group received the CBT intervention. Participants completed measures at entry, and at 2 and 6 months, consisting of three validated age-appropriate FAQL questionnaires (FAQLQ-PF-CF-TF), which include questions on anxiety, avoidance, and fearfulness caused by food allergy. A multivariate analysis of variance (MANOVA) with a 2 (experimental, control) × 2 (time) design was used. Data was analysed via SPSS 18.0.

Results: Groups had equivalent score at entry but significant differences were found between the intervention and control groups at times 2 and 3. Furthermore, the intervention group showed improved scores (two standard deviations) at 6 months post intervention, on the overall score of the FAQL psychometric measures administered at baseline [$F_{2.67} = 5.791$, $P < 0.05$].

Conclusion: Any treatment must be evidence-based, using gold standard designs, to ensure efficacy. This study is the first to have assessed the efficacy of an intervention developed to moderate the psychological impact of food allergy, using a controlled design. Our findings may impact on clinical care guidelines and provide a sound foundation for intervention practice.

138

Prolonged improvement in food allergy-related quality of life after withdrawal of 24 h access to expert advice on anaphylaxis management: follow up of a randomised control trial

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Background: Despite adequate education in their use, children with food allergy, and their carers are often unsure when to use prescribed adrenaline auto-injectors (AAIs). Non-use of AAIs may worsen outcomes of allergic reactions and patients' quality of life (QoL) may be low.

Method: We recontacted families 6 months after closure of a randomised trial of 24-hur access to expert advice on how to treat allergic reactions in the community. We had randomised 52 children (<16 years) with food allergy, trained in AAI use, in a 1:1 ratio to routine care (office hour access) or intervention (24 h telephone access to clinic staff) and measured their food allergy-related QoL (FAQL) using validated self administered FAQL Questionnaires (a low FAQLQ score reflects better FAQL). The primary outcome measure of interest had been a change in FAQLQ scores, at 1 and 6 months post-randomisation. Follow up FAQLQs were studied, 6 months after the intervention was withdrawn.

Results: FAQLQ scores, equal at baseline, changed over time with a significant difference evident at 6 months but not at 1 month between study arms ($F = 6.376$; $P < 0.05$). Twenty-five participants returned FAQLQs 6 months post study. FAQLQ had remained stable in both the control group (mean score = 3.1) and the intervention group (mean score 1.0, $P < 0.05$).

Conclusion: We have made the novel, first-in-field, finding that the significant positive effect of access to expert advice on anaphylaxis management (though the advice itself is rarely needed) persisted for at least 6 months after the intervention was withdrawn. This may impact on clinical care guidelines and offers further novel research opportunities in an area of allergy practice where clinical trials are so difficult to design.

Oral Abstract Session 24

Mechanisms of immunotherapy, T cells and beyond

139

A novel subset of regulatory T cells: interleukin-35 producing T regs (iTR35) are induced after grass pollen sublingual immunotherapy

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Background: Sublingual grass pollen-specific immunotherapy (SLIT) involves immunomodulation of Th2 responses and the induction of IL-10+ (Tr1) and/or TGF-b+CD4+CD25+ (Th3) regulatory T cells (T regs). IL-35-producing regulatory T cells (iTR35) have been recently reported as a novel subset of regulatory T cells. We hypothesised that IL-35 suppresses grass pollen-driven Th2 responses following *ex-vivo* allergen stimulation. We further hypothesised that iTR35 cells are induced following grass pollen-SLIT.

Method: T effector cells (CD4+CD25−) obtained from grass pollen allergics ($n = 12$) were purified and enriched from peripheral blood mononuclear cells by magnetic separation. CD4+CD25− T cells were co-cultured with irradiated antigen-presenting cells using 5 μg/ml of *Phleum pratense* in the presence/absence 10 ng/ml of recombinant human IL-35. T cell proliferative responses were measured by 3H-thymidine incorporation. Cytokine gene expression and protein levels were assessed by RT-PCR and Luminex MagPix assay, respectively. Proportions of FoxP3+, IL-10+ regulatory T cells and iTR35 were determined in non-atopics (NA, $n = 12$), untreated allergics (SAR, $n = 12$) and SLIT-treated patients (SLIT, $n = 7$).

Results: IL-35 significantly suppressed *Phleum pratense*-driven CD4+CD25− T cell proliferative responses ($n = 12$; $P < 0.001$). This suppression was associated with reduced IL-4 ($P = 0.002$), IL-5 ($P = 0.0001$), IL-9 ($P = 0.0002$), IL-13 ($P = 0.0001$). Increases in IFN-g ($P = 0.0010$) and IL-10 ($P = 0.0002$) were also demonstrated. Furthermore, *in vitro*-generated iTR35 cells obtained from naïve subjects following co-culture with allergen in the presence exogenous IL-35 (10 ng/ml) over 9 days suppressed allergen-driven memory CD4+CD45RO+ proliferative responses ($P = 0.006$) and IL-4 ($P = 0.0001$) and IL-5 ($P = 0.002$). iTR35 cells, IL-10+ and FoxP3+ T regs were lower in SAR compared to NA ($P = 0.0001$; 0.0002; $P = 0.0001$). SLIT was associated with increased numbers of iTR35 ($P = 0.001$) and IL-10+ Treg ($P = 0.001$) cells compared to controls, whereas FoxP3+ Tregs were not elevated in SLIT-treated subjects. Furthermore, an elevated expression of IL-35 (IL-12p35 ($P = 0.001$), EBI3 ($P = 0.002$) and Foxp3 ($P = 0.001$) mRNA was observed in allergen-stimulated PBMCs in SLIT group compared to controls.

Conclusions: iTR35 cells suppress grass pollen-driven Th2 responses and are induced following grass SLIT. Their role in the induction of immunological and clinical tolerance following SLIT remain to be further determined.

140

Changes in frequency of various regulatory T cells during rush oral immunotherapy for food allergy

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Background: Oral immunotherapy is considered as one of the curative treatments for food allergy. However, the detailed mechanisms of the effect of the therapy such as induction of peripheral tolerance through the generation of regulatory T cells are still unclear. In this study, we focused on changes in frequency of various regulatory T cells during rush oral immunotherapy for food allergy.

Method: Nine children (age, 5–10 years) with IgE-mediated cow's milk allergy confirmed by positive double- blind, placebo-controlled food challenge received oral immunotherapy. Informed consent was obtained from their parents or guardians.

After an initial escalation phase for 2–3 weeks, all patients achieved a daily maintenance dose (200 ml). We collected bloods from the patients before the initial phase, on day 7, after the initial phase and after 2 months of the maintenance therapy. We analyzed the changes in frequency of Foxp3+ regulatory T cells ($CD25^{high}Foxp3^+Helios^+CD4^+$ T cells and $CD25^{high}Foxp3^+Helios^-CD4^+$ T cells) and IL-10-producing $CD4^+$ T cells ($CD25^-LAP^+CD4^+$ T cells, $NKG2D^+CD4^+$ T cells and $CD25^-CD127^-CD4^+$ T cells).

Results: On day 7, the frequency of $CD25^{high}Foxp3^+Helios^+CD4^+$ T cells transiently decreased, while the frequency of $CD25^{high}Foxp3^+Helios^-CD4^+$ T cells transiently increased. The frequency of both types of $Foxp3^+$ $CD4^+$ cells recovered after the initial phase. On the other hand, the frequency of $CD25^-CD127^-CD4^+$ T cells significantly gradually increased during oral immunotherapy. The frequency of $CD25^-LAP^+CD4^+$ T cells or $NKG2D^+CD4^+$ T cells was not changed.

Conclusion: Our data suggest that $CD25^-CD127^-CD4^+$ T cells may have a role in the effects of oral immunotherapy for food allergy.

141

Immunological profile of the induction of tolerance in peanut and tree nuts food allergy in children: a pilot study

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Background: The lymphocytes and the cytokines (soluble mediators) play an essential role in food allergy (AA) mediated by IgE and their modulation by immune cells is associated with differentiation of naive T cells to the way helper2 T (Th2). The rise of Immunoglobulins (Ig) Ig G4 was observed in the response of allergic immune tolerance.

Method: Fifty-nine children (mean age (SD): 8.4 years (±3.7) years, 36 boys) were included in the study and divided into four groups: Group 1 (G1, $n = 11$) ongoing AA

eviction, Group 2 (G2, $n = 11$) who had an AA oral provocation test 1 week before blood sampling, Group 3 (G3, $n = 30$) who had an AA during a protocol of oral immune tolerance (OIT) induction protocol and a control group (T: $n = 7$) without AA or atopy. Serum concentrations of lymphocytes were analyzed. Cytokines: IL-1β, IL-2, IL-4, IL-5, IL-10, IL-12 p70, IFN-g, TNF-α and TNF-β were measured by multiplexed analysis by flow cytometry.

Immunoglobulin G4 (Ig4) and L and P selectines were also analyzed in all groups except two patients in G3 ($n = 28$).

Results: A significant difference in concentrations of IL-10 and IL-12 was found between the four groups ($P = 0.02$ and $P = 0.002$, respectively). This difference is due to an increase of IL-10 and IL-12 concentration from the group ITO (G3) with respect to the other groups ($P = 0.09$ and $P = 0.059$ respectively). In G3: Me = 131.63, IQ = [15.05, 146.55] for IL-10 and Me = 20.27, IQ = [0, 43.36] for IL-12.

IgG4 are increased in G3 (OIT): Me = 1.2 mg/l, IQ = [0.49, 3.19], the comparison with the G1 (Me = 0.2) and G2 (Me = 0.17) showed a significant increase ($P = 0.0019$).

Additional inclusions are provided to confirm the results.

The role of cellular and humoral immune function and of adhesion molecules, are important for understanding the mechanisms modulating intestinal mucosa inflammation in obtaining immune tolerance towards the allergen-specific protocols during gradually oral tolerance induced or after allergen exposure.

Conclusion: Patients receiving OIT protocol have a Th1 cytokine profile, an increase in the antiinflammatory cytokine IL-10, and rise of Immunoglobulin G4 compared to allergic children ongoing eviction or non-allergic children.

142

Participation of invariant natural killer T cells in allergen-specific immunotherapy: regulatory or pathologic role?

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Human invariant natural killer T (iNKT) cells are characterised by a unique TCR composed of an invariant alpha-chain combined with a beta-chain and co-expression of NK cell receptors. Although their critical role in regulating the development of allergy, their significance in asthma or atopic dermatitis remains controversial, and so far their involvement in allergic rhinitis is poorly documented as well as their participation in allergen-specific immunotherapy (IT). The aim was to characterise the iNKT population in peripheral blood from allergic rhinitis (AR) patients to house dust mite (HDM) receiving immunotherapy compared with non-atopic healthy controls.

Method: PBMCs were obtained from 19 AR patients to HDM and 17 controls at basal, 1, 3, 6 and 12 months after the beginning of IT. We assessed the frequency of peripheral blood iNKT cells measured as CD3+ cells co-expressing TCRVα24-TCRVβ11 by flow cytometry.

Results: The mean of relative iNKT cells counts was significantly increased in AR patients vs controls ($P = 0.001$). The proportion of CD8+ iNKT cells decreased at 6 months of treatment ($P = 0.016$). Those for double negative iNKT cells increased along IT in AR patients, but no changes were observed in the frequency of total iNKT cells. Moreover, AR patients showed an increase in CD56+ iNKT cells compared to controls ($P = 0.003$), in addiction this subpopulation increased along treatment. In the other hand, the proportion of the earliest activation marker, CD69, in iNKT cells decreased strongly in the first month ($P = 0.011$), and increased slightly at 3 months but not over basal levels. Besides, the mean of relative CD25+ iNKT cells count was significantly increased in AR patients after IT in all time intervals considered. Finally, a positive association between iNKT and B cells in allergic patients was found ($r = 0.77$).

Conclusion: Increased iNKT cells in AR patients to HDM might play an essential role in the allergic immune response. CD69+ and CD25+ iNKT cells may serve as early-indicator cells in allergen-specific immunotherapy. This could be useful for developing more effective vaccines and strategies to regulate this AR and to understand the mechanisms of immunological memory.

143

Inhibition of Kv1.3 and IKCa1 lymphocyte potassium channels as a novel therapeutic strategy in T helper cell mediated diseases

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Background: Imbalance between the function of the four main T helper subsets (Th1, Th2, Th17 and regulatory T (Treg) cells) plays an important role in the pathogenesis of immune mediated disorders, such as autoimmunity and allergy. The transient increase of the cytoplasmic free calcium level is a cornerstone of short-term lymphocyte activation and functionality. Voltage sensitive Kv1.3 and calcium-dependent IKCa1 potassium channels are important regulators of the maintenance of calcium influx during lymphocyte activation since they regulate the electrochemical potential gradient between the intra- and extracellular spaces. They present a possible target for selective immunomodulation, since their inhibitory properties are supposed to be different in the above subsets. We aimed to investigate calcium influx kinetics and its sensitivity to the inhibition of lymphocyte potassium channels in the above T helper subsets.

Methods: We took peripheral blood samples from 11 healthy individuals and evaluated calcium influx kinetics following activation with phytohemagglutinin in Th1 (CD4+ CXCR3+), Th2 (CD4+ CCR4+), Th17 (CD4+ CCR4+ CCR6+) and Treg (CD4+ CD25 high) cells applying a kinetic flow cytometry approach. We also assessed the alteration of calcium influx induced by specific inhibitors of Kv1.3 and IKCa1 potassium channels (margatoxin and triarylmethane, respectively). Furthermore, we determined the expression of Kv1.3 channels in the above subsets.

Results: The highest cytoplasmic calcium concentration was observed in Th1 cells upon stimulation. In contrast, Treg cells are characterised by the lowest level of calcium influx that lasts longer in line with the physiological regulatory role of this subset. In Th1 and Th17 cells, the specific inhibitors of both investigated potassium channels resulted in the decrease of calcium influx. However, in Th2 cells only the inhibition of Kv1.3 channels proved to be effective, while in Treg cells none of the specific inhibitors had an effect on lymphocyte calcium influx.

Conclusion: Upon the inhibition of IKCa1 channels, the short-term activation of Th1 and Th17 cells is specifically decreased without affecting the Th2 and Treg

subsets, indicating that selective immunomodulation can be reached in healthy individuals under experimental conditions. Further studies are needed to evaluate the potential therapeutic application of IKCa1 inhibitors in immune-mediated disorders.

144
IgG4 memory elicited by a single course of immunotherapy with Bet v 1 COPs persists up to the fourth season post treatment

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Background: Allergen specific immunotherapy with poorly standardised-whole pollen extract is of long duration and may induce anaphylaxis. Specific immunotherapy may be improved by using non-IgE binding, hypoallergenic, molecules including Contiguous Overlapping Peptides (COPs). AllerT™, an equimolar mix of three COPs derived from Bet v 1 showed good safety and immunogenicity in a placebo-controlled phase I/IIa clinical trial in volunteers with birch pollen allergic rhinitis and asthma. The possible long term effect of AllerT was analysed after two and four seasons without any further treatment.

Method: Prior to the 2009 pollen season, AllerT reconstituted with the adjuvant Aluminium hydroxide was injected subcutaneously five times at 1–4 weeks intervals into 15 adult subjects. Control subjects (n = 5) received only adjuvant. Blood was collected after treatment (February–March 2009) as well as after the second (July 2010) and fourth (August 2012) birch pollen season in two open studies. IgE and IgG4 levels specific for Bet v 1 as well as each individual COP were quantified by ELISA in samples from both treated and placebo subjects.

Results: AllerT treatment increased cytokines, including IL-10, preceding a more than 20-fold mean increase in anti-Bet v 1 IgG4. Anti-Bet v 1 IgG4 levels increased in all treated subjects, whereas IgG4 against injected COP peptides were detected in only few subjects and at low levels. Two months after the second season post-treatment and 3 month after the fourth season, serum Bet v 1 specific IgG4 response remained increased by 4–5-fold over pre-treatment whereas post-seasonal Bet v1 specific IgE titres were similar to baseline values. IgG4 values in treated subjects differed significantly from placebo.

Conclusion: AllerT induced an increase in Bet v 1 specific IgG4 in all treated subjects. AllerT COPs seem to boost pre-existing anti-Bet v 1 B-cell responses possibly via T-cell activation, since anti-COPs IgG4 increased only marginally. Immunotherapy with a mixture of three COPs derived from Bet v 1 (AllerT) is safe and immunogenic, and leads to long term IgG4 immunological memory as seen by significant post-seasonal levels.

Oral Abstract Session 25

The efficacy of allergen-specific immunotherapy

145

Cat-PAD demonstrates sustained, consistent treatment effect on allergic rhinoconjunctivitis in individual cat-allergic patients 1 and 2 years after four intradermal injections

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Background: A population of cat-allergic patients treated with Cat-PAD in an Environmental Exposure Chamber (EEC) study showed a persistent treatment effect 1 year (Patel et al., JACI 2013) and 2 years (Hafner et al., AAAAI 2013) after only four intradermal injections. Here we demonstrate that the treatment effect observed is due to sustained consistent treatment effects in individual patients, not due to variable responses in different patients at each EEC.

Methods: Originally 202 patients were randomised to 6 nmol Cat-PAD 4 weeks (wk) apart for 3 months (4 × 6 nmol), 3 nmol Cat-PAD 2 wk apart for 3 months (8 × 3 nmol), or placebo. EEC challenges were performed at baseline and 18–22 week after the first dose. Eighty-nine of these patients had a further EEC challenge at 50–54 week, and of these, 51 patients had an EEC challenge at 100–104 week. EEC challenge consisted of four consecutive days of 3-h allergen exposure (Fel d1 circa 50 ng/m^3). Patients recorded Total Rhinoconjunctivitis Symptom Score (TRSS) every 30 min. A *post-hoc* analysis was performed to evaluate the correlation between Change from Baseline in mean TRSS on Day 1–4 (CB-TRSS) at 18–22 weeks vs CB-TRSS at 50–54 week and 100–104 week for each patient.

Results: For the 89 patients who returned for an EEC at 50–54 week, the treatment effect for each patient was more strongly correlated with their response at 18–22 week with 4 × 6 nmol [regression slope (RS) = 0.85, correlation coefficient (r) = 0.74] than either 8 × 3 nmol (RS = 0.63, r = 0.61) or placebo (RS = 0.62, r = 0.62). For the 51 patients returning at 100–104 week, the treatment effect on 4 × 6 nmol increased with time (RS = 1.36, r = 0.89); 8 × 3 nmol showed similar effect (RS = 0.75, r = 0.70) to placebo (RS = 0.84, r = 0.76). Patients in the 4 × 6 nmol group demonstrated consistent improvements in symptomatology at both 50–54 and 100–104 weeks; the responses in patients in the 8 × 3 nmol and placebo arms did not show consistent improvement.

Conclusions: Cat-PAD 4 × 6 nmol induces a sustained consistent treatment effect in cat-allergic patients after only four injections over 12 week. In contrast, 8 × 3 nmol has a less consistent effect that wanes with time, suggesting a possible threshold dose effect. Cat-PAD is the first in a new class of synthetic peptide immuno-regulatory epitopes conferring sustained treatment benefit in individual patients with chronic rhinoconjunctivitis due to cat allergy at 1 and 2 years after short-term administration, indicating true disease modification.

146

The influence of pollen exposure on efficacy measurements in grass allergy immunotherapy trials

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Background: Magnitude of symptoms for a grass allergic subject depends on seasonal grass pollen exposure. The objective of this study was to evaluate the relationship between efficacy endpoints and grass pollen exposure based on pooled data from multiple regional trials across several years, with a grass allergy immunotherapy tablet (AIT).

Method: The *post-hoc* analysis included pooled data from six North American and European randomised, placebo-controlled grass AIT trials (1–6) including 2363 paediatric and adults subjects (1198 active and 1165 placebo).

Subjects with grass-pollen induced rhinoconjunctivitis were randomised to grass AIT or placebo in trials that had duration of 18–29 weeks (five one-season trials) or up to 5 years (1 trial with 3 years of treatment and 2 years of follow-up). Subjects filled in daily diaries with rhinoconjunctivitis symptoms (four nasal and two ocular symptoms scored on a 0–3 scale with 3 = severe symptoms) and symptomatic medications use during the grass pollen season. Pooled data were analysed by daily symptom (DSS), medication (DMS), and total combined symptom and medication score (TCS = DSS + DMS). The DSS, DMS and TCS was modelled by a generalised additive model (gam; smoothing spline with df = 5) on daily grass pollen counts (7). Daily grass pollen counts were obtained from regional pollen stations in the vicinity of the trial sites. The grass pollen seasons were defined by boundaries of three consecutive days with counts greater or less than 10 grains/m^3.

Results: The magnitude of the treatment effect based on DSS, DMS and TCS was greater with higher pollen exposure ($P < 0.001$).

The average grass pollen exposure over the first 20 days of the season varied from 31 to 82 grains/m^3. Overall, the relative treatment effect ((placebo-active)/placebo) in terms of TCS for each trial (including each year of the 5-year trial) was correlated with the average grass pollen exposure during the first period of the season. The predicted percent reduction in TCS = 12% + 0.35% × pollen count (slope significantly different from 0, $P = 0.003$; $R^2 = 0.7$). Similar relationships were found for the DMS and DSS.

Conclusion: In seasonal allergy trials with grass AIT, the observed treatment effect is highly dependent on pollen exposure. Regardless of the clinical parameter assessed, the magnitude of the treatment effect was greater with higher pollen exposure. This is an important relationship that must be considered when interpreting individual clinical trial results.

147

Evaluation of the relationship between EQ-5D health utilities and clinical efficacy endpoints in the treatment of seasonal grass pollen induced rhinoconjunctivitis

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Background: Standard clinical efficacy endpoints in allergen immunotherapy trials include the daily Rhinoconjunctivitis Symptom Score (RSS) and daily Rhinoconjunctivitis Medication Score (RMS). However, measurement of subjects' quality of life in terms of health utility (e.g. EQ-5D index) is also necessary to characterise changes in health status. In this study, we aimed to determine a relationship between standard efficacy endpoints and the EQ-5D health utility.

Method: Data from a 5-year randomised, parallel-group, double-blind, placebo-controlled, phase III trial (GT-08, ALK, Denmark) in subjects with seasonal grass pollen induced rhinoconjunctivitis were analysed. During each pollen season, subjects recorded their RSS and RMS on a daily basis and the EQ-5D index was recorded weekly. EQ-5D index responses were converted to utilities using the UK tariff. EQ-5D utilities often follow a one-inflated distributed with subjects frequently reporting 'perfect health' (EQ-5D utility = 1) while the remaining utilities are distributed between 0 and 1. To account for this distribution a two-stage model was constructed:

1 binomial modelling of subjects with 'perfect health' (EQ-5D utility = 1) and
2 Gaussian modelling of EQ-5D utilities < 1 (subjects with 'imperfect health').

In both cases generalised linear mixed modelling was used to explore the fixed effects of RSS, RMS, gender, age and asthma status and the random effects of subject, year and country. All statistical modelling was performed with R (R Foundation for Statistical Computing, Vienna, Austria).

Results: Five hundred and sixty-eight subjects, enrolled in the first year, provided diary data. A total of 16 690 weekly EQ-5D observations were recorded over the trial period. The binomial model showed the daily RSS and RMS to be the two most important predictors of 'perfect health' (RSS: $DOR_{per\ unit} = 0.729$; $P < 0.001$; $DRMS_{per\ unit}$: OR = 0.841; $P < 0.001$) – implying that low RSS/RMS scores increase the likelihood of reporting a 'perfect health'. The Gaussian model of subjects with 'imperfect health' (EQ-5D utility <1) confirmed this correlation.

Conclusion: In a clinical trial including subjects with seasonal grass pollen induced rhinoconjunctivitis, the daily rhinoconjunctivitis symptom and medication scores were found to be suitable as predictors of the EQ-5D health utility. A low score increases the likelihood of reporting a 'perfect health' as measured by EQ-5D utilities.

148

An evaluation of data on the relative clinical impact of sublingual allergen immunotherapy tablets and symptomatic medications in grass-pollen-induced allergic rhinoconjunctivitis

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Background: Despite unambiguous evidence on the ability of sublingual immunotherapy (SLIT) tablets to markedly relieve symptoms of pollen-induced seasonal allergic rhinitis (SAR) from meta-analysis and large, double-blinded, placebo-controlled (DBPC) trials, reports on the tablets' high 'relative clinical impact' (RCI) are often questioned. We therefore determined the RCIs of five-grass and single-grass pollen SLIT tablets and 'symptomatic' medications (oral H1-antihistamines, nasal corticosteroids and a leukotriene receptor antagonist) having received marketing authorization within the last 15 years for SAR in adults and/or children.

Method: We identified 49 single-season DBPC trials with at least 100 participants in the smallest treatment arm. For each drug class, we performed a meta-analysis (Hedges' g) of the symptom scores. The RCI (as defined by the World Allergy Organization) in each arm was calculated from the post-treatment or season-long nasal or total symptom scores: $100 \times (score_{Placebo} - score_{Active})/score_{Placebo}$.

Results: Twenty-six symptomatic medication trials (including one in children only) and nine SLIT tablet trials (including three in children only) met our criteria. Meta-analysis of over 17 800 cases confirmed the presence of a highly significant overall treatment effect for each individual drug class. A number of methodological factors are likely to mask the true RCI in trials of SLIT tablets, such as long trial durations, non-uniformly high disease activity on randomisation and authorised rescue medication use in SLIT tablet trials but not in pharmacotherapy trials. However, even with these methodological disadvantages, the RCIs for SLIT tablets (ranging from 16% to 30%) in SAR were at least similar to or even greater than the values for symptomatic medications (3–26% for second-generation H1-antihistamines, 7–37% for nasal corticosteroids and 3–10% for montelukast).

Conclusion: Despite the presence of methodological factors in clinical trials that may lead to underestimate allergen immunotherapy effect size, grass pollen SLIT tablets appears to have a greater RCI than second-generation H1-antihistamine and montelukast and much the same RCI as nasal corticosteroids in poorly controlled patients with moderate-to-severe SAR.

149

New objective method to measure skin test results within clinical trials

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Background: The difference of the late phase reaction (LPR) using intracutaneous tests (ICT) before and after immunotherapy has been used in clinical trials as an efficacy endpoint. Results of read-out and interpretation are subject to high inter- and intra-personal variations. This might be a problem especially in multicentre clinical trials Here we are introducing a new method to objectively measure ICT results. Furthermore we present kinetics of the LPR of the ICT using different allergen concentrations.

Method: In the first part of an ongoing phase II study with ACAROID® (EudraCT-No.: 2011-02248-29) we assessed the optimal dose and time point for ICT reading. The wheal/swelling after intracutanous injections with *Dermatophagoides pteronyssinus* was evaluated by using a new opetical device. This camera based and software assisted method provides a measurement with a height resolution of >20 µm. Within the gauge field of 160 × 100 × 90 mm the area and volume of the skin reaction can be calculated at inevitable time points.

We screened 28 patients with rhinitis or asthma caused by house dust mites and performed an ICT with three different doses (500, 2500 and 5000 SBE/ml) in 16 patients. The kinetic of the skin reaction

was measured at baseline and 2, 4, 6 and 8 h after injection.

Results: The mean area of the LPR showed a maximum after 6 h with 33.7, 21.0 and 11.4 cm^2 for the concentrations of 5000, 2500 and 500 SBE/ml. But the volumes of the wheal for the two highest concentrations had already their maximum after 4 h whereas for the lowest concentrations a saturation kinetic was achieved.

Conclusion:

1 6 h after ICT injection seems to be the most promising time point to measure the area of the LPR
2 This new method also allows evaluation of the volume of the LPR. This may provide an even more interesting information as it reflects the natural three dimensional reaction.
3 This method may improve the reliability of ICT measurements in clinical trials and maybe even in daily practice.
4 Outlook: The results from this pilot phase encourage us to use the new method when applying the ICT as efficacy endpoint in phase II studies.

150

Meta-analysis of allergen immunotherapy for treatment of grass pollen allergies indicates sublingual immunotherapy tablet is comparable to subcutaneous immunotherapy

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Background: Subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) have been shown to effectively treat grass pollen allergies. Network meta-analysis was conducted to estimate relative efficacy of SLIT-tablets for treatment of grass pollen allergy compared to SCIT and SLIT-drops.

Method: A literature search was conducted of Medline, Embase, and Cochrane Library, English language publications, through June 2012. Only randomised, double-blind, placebo controlled clinical trials of SCIT, SLIT-drops and SLIT-tablets for grass pollen were included. Articles were cross-checked against previous meta-analyses and reviews. Bayesian network meta-analyses estimated standardised mean difference (SMD) across three immunotherapy modalities on allergic rhinoconjunctivitis symptom and/or medication score data. Heterogeneity assessed with I^2 statistic for all direct comparison data. Random effects model accounted for heterogeneity across studies.

Results: Thirty-three studies (11 SLIT-tablets, 14 SLIT-drops, eight SCIT) of commercially available products were included in meta-analyses for symptom scores; 29 studies (10 SLIT-tablets, 13 SLIT-drops, six SCIT) for medication scores.

Moderate to substantial heterogeneity was observed among SLIT-drops studies (64%) and moderate heterogeneity observed among SCIT studies (42%) for symptom scores. For medication scores, moderate to substantial heterogeneity among SCIT studies (63%) and considerable heterogeneity among SLIT-drops studies (89%) was observed. Little to no heterogeneity among SLIT-tablet studies for symptom (32%) and medication scores (0%).

Random effects model results revealed no statistically significant differences in SMD′s (95%CI) for symptom scores [−0.0309 (−0.266, 0.213)] or medication scores (0.120 [−0.420, 0.672)] between SLIT-tablets and SCIT. SLIT-tablets comparisons with SLIT-drops demonstrated statistically significant lower SMD for SLIT-tablets symptom scores [−0.246 (−0.461, −0.034)] as compared to SLIT-drops indicating better symptom improvement for SLIT-tablet patients; no statistically significant difference in medication scores was observed [0.15 (95%Cl −0.30, 0.60)].

Conclusion: These comparisons of published trial results for commercialized grass pollen immunotherapy indicate comparable reductions in allergic rhinoconjunctivitis symptoms and supplemental medication use for SLIT-tablets and SCIT. SLIT-drops appear to be less effective in symptom reduction than SCIT or SLIT-tablets.

Oral Abstract Session 26

Novel insights into the genetics and epigenetics of asthma

151

Sex- and age- dependent DNA methylation at the 17q12-q21 locus is associated with childhood asthma

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Background: Chromosomal region 17q12-q21 is one of the best-replicated genome-wide association study (GWAS) hits and associated with childhood onset asthma[1,2]. However, the mechanism by which the genetic association is restricted to childhood onset disease is unclear. As there are more boys than girls among asthmatic children, therefore we hypothesised that the genetic association was sex-specific.
Method and results: This hypothesis was tested in the Saguenay-Lac-Saint-Jean familial collection that included 1214 samples from 240 extended families. The TDT test showed that the 17q12-q21 genetic association was statistically significant among male, but not among female asthmatic subjects. We next hypothesised that the bias in the genetic association resulted from sex-specific and/or age-dependent DNA methylation at regulatory regions and determined the methylation profiles of five 17q12-q21 gene promoters using the bisulfite sequencing methylation assay. We identified a single regulatory region within the *zona pellucida* binding protein 2 (*ZPBP2*) gene promoter, which showed statistically significant differences between males and females with respect to DNA methylation (26% in males and 35% in females). DNA methylation also varied with age and was higher in adult men compared to boys. We have recently identified two functionally important polymorphisms[3,4], both within the *ZPBP2* gene that influence expression levels of neighboring genes. Combined with the results of the present work, these data converge pointing to the same region within the *ZPBP2* gene as a critical region for both gene expression regulation and predisposition to asthma.
Conclusion: Our data show that sex and age-dependent DNA methylation may act as a modifier of genetic effects and influence the results of genetic association studies.

152

Environment–environment interaction between bronchiolitis and PM_{10} exposure could be modified by *IL-13* polymorphism in the development of childhood asthma

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Background: The aim of this study was to investigate the synergistic environment-environment interaction between bronchiolitis and particulate matter 10 (PM_{10}) exposure regarding to the genotypes of interleukin-13 (*IL-13*) polymorphism in the development of asthma in pre-school children.
Method: A modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was used to survey 919 nursery school children from Seoul, Korea. *IL-13* (rs20541) polymorphism was genotyped by TaqMan assay. Individual exposure level to PM_{10} was estimated by kriging method in geometric information system (ArcGIS 9.3).
Results: The lifetime prevalence of asthma diagnosis was 8.7%. Parental history of asthma [adjusted odds ratio (aOR), 3.60; 95% confidence interval (CI), 1.66–7.76], parental history of AR (aOR, 3.48; 95% CI, 1.95–6.19), and bronchiolitis in the first 2 years of life (aOR, 3.94; 95% CI, 2.27–6.84) were independent risk factors for asthma diagnosis. However, PM_{10} exposure of recent 5 years was not independent risk factor for asthma diagnosis. Asthma diagnosis was presented no higher in subjects with *IL-13 +2044 GG* and bronchiolitis who were exposed to high PM_{10} than those with *IL-13 GG* and bronchiolitis who were exposed to low PM_{10}. However, Asthma diagnosis was presented higher in subjects with *IL-13 +2044 GA + AA* and bronchiolitis who were exposed to high PM_{10} than those with *IL-13 GA + AA* and bronchiolitis who were exposed to low PM_{10}. High PM_{10} and bronchiolitis with *GA + AA* type of *IL-13* polymorphism showed an increased risk of asthma (aOR, 17.77; 95% CI, 3.81–82.91).
Conclusion: The combined effect between bronchiolitis and PM_{10} exposure in the development of asthma could be modified by *IL-13* polymorphism in pre-school children.

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153

Interactive effects between polymorphic markers on 17q21 locus and *GSTP1* for childhood asthma susceptibility

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Background: Oxidative stress plays key roles in asthma pathogenesis. Glutathione S-transferase P1 (GSTP1), an abundant isoform of glutathione S-transferases (GSTs) in lung epithelium, is important in the cellular protection against oxidative stress. Gasdermin A (*GSDMA*) on chromosome 17q21 is a susceptibility locus identified by asthma genome-wide association study, and is consistently replicated in different ethnic groups. This study explored both single-locus effects and multi-locus interactions for GSTs and *GSDMA* on asthma risk and spirometric variables in Hong Kong children.

Methods: Nine hundred and thirty-five asthma patients aged 14.6 ± 4.2 years and 1225 non-allergic controls aged 15.1 ± 4.1 years were recruited. Pre-bronchodilator spirometry was performed according to international guidelines to measure subjects' forced expiratory volume in 1-s (FEV_1) and forced vital capacity (FVC). Single-nucleotide polymorphisms (SNPs) in GSTs, including *GSTP1* (Ile105Val; rs1695), *GSTM1* (null; rs10712361) and *GSTT1* (null; rs10549055), and *GSDMA* (Arg18Gln; rs3894194) were genotyped using Sequenom iPLEX Gold assay. Genetic associations of these SNPs with asthma diagnosis and subphenotypes were analyzed by multivariate regression. The epistatic interactions between these genes for asthma traits were examined using generalised multifactor dimensionality reduction.

Results: Sequenom assay design was successful for *GSTP1* Ile105Val but not the other GSTs. Genotyping was obtained in ≥99.6% for this SNP and *GSDMA* Arg18Gln, both of which followed Hardy-Weinberg equilibrium. *GSDMA* codon 18 Arg/Arg genotype was protective against asthma (odds ratio 0.69, 95% confidence interval 0.52–0.92; $P = 0.012$). Linear regression revealed *GSTP1* Ile105Val to be associated with FEV_1 ($P = 0.030$ in all asthmatics and 0.006 in asthmatic boys) and FEV_1/FVC ($P = 0.046$ and 0.038 respectively). Gender-specific epistatic interaction was also detected between *GSTP1* and *GSDMA* for FVC in asthmatic girls (testing accuracy 61.3%, cross-validation consistency 10/10; $P = 0.044$) but not boys.

Conclusions: *GSDMA* codon 18 Arg/Arg genotype is associated with a decreased risk for childhood asthma, and *GSTP1* is associated with FEV_1 in asthmatics especially among males. There is epistatic interaction between *GSTP1* and *GSDMA* that modulates FVC among asthmatic patients in a gender-specific manner.

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154

Evaluation of the gucocorticoid receptor α and β gene expression levels of the perimononuclear cells for vitamın D insufficient children with asthma

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Background: Vitamin D (VitD) is related with the composition of immune system, development of lung and response to treatment of asthma. To date, different mechanisms were suggested for the relationship between VitD and asthma, however, interaction of VitD with glucocorticoid receptors (GR) have not been studied yet. In this study, our aim was to investigate the clinical response, relative gene expressions (RGE) of GR-α and β after VitD treatment in children with asthma and VitD insufficiency.

Method: Twenty-five patients with asthma composed the asthma group, 14 healthy children composed the control group who all were followed in Ondokuz Mayis University Pediatric Immunology and Allergy Unit, aged between 8 and 16 years and had VitD deficiency (25(OH)D level under 30 ng/ml). Vitamin D with a dose of 300 000 units was given orally to all the children at first visit. Two blood samples were taken from all children at admission and after 1 month to evaluate the serum 25(OH)D level and GR-α and β RGE levels of the peripheral mononuclear cells.

Results: GR-α and β RGE did not change significantly between first and second visits in the asthma group. GR-β RGE decreased significantly at second visit in the control group ($P = 0.05$). GR-α RGE did not change between the two groups, however the decrease of GR-β RGE between two periods was statistically significant in the control group ($P = 0.002$). GR-β RGE was significantly higher at admission in the control group when compared with the asthma group ($P = 0.006$), however the difference disappeared in the second visit ($P = 0.09$). Positive correlation was found between 25(OH)D level and IgE(log10) in the patient group ($r = 0.44$; $P = 0.03$), while it was not detected in the control group ($r = -0.42$; $P = 0.13$) at first visit.

Conclusion: Basal GR-β RGE was lower in the asthma group when compared with the control group; GR-α RGE was not increased by VitD in the asthma group as control group, and VitD decreased GR-β RGE in the control group more than asthma group. These findings show that VitD is associated with GR RGE for children with asthma.

155

Genetic variants in endotoxin signaling pathway and endotoxin exposure: *in vitro* IgE synthesis and replication in a birth cohort study

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Background: Variants in genes involved in the endotoxin signaling pathway may be important in determining the effect of endotoxin exposure on asthma and atopy. We aimed to: (i) Determine single nucleotide polymorphisms (SNPs) in the endotoxin signaling pathway that influence IgE synthesis *in-vitro*; (ii) Replicate the significant findings from *in-vitro* experiments *in vivo*, by examining the relationship between genetic variants and environmental endotoxin exposure in relation to asthma and atopy.

Methods: In *in-vitro* experiments, peripheral blood mononuclear cells from 45 asthmatic children from Turkey were stimulated with 2 and 200 ng/ml of lipopolysaccharide (LPS), and IgE was measured in the culture supernatants. We genotyped children for 121 SNPs in 30 genes involved in the endotoxin signaling pathway, and determined genetic variants in which there was a dose-response IgE synthesis after *in-vitro* LPS stimulation. We then investigated whether our *in-vitro* findings could be replicated *in vivo*, by examining the interaction between these genetic variants and domestic environmental endotoxin exposure with allergic sensitisation, wheeze and airway hyper-responsiveness in 1084 children in a population-based birth cohort from the UK. Endotoxin was measured in dust samples collected from homes in pre-school age, and clinical outcomes (current wheeze, methacholine challenge test, skin tests and measurement of sIgE) assessed when children were aged 8 and 11 years.

Results: Twenty-one SNPs in nine genes (*CD14*, *TLR4*, *IRF3*, *TRAF-6*, *TIRAP*, *TRIF*, *IKK-1*, *ST2*, *SOCS1*) were related to *in-vitro* IgE synthesis following LPS stimulation *in-vitro*, of which six were in high linkage disequilibrium. We replicated the following significant interactions between genotype and environmental endotoxin exposure in relation to clinical outcomes: for symptomatic airway hyperresponsiveness, *CD14*-rs2915863 and *TRIF*-rs4807000 atage 8 and age 11, and *CD14*-rs2569191 at age 11 years; for current wheeze, *ST-2*-rs17639215 at age 8 and 11 years, *IKK-1*-rs2230804 and *TRIF*-rs4807000 at age 11 years; for positive skin prick tests, *CD14-2915863* and *TRAF-6*-rs5030411 at age 11; for positive sIgE, *CD14*-rs2569192 and *IKK-1*-rs2230804 at age 8 years.

Conclusion: We determined genetic variants in nine genes in the endotoxin signaling pathway that influence IgE synthesis *in-vitro*, and confirmed that a number of these variants interact with environmental endotoxin exposure in modulating the risk asthma and atopy.

156
Interaction between DNA methylation and genotype in Th2 pathway genes, association with asthma and adolescent transition of asthma between ages 10 and 18 years

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Background: Gene activity is determined by interactions between transcription factors, gene promoters, and epigenetic changes, including DNA methylation (DNA-M). We hypothesise that single nucleotide polymorphisms (SNPs) and DNA-M in *IL4, IL4R, GATA3, STAT6,* and *IL13* genes in the Th2 cytokine pathway interact to affect the risk of asthma and transition of asthma status among children aged 10 and 18.

Method: A random subsample of 245 women in a whole-population birth cohort ($n = 1456$), established on the Isle of Wight, UK in 1989, were studied. Haplotype-tagging SNPs were genotyped and DNA-M in each gene was assessed at age 18 in DNA derived from peripheral blood leukocytes using the Illumina GoldenGate Genotyping and Infinium HumanMethylation450 BeadChip assays, respectively. Outcomes were asthma affection status at ages 10 and 18 years, and positive and negative transitions of affection status between 10 and 18. Logistic regressions were applied to examine the interaction effects of methylation and SNPs on asthma. Bootstrapping was used to evaluate the quality of selection. Among the selected CpG sites, Kruskal–Wallis tests were used to test differences in methylation between different asthma transitions. Multiple testing was corrected using the Bonferroni method.

Results: The pathway-based selection process of fitting 1472 logistic regression models yielded 10 CpG sites and 12 SNPs in *IL4, IL4R,* and *GATA3* significantly associated with asthma status at age 18. At age 10, 12 CpG sites and 11 SNPs in the same genes were selected, among which three CpG sites and seven SNPs agreed with the age 18 selection. Of the selected CpG sites and SNPs, some were potential risk factors and others seemed to be protective. For instance, for *GATA3*, increased methylation of cg01166071 is a risk factor for asthma at age 18, with the risk being higher among subjects heterozygous for rs1058240 (log-OR = 98.40, $P = 0.008$). Utilizing Kruskal–Wallis test, only cg12377972 was statistically significantly associated with positive transition of asthma status ($P = 0.0093$).

Conclusions: The findings emphasized the importance of considering the effects of both methylation and SNPs on asthma. Furthermore, the results indicated two possibilities regarding the effect of methylation on the status of asthma affection:

1 dynamic methylation with concurrent effects and
2 stable methylation with delayed methylation effects.

Oral Abstract Session 27

Mechanisms of inflammation and potential biomarkers of asthma

157
Real time *in vivo* imaging of eosinophil and neutrophil migration in healthy and asthmatic volunteers

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Background: Lung inflammation is now recognised as a leading pathological process in the development and exacerbation of asthma. Here we present a study looking at purified eosinophils and neutrophils, largely thought to be responsible for asthmatic lung inflammation, migrating *in vivo* in real time through healthy and asthmatic lungs.
Method: One hundred and five millilitre of venous blood was obtained from healthy (4) and asthmatic (4) volunteers on two separate occasions. Granulocytes were separated using gradient Ficoll-Paque PLU 1.084 centrifugation. Superparamagnetic particles coupled to a monoclonal antibody against CD16, a surface marker present in neutrophils, were incubated with the granulocytes (mixed eosinophils and neutrophils). CliniMACS system (Miltenyi biotec, Bergisch-Gladbach, Germany) was used to obtain highly purified (>93% pure) human blood eosinophils (negative selection) or neutrophils (>97%, positive selection). Purified cells were labelled with Tc-99 m HMPAO (Ceretec, GE Healthcare) under aseptic cGMP conditions and 75–100 MBq of labelled cells were administered intravenously. Dynamic lung images were acquired for the first 30 min. Further static scans of 5 min each were acquired at 1, 2 and 4 h.
Results: Lung migration of eosinophils differed significantly from that of neutrophils in both groups of patients. Migration of eosinophils in healthy volunteers followed a monoexponential clearance with $T_{1/2}$ of 4.16 min whilst neutrophils $T_{1/2}$ measured 13.72 min ($P = 0.0019$). Similar results were obtained for eosinophils and neutrophils in stable asthmatics; $T_{1/2}$ 6.10 min measured for eosinophils and 14.01 min for neutrophils, P-value = 0.0246. There were no statistically significant differences observed between healthy volunteers and stable asthmatics (eosinophils $P = 0.14$ and neutrophils $P = 0.45$).
Conclusion: This study gives an insight into the process of pure cell migration *in vivo* in human lung. It highlights the complexity of the immune system where differences exist even at the level of migratory patterns; eosinophils and neutrophils, in spite of being of similar size and shape, display distinct patterns of lung kinetics. These differences are preserved in healthy volunteers and stable asthmatic. This technique provides the opportunity for rapid throughput screening of novel therapeutic agents designed to alter leukocyte migration and forms the basis of future spaciotemporal studies of pure cell kinetics.

158
Altered microRNA expression profile during epithelial wound repair in bronchial epithelial cells

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Background: Airway epithelial cells provide a protective barrier against environmental particles including potential pathogens. Epithelial repair in response to tissue damage is abnormal in asthmatic airway epithelium in comparison to repair of normal epithelium after damage. The complex mechanism coordinating the regulation of these processes involved in wound repair requires the phased expression of networks of genes. One biological mechanism that plays a critical role in the coordinate regulation of gene expression is the expression of small non-coding RNA molecules termed microRNAs (miRNAs).
Method: To investigate the possible involvement of miRNA in epithelial repair, we analyzed miRNA expression profiles during epithelial repair in a cell culture model using TaqMan-based quantitative real-time PCR in a TaqMan Low Density Array format. The expression of 754 miRNA genes at seven time points of wound repair in a 48-h period was profiled using the bronchial epithelial cell line 16HBE14o$^-$.
Results: The expression levels of numerous miRNAs were found to be altered during the wound repair process, and these miRNA genes were clustered into three different patterns of expression, which further regulate several biological pathways involved in wound repair. Moreover, it was observed that some miRNA genes are significantly altered only at one time point indicating their involvement in a specific stage of epithelial wound repair.
Conclusion: In summary, miRNA expression is modulated in the normal repair processes in airway epithelium *in vitro* suggesting a potential role in regulation of wound repair.

159
Allergic eosinophilic granulomatosis with polyangiitis: evidence for disease subtypes?

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Background: Allergic Eosinophilic Granulomatosis with Polyangiitis (EGPA) is a systemic necrotizing vasculitis which occurs in patients with asthma, nasal disease, blood and tissue eosinophilia. Recently, the availability of novel therapies such as humanized anti-IL-5 mAbs, has made it increasingly important to accurately characterise the 'vasculitic' and the 'eosinophilic' manifestations of the disease.
Aim: (i) to describe the occurrence of 'vasculitic' and 'eosinophilic' EGPA manifestations in a single center cohort of patients, focusing in particular on pulmonary involvement (ii) to compare lung function, asthma severity/control, airway inflammation markers and quality of life in patients with a predominant 'vasculitic phenotype' (VP) and in those with an 'eosinophilic phenotype' (EP).
Methods: Thirty-six patients with EGPA were enrolled in this cross-sectional study. All patients were assessed for lung function and bronchial hyperreactivity; asthma

severity was evaluated according to GINA guidelines and asthma control by ACT. Sputum eosinophil percentages, exhaled nitric oxide (eNO) and blood biomarkers were assessed, including: eosinophilic count, serum IL-2-4-5, eosinophil cationic protein and anti-neutrophil cytoplasmic autoantibody (ANCA). Systemic and inhaled therapy, BVAS (Birmingham Vasculitis Activity Score) and VDI (Vasculitis Damage Index), the short form (SF)-36 and the Asthma Quality of Life Questionnaire (AQLQs) were recorded.

Results: Twenty patient with VP and 16 with EP were enrolled. Patients with VP were older ($P < 0.05$), had a longer disease duration ($P < 0.006$) and showed a higher positivity for ANCA-MPO ($P < 0.05$). The 'eosinophilic' subset presented a higher eosinophilic airway inflammation ($P < 0.05$) (despite a higher dose of inhaled ICS) and higher incidence of lung infiltrates ($P < 0.003$) and bronchiectasis ($P < 0.005$). No significant differences were observed in lung function, level of asthma control, disease relapses, therapeutic approaches, and quality of life (AQLQs was in mean 4.9 ± 1 and was correlated with the ISF-36 score ($P < 0.003$).

Conclusions: This study confirms the presence of two different pathogenetic and clinical subsets in EGPA. Higher frequency of lung infiltrates and higher levels of airway inflammatory markers were documented in the EGPA 'eosinophilic' subset, shedding a new light for targeted therapies in EGPA.

160
Up-regulation of thymic stromal lymphopoietin receptor on myeloid dendritic cells from atopic asthmatics

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Background: It was shown in mice that dendritic cells (DCs) are essential to initiate immune responses associated with asthma inception. Since involvement of DCs in human asthma remains less clear, our study was undertaken to identify potential aberrant phenotype of DCs from atopic asthmatics as compared to control subjects, as well as their functional correlates to T-cell regulation.

Methods: Buffy coats were obtained from atopic asthmatics (AA, $n = 14$) and non-atopic controls (CT, $n = 15$). Myeloid (mDCs) and plasmacytoid (pDCs) were isolated by immunomagnetic purification. DCs were cultured overnight with or without TLR4 (LPS, 1 μg/ml) or TLR9 (CpG-DNA 1 μM) stimulation, before phenotype analysis (flow cytometry) and cytokine assays (ELISA). To explore the thymic stromal lymphopoietin (TSLP) pathway, we cultured mDCs for 2 days with TSLP, Der p allergen, LPS or a combination, and then cultured mDCs for 5 days with allogeneic CD4+ T cells from non-atopic donors in a paired manner (same T cells cultured with DCs from asthma or control).

Results: Both mDCs and pDCs from AA patients showed a higher expression of FcεRIα compared to controls. In addition, CCR7 – involved in the migration of DCs to regional lymph nodes – and TSLP receptor (TSLP-R) were also significantly upregulated on their mDCs. In contrast, immune costimulator ligand (ICOS-L), involved in Treg induction, was reduced on DCs from AA patients.

TNF-α secretion by resting mDCs from AA patients was higher compared to controls (0.29 ± 0.12 vs 0.09 ± 0.12 ng/ml; mean ± SD, $P = 0.007$). Upon TLR4 ligation, mDCs from AA patients secreted less IL-10 (3.157 ± 4.292 vs 6.775 ± 3.651 ng/ml; mean ± SD, $P = 0.0274$) and IL-12 p40 (0.99 ± 0.38 vs 4.84 ± 3.75 ng/ml; mean ± SD, $P = 0.0047$) than mDCs from controls. Moreover, TSLP-primed mDCs from AA triggered increased IL-5, IL-13 and TNF-α responses by T cells.

Conclusions: Our data suggest that (human) DCs from atopic asthmatics display a more mature phenotype and are primed to migrate and to promote Th2-mediated allergic inflammation, and further highlight the TSLP pathway as a potential therapeutic target in human asthma.

161
Mitochondrial reactive oxidative species is implicated in the pathogenesis of allergic asthmatic inflammation via regulation of NLRP3 inflammasome

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Background: Mitochondrial dysfunction is well known as a cause of mitochondria-related diseases. A major mechanism underlying the development of mitochondria-related diseases is thought to be an increase in intracellular oxidative stress. Recent studies have suggested that abnormality in mitochondria is associated with development of asthma. However, the precise role of mitochondrial dysfunction in asthmatic inflammatory diseases is not well understood.

Method: In this study, we used a newly developed mitochondrial reactive oxygen species (ROS) inhibitor, Necrox-5 to evaluate the role of mitochondrial ROS in the induction and/or maintaining of bronchial asthma, focusing on the relationship between mitochondrial ROS and NLRP3 inflammasome activation using a neutrophilic asthma murine model.

Results: Administration of Necrox-5 reduced the increase of mitochondrial ROS generation in airway inflammatory cells as well as bronchial epithelial cells, the NLRP3 inflammasome activation, the pathophysiological features of mice sensitised with ovalbumin (OVA) and lipopolysaccharide (LPS) and then challenged with OVA (OVA_{LPS}-OVA mice). Our results also showed that Necrox-5 suppressed the nuclear translocation of NF-kB and the increased expression of various inflammatory mediators in the lung. Lastly, neutralisation of IL-1b substantially reduced the airway inflammation and hyperresponsiveness in OVA_{LPS}-OVA mice.

Conclusion: These findings suggest that mitochondrial ROS play a critical role in the pathogenesis of neutrophil-dominant allergic airway inflammation through the modulation of NLRP3 inflammasome activation, providing a novel concept of the therapeutic strategy for allergen-induced airway disorders.

162
Role of IL-17 in the recruitment of B cells into bronchial tissue of severe asthmatic patients

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Background: Asthma is a chronic inflammatory disorder of the lung airways that is associated with airway remodeling and hyperresponsiveness (AHR). IgE plays an important role in triggering inflammatory responses responsible for asthma symptoms and pathogenesis. IgE levels increases in asthmatic patients both systematically and in lung tissues. However, the source behind this increase in IgE is still debatable. B cells play an important role in asthma development mostly via the production of IgE. In this proposal, we hypothesised that IgE is increased in lung tissue of asthmatic patients due to increased infiltration of B cells to this tissue. We have recently reported elevated expression of IL-17 in severe asthma. So we suggested that IL-17 is involved in the migration of B cells to the mucosal surface of the airways.

Method: We determined the number and pattern of infiltrated B cells into lung tissues of asthmatic compared to healthy subjects. Bronchial biopsies from asthmatic vs healthy subjects were stained for B cells marker (CD20) using Immunohistochemistry. Migration of B cells towards Th-17 cytokines were examined using Boyden Chamber migration assay. Mechanism of IL-17 induced B cell migration were tested using MAP kinase inhibitors to determine IL-17 induced MAP kinase pathways involved in this process.

Results: The number of CD20 positive cells in asthmatic biopsies was significantly higher than those in healthy subjects. Interestingly, we have also observed an increase in lymph follicle numbers in asthmatic airways compared to healthy subjects although this increase did not reach significance. Most of the lymph follicles were B cells follicles (CD20 positive cells) and were formed close to the epithelial layer.

Although B cells were shown to migrate *in vitro* towards both IL-17A and IL-17F, lower concentrations of IL-17F, compared to IL-17A, were sufficient to induce migration. Blocking IL-17 signaling using either anti-IL-17R antibodies or p38 MAP kinase inhibitors prevented *in vitro* migration of B cell towards IL-17.

Conclusion: These results indicated that IL-17 might drive the migration of B cells in the lung tissues of asthmatic patients. Activation of p38 MAP kinase seems to be required for IL-17 activity on B cells.

Oral Abstract Session 28

Food allergy: from sensitisation to manifestation

163
Investigation of delayed anaphylaxis to mammalian meat after food challenge in subjects with IgE to alpha-gal

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Background: Following the first reports of IgE antibodies to galactose-alpha-1,3-galactose (alpha-gal), we identified patients who presented with urticaria or anaphylaxis without an immediate cause who also had IgE antibodies to alpha-gal. This oligosaccharide is a blood group substance of the non-primate mammals and is present on both lipids and proteins. Evaluating 200 cases who had been to an ED with urticaria or anaphylaxis, over 80% had a history of eating meat in the 3–6 h before the start of the reaction.
Method: To investigate the delay in the food reactions, we carried out challenges (in 11 allergic subjects and 10 controls) using 50 g of pork or beef. The patients were monitored clinically by measurement of serum tryptase and histamine and by following *in vivo* basophil activation (as CD63 expression) for 6 h.
Results: Eight of 11 cases with IgE antibodies developed urticaria 2–5 h (mean 3.7) after meat challenge. Seven cases required treatment with oral anti-histamine and two subjects received epinephrine. None of the ten controls had symptoms or urticaria. In three of the allergic subjects, there was a significant rise in tryptase at the same time as the urticaria; none of the controls had a rise in tryptase. Among the patients, upregulation of CD63 was observed at the same time as urticaria, but was seen in two cases that did not have a reaction. CD63 increase was also seen in five controls who had no symptoms. Elevation in plasma histamine was observed in the cases but was not consistent.
Conclusion: Challenge studies confirmed the delayed time course reported by patients with IgE to alpha-gal in that urticaria and pruritis started 2–5 h after eating meat containing alpha-gal. Changes in tryptase and basophil activation reflected a similar time course to the appearance of symptoms. Activation of basophils may reflect a direct action of VLDL on basophils. The results suggest that basophil activation alone is not sufficient to give rise to systemic symptoms. The results are in keeping with a model where the delay in symptoms reflects the time taken for absorption of glycolipids and their modification into a form (e.g., LDL) that can activate tissue resident mast cells.

164
Not always omega-5 gliadin: specific IgE profiles in patients with wheat-dependent anaphylaxis

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Background: Wheat allergy as a cause of severe anaphylaxis is frequent. Thus, we aimed the value of extended serologic testing in a defined patient population with a suspicion of wheat-dependent anaphylaxis.
Method: We analyzed 59 patients with a corresponding diagnosis from 2009 to 2012. Patients received a skin prick test; total IgE and sIgE against wheat flour and omega-5 gliadin were measured followed by double-blind placebo-controlled food challenges. We measured sIgE against total gliadins, wheat lipid transfer protein (LTP) and alpha-amylase inhibitor.
Results: Fifty-six patients (34 women, 22 men) with a median age of 35 [14–71] years were challenged with wheat and/or wheat in combination with cofactors like exercise (71 times), additives (65 times), alcohol (29 times), additional food allergens (27 times) and acetylsalicylic acid (eight times). In 13/56 patients the challenge test was positive. Of those 13 positive patients eight were only positive if wheat was challenged in combination with cofactors. The sIgEs against wheat flour [− challenge: 1.39 (0.35–14.5) vs + challenge 1.29 (0.52–4.90)], total gliadins [− challenge 1.85 (0.57–20.9) vs + challenge 1.56 (0.74–7.01)] and omega-5 gliadin [− challenge 6.33 (0.35–47.6) vs + challenge 5.47 (4.12–9.59)] differed not significantly between the patient groups.

In the group with a positive challenge result total gliadin and omega-5 gliadin sIgE were only found in patients challenged with wheat in combination with cofactors.

Four patients were positive for gliadins without sIgE against omega-5 gliadin. One patient had a positive challenge test to wheat in combination with cofactors. Three of them had negative challenge tests and were released without the advice of a wheat-free diet; however, two experienced symptoms again.

Wheat LTP sIgE was positive in two patients. The alpha-amylase inhibitor was positive in four of 12 patients. These four had no sIgE to any gliadin; three of them had a positive challenge test with wheat alone.
Conclusion: We conclude that beside the omega-5 gliadin other gliadins may be important in wheat-dependent anaphylaxis, the role of cofactors for sensitisation and elicitation of wheat allergy will need further clarification.

165
The allergenic capacity of the 11S globulin Sin a 2 is related to its structural and immunological stability

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Background: The 11S globulin Sin a 2 is a marker to predict severity of symptoms after mustard ingestion and is involved in IgE-cross reactivity with tree nuts and peanut. Herein, we studied the structural and immunological integrity of Sin a 2 after gastric and duodenal *in vitro* digestions and the mechanisms of interaction of this allergen with human monocyte-derived dendritic cells (hmoDCs).
Methods: Sin a 2 was purified from mustard according to established methods. Digestions were performed in simulated gastric or intestinal fluid in presence of different lipid vesicles. Endolysosomal proteolysis was carried out with enzymes directly isolated from hmoDCs. The proteolytic products were analysed by SDS-PAGE, Coomassie blue staining or immunoblotting with anti-Sin a 2 serum or

sera from mustard allergic patients. Circular dichroism was used to quantify secondary structure content. The interaction with hmoDCs was assessed by flow cytometry and confocal microscopy.

Results: Sin a 2 was not resistant to the action of gastric enzymes but the presence of phosphatidylglycerol vesicles or lipids derived from the mustard food matrix avoided the gastric digestion of the allergen. Under these conditions, Sin a 2 remained immunologically active after 1 h of treatment. Sin a 2 was capable of binding *in vitro* to phosphatidylglycerol but not to phosphatidylcholine vesicles and this interaction was dependent on the pH. Although thermal processing and duodenal digestion did not alter the overall integrity and the IgE-binding capacity of Sin a 2, modifications on the secondary structure content of the allergen were observed. The 11S globulin Sin a 2 was able to specifically bind to hmoDCs. The allergen was uptaken by hmoDCs and subsequently degraded by endolysosomal proteases in a time-dependent manner. Sin a 2 exhibited a limited degree of proteolysis after treatment with endolysosomal enzymes which contributes to enhance its immunogenic capacity.

Conclusion: The structural features of the 11S globulin Sin a 2 and the presence of food matrix components allow this allergen to reach the intestinal mucosa in an immunological active form, where is able to specifically interact with hmoDCs, a key event leading to sensitisation and/or triggering of symptoms.

166
Cutaneous or respiratory peanut exposures induce sensitisation and allow a further oral sensitisation without Th2 adjuvant

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Background: The primary site of allergen exposure seems to play a role in the development of food allergy and some pieces of evidence suggest that non-gastrointestinal routes can lead to *de novo* sensitisation.

Aim: To assess the impact of respiratory or short-term cutaneous exposures to the purified major peanut allergen Ara h 1 or to roasted peanut extract (PE) on allergic sensitisation to Ara h 1 and then to investigate the impact of such pre-exposures on subsequent oral administration of PE.

Method: Female Balb/c mice were exposed to 100 μg of purified Ara h 1 or PE containing an equivalent amount of Ara h 1 on intact skin (i.e. not using a barrier-disrupted skin) for 40 min, six times at weekly interval (n = 20/group). Other mice were exposed intra nasally with to either 50 μg of Ara h 1 or the equivalent PE following the same timing. *Ex vivo* stimulation by Ara h 1 of cells from draining lymph nodes and spleen was performed after cutaneous or respiratory exposure (n = 4/group). Then, remaining pre-exposed mice received intra-gastric administrations of PE by gavage with or without the Th2 mucosal adjuvant cholera toxin (CT; six times, at weekly interval, n = 7–8/group). Specific antibodies to Ara h 1 were assayed on sera collected at different time points.

Results: Six skin applications of Ara h 1 led to significant Ara h 1 specific IgG1 production whereas PE was far less effective. After intranasal exposure, both administrations of Ara h 1 and PE led to an increasing Ara h 1 specific IgG1 production. In both cases, local and systemic production of Th2 cytokines was detected.

As expected, control mice (i.e. PBS pre-exposure) were sensitised only if they received gavages of PE with CT. Ara h 1 pre-exposition *via* the intra-nasal or cutaneous route led to the induction of specific IgE and IgG1 that was earlier than observed for control mice and that did not require the use of CT adjuvant. Sensitisation in absence of adjuvant was also observed in mice pre-exposed to PE *via* the intra-nasal route.

Conclusion: Cutaneous or respiratory exposure to a purified allergen or to a complex matrix such as a whole food extract may cause *de novo* sensitisation. Interestingly, such exposures promote early further sensitisation *via* the oral route without the need for Th2 mucosal adjuvant. Taken together, these results demonstrate the important role of environmental exposure in food allergy.

167
Peptide array based analysis of IgE and IgG4 epitopes for evaluation and prediction of milk allergy outgrow

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Background: Recently, peptide arrays have become powerful tools for the analysis of IgE and IgG4 epitope recognition profiles in clinical studies. For evaluation and prediction of clinical outcome of milk allergy patients, epitope analysis was performed using the milk peptide array.

Method: Milk peptide array consisting of a synthetic linear peptide library of six major cow's milk allergens with 16 amino acids overlapping by 13 (3-offset) was constructed using the piezoelectric ceramic micropump. IgE and IgG4 binding analysis was performed using the blood sera of allergy patients diluted by 10 fold with PBS-T containing 1% (w/v) ovalbumin. The binding of IgE and IgG4 were detected using the fluorescence-labeled anti-IgE and anti-IgG4, and fluorescent image was obtained using the microarray scanner. The fluorescence signal of each peptide spots was digitized and transformed to a z score for identification of epitopes that can distinguish allergic symptoms.

Results: The IgE and IgG4 recognition pattern of milk persistent allergy patients and outgrown children was evaluated. Higher numbers of IgE epitopes were found in α_{S1}-casein and characteristic IgE-binding sites were identified. Also, IgE epitopes suggestive of clinical outcome of allergy were found from the time series data analysis. The IgE and IgG4 recognition pattern of milk persistent allergy patients and outgrown children was evaluated. Higher numbers of IgE epitopes were found in α_{S1}-casein and characteristic IgE-binding sites were identified. Also, IgE epitopes suggestive of clinical outcome of allergy were found from the time series data analysis.

Conclusion: This study represents a preliminary step toward finding better diagnostic tools for analysis of food allergy and identified epitopes would be a promising peptide array contents that could indicate allergy outgrow.

168
Dietary long chain n-3 polyunsaturated fatty acid induced regulatory T cells contribute to the prevention of oral sensitisation to cow's milk protein in mice

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Background: Cow's milk allergy is one of the most common food allergies in children. Recently, we have shown that dietary supplementation with long chain n-3 polyunsaturated fatty acids (n-3 LCPUFA) largely prevent allergic sensitisation in a murine model for cow's milk allergy. Aim of this study was to assess the role of regulatory T cells (Treg) in the prevention of food allerby by n-3 LCPUFA.

Methods: Donor mice were fed a control or fish oil diet before and during oral sensitisation with whey. Acute allergic skin response, serum immunoglobulins as well as dendritic cell (DC) and T-cell subsets in mesenteric lymph nodes (MLN), spleen and/or small intestine were assessed. Besides serum transfer experiments, splenocytes of whey-sensitised donor mice fed either the control or fish oil diet were adoptively transferred to naïve recipient mice. Recipient mice received splenocytes either or not *ex vivo* depleted of CD25+ cells, or MACS isolated CD4+CD25+ Treg. Recipient mice were sham- or whey-sensitised and fed control diet.

Results: The acute skin response ($P < 0.001$) as well as whey-IgE ($P < 0.05$) and -IgG1 ($P < 0.001$) levels were reduced in sensitised donor mice fed the fish oil diet as compared to the control diet. Serum transfer confirmed the Th2 type humoral response to be suppressed since sera of fish oil fed sensitised mice had a diminished capacity to induce an allergic response in naïve recipient mice compared to control sera ($P < 0.001$). Furthermore, the acute skin response was diminished upon passive sensitisation with hyperimmune serum in fish oil fed naïve recipient mice ($P < 0.001$) indicating suppression of the effector response by n-3 LCPUFA. Fish oil fed whey-sensitised donor mice showed an increased percentage of Treg inducing CD11b+CD103+CD8α- DC in MLN ($P < 0.05$) in association with enhanced FoxP3+ Treg in spleen ($P < 0.05$) and intestine ($P < 0.05$) compared to sham mice. In whey-sensitised recipient mice transferred with splenocytes from whey-sensitised fish oil fed donor mice, the acute allergic skin response was similar to sham-sensitised recipients. *Ex vivo* depletion of Treg prevented this transfer of tolerance. Transfer of CD4+CD25+ Treg (85% was FoxP3+) from fish oil fed whey sensitised donors prevented the acute allergic skin response most pronounced.

Conclusion: FoxP3+ Treg play an important role in whey allergy prevention by n-3 LCPUFA.

Oral Abstract Session 29

Allergic inflammation

169

Morphometric to proteomic combined investigation improves information on severe persistent asthma

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Background: Asthma is a complex disorder characterised by airway inflammation and reversible airflow obstruction. It is further distinguished by multiple phenotypes that might differ based on age of onset, risk and triggering factors and patterns of severity both during acute exacerbations and, on a more chronic basis, as reflected by variably reversible loss of lung function. Severe persistent asthma causes a substantial morbidity and mortality burden and is frequently not-well controlled despite intensive guideline-based therapy. Asthma is characterised by airway inflammation, bronchial hyperresponsiveness and airway remodeling. Omalizumab is a monoclonal anti-IgE antibody synthesised for therapy of asthmatic patients with inadequately controlled severe persistent allergic asthma despite optimal controller treatment. Aim of the study was determination of differential expression levels of proteins (target/marker protein), identification of protein functions interrelationships of proteins in the bronchial tissue in patients with severe persistent asthma treated with omalizumab.
Methods: Eleven patients suffering from severe persistent atopic (IgE-mediated) asthma were recruited. All patients were treated with omalizumab. Two biopsies were obtained from each patient using a flexible bronchoscope before and post treatment with omalizumab. Reticular basement membrane thickness was measured by light microscope image analysis and proteins extracted from FPPE tissues were analyzed using MudPIT innovative proteomic approach.
Results: MudPIT proteomic approach allowed to identify more than 800 distinct proteins, and MAPROMA software permitted characterisation of differentially expressed proteins comparing both pre- vs post-treatment and Responder vs non-Responder patients. Specifically, based on metabolic pathways, potential biomarkers resulted mainly correlate to extracellular matrice, cytoskeleton, folding and glycolysis. Finally, proteomic profiles allowed sample 'stratification' and a good correlation with the morphometric data.
Conclusion: The large scale analysis of proteome of asthma severe affected patients, combined with computational tools and morphometric analysis, is a good approach for reliable patient stratification and for identification of perturbed protein pathways. In addition, combining proteomic and morphometric analysis it has been possible to correlate histological and molecular aspects, and this allows to improve knowledge of asthma severe.

170

Exosomes secretion by eosinophils: a possible role in asthma progression

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Background: Eosinophils secrete cytotoxic granules which are involved in initiation and propagation of diverse inflammatory responses as asthma. Our hypothesis is that some of these granules are exosomes which contain special lipid and protein compositions and carry mRNAs and miRNAs which can transfer to the recipient cell and participate in the progression of these diseases. Our aims are to characterise the eosinophils exosomes and to investigate their role in asthma.
Method: Exosomes derived from eosinophils were characterised by WB developed by CD63, nanoparticle tracking analysis (NTA) to estimate the size distribution and concentration, flow cytometry and by electron microscopy images. Furthermore, we study the capacity of eosinophils to generate intracellular precursors of exosomes (multivesicular bodies, MVBs) by electron, confocal and fluorescence microscopy and flow cytometry analysis. Eosinophils were labelled with the specific marker of MVBs (LBPA), and the reporter of endosomal vesicles (CD63).
Results: We found that eosinophils can produce and secrete exosomes. Exosomes purified from eosinophils from healthy and asthmatic subjects were CD63+. The amount of exosomes was increased after stimulation with INF-γ. NTA showed that the size of eosinophils exosomes was into the characteristic exosomal range (80–160 nm). The flow cytometry of these samples showed that they were CD63+ structures, different from apoptotic bodies (DNA^-). We also found that the exosomes production from asthmatic subjects was higher than healthy subjects.

We observed that eosinophils generate intracellular MVBs. It was confirmed by the colocalisation of LBPA and CD63 in these vesicles. This result was corroborated by electron microscopy images. The fluorescence microscopy and flow cytometry study of the cells showed that MVBs increase after stimulation with INF-γ.
Conclusion: Our findings provide the first evidence that eosinophils produce MVBs and secrete exosomes. It is possible that they have an important implication in the progression of asthma. Moreover, they also could be considered as a future biomarker.

171

Anti-allergic effect of anti-Siglec-F through reduction of eosinophilic inflammation in murine allergic rhinitis

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Background: Sialic acid-binding Ig-like lectin-F (Siglec-F) in mice and its functional paralog Siglec-8 in humans are transmembrane receptors that play a role in the apoptosis of eosinophils. We aimed to evaluate the therapeutic potential of anti-Siglec-F antibodies in a murine model of allergic rhinitis.
Method: Twenty-eight BALB/c mice were used. In Group A (control group, $n = 7$), mice were sensitised and challenged with

saline. In Group B (ovalbumin (OVA) challenge group, $n = 7$), OVA was used for intraperitoneal sensitisation and intranasal challenge. Mice in Group C (Control IgG group, $n = 7$) or those in Group D (anti-Siglec-F group, $n = 7$) had been given rabbit control IgG or anti-Siglec-F antibody injections, respectively. We assessed the number of nose-scratching events; serum total/OVA-specific IgE; the number of eosinophils, neutrophils and lymphocytes in bronchoalveolar lavage (BAL) fluid; histopathologic changes in nasal cavity tissues; and the levels of IL-4, IL-5 and IL-13 in BAL fluid.

Results: Mice in Group D had significantly less nose scratching. Serum total and OVA-specific IgE were not significantly changed. The number of eosinophils in BAL fluid and in the lamina propria of the nasal cavity mucosa was significantly decreased with anti-Siglec-F antibody treatment. The levels of Th2 cytokines such as IL-4, IL-5 and IL-13 were also significantly decreased with anti-Siglec-F antibody treatment.

Conclusion: Anti-Siglec-F antibody has beneficial effects in a mouse model of experimental allergic rhinitis.

172
Regulation of neutrophil-mediated antimicrobial immunity by type 2 inflammation

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Background: Neutrophils are innate immune cells constituting a rapid first line of defense against bacterial infections. Interestingly, allergic patients show an increased susceptibility towards certain bacteria usually contained by neutrophils. Moreover, acute type 2 inflammation, as seen in allergic conditions or during infection with parasites, displays a striking lack of neutrophils, suggesting that active or passive mechanisms might inhibit neutrophils during type 2 responses.

Methods: We used *in vivo* and *in vitro* systems for measuring functional aspects of murine and human neutrophils, including cytokine-deficient and mixed bone marrow chimeric mice infected with different relevant human pathogens, such as *Salmonella thyphimurium*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes*, as well as neutrophil migration and cytotoxicity assays.

Results: We found that skewing of the immune system towards type 2 immunity led to dominant impairment of several functional aspects of murine and human neutrophils. As a result, animals prone to type 2 immunity rapidly succumbed to several pathogens usually contained by neutrophils, whereas inhibition of pathways required for type 2 responses led to the survival of mice following a challenge with the same pathogens. Immunity against these pathogens was mediated chiefly by neutrophils, and not by other innate immune cells, nor T, B or natural killer cells, as demonstrated by using cell-specific knockout animals and depleting antibodies. We found that type 2 inflammation inhibited neutrophil migration and recruitment from the bone marrow to the circulation, and neutrophil anti-bacterial cytotoxicity.

Conclusions: Based on these data, we postulate that type 2 inflammation regulates neutrophil function and migration, thus impacting innate anti-bacterial immunity, which might explain why patients with allergic conditions are prone to infections with certain bacteria. Moreover, these data reveal novel targets for tackling neutrophils during inflammatory pathologies.

173
Specific targeting of human allergic effector cells using gold nanoconjugates

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Background: Several intracellular signalling inhibitors (e.g. calcineurin and Syk inhibitors) are effective at blocking allergic mediator release from mast cells and basophils but rarely used systemically due to ubiquitous expressions of these signalling proteins. This problem would be solved if we could specifically target mast cells and basophils with these inhibitors. We recently discovered that gold nanoparticles (AuNPs) are a biocompatible platform for non-toxic delivery of such agents due to their unique physicochemical properties [1]. Since AuNPs permit ready conjugation with both anti-allergic drugs and antibodies that recognise mast cells and basophils our aims were to assess specific targeting of allergic effector cell function using AuNPs conjugated with signal transduction inhibitors.

Methods: Purified human basophils and LAD2 human mast cells [2] were used for investigations of AuNPs conjugated to anti-CD203c using a Biacore amine-coupling kit. Specificity of binding was determined using anti-CD203c-conjugated AuNPs that were immobilised onto 96-well ELISA plates using LPS (coupled to AuNPs with 1–2 molecules per particle) and anti-lipid A antibodies coating the wells. Glutathione-SH fusion was used to generate AuNPs conjugated with the calcineurin inhibitor ascomycin.

Results: AuNPs alone did not affect human basophil mediator release or viability. However, AuNPs conjugated with anti-CD203c readily -and specifically- bound to basophils. When conjugated with ascomycin, these AuNPs strikingly inhibited human basophil degranulation at effective concentrations of the inhibitor of 5 nM (20-fold more potent than ascomycin alone). Similar results were obtained using mast cells and unpurified basophils present in mixed leukocyte preparations, suggesting specific targeting of these cells.

Conclusions: We demonstrate successful targeting of allergic effector cells using gold nanoconjugates. This technology is of potential interest for the therapeutic use of highly effective signalling inhibitors in allergy without side-effects. The principle can be applied to a large number of agents (drugs, toxins, siRNA) and to a variety of experimental and clinical settings.

174
Major basic protein enhances apoptosis and production of GM-CSF and IL-17 in bronchial epithelial cells infected with respiratory syncytial virus

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Background: Respiratory syncytial virus (RSV) is an important exacerbating factor of wheezing and acute asthma. However, the precise mechanisms responsible for viral infection-induced exacerbations of asthma are uncertain. To elucidate the role of eosinophilic inflammation in the pathogenesis of virus-induced asthma, we investigated the effects of eosinophil granule proteins on bronchial epithelial cells infected with RSV.

Method: Morphological changes and cytopathic effects in human type II pulmonary alveolar epithelial cells, A549, infected with RSV and/or eosinophil granule proteins such as major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN) were observed by microscopy. Apoptosis/necrosis was evaluated by flow cytometric analysis,

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and trypan blue exclusion test. We also measured 17 types of cytokines and chemokines in A549 cell supernatants and eight types of phosphorylated proteins in A549 cells infected with RSV.

Results: Although RSV alone did not affect cytopathic effects of A549 cells, high concentrations of MBP or combination of four granule proteins resulted in the effects. MBP or EPO, but not ECP or EDN, induced the cytotoxicity of A549 cells infected with RSV. MBP enhanced the apoptosis/necrosis in A549 cells infected RSV. In A549 cells treated with MBP alone, productions of interleukin (IL)-2, 4, 5, 7, 10, 12, 13, 17, interferon (IFN)-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and macrophage inflammatory protein (MIP)-1β were significantly increased compared with controls. Notably, levels of GM-CSF and IL-17 in both RSV and MBP-treated cells were significantly greater than in those treated with MBP alone. Furthermore, MBP enhanced the phosphorylation of the extracellular signal-regulated kinase (ERK) 1/2, p38 mitogen-activated protein kinase (MAPK), Jun-N-terminal protein kinase (JNK), and signal transducer and activator of transcription (STAT) 3 in A549 cells infected with RSV.

Conclusion: Eosinophil granule proteins, specifically MBP, damage bronchial epithelial cells infected with RSV and that GM-CSF and IL-17 productions and MAPK family are involved in these responses. These results suggest that eosinophilic inflammation may be closely associated with the pathophysiology of RSV-induced acute exacerbations of asthma.

Oral Abstract Session 30

New insights into the pathomechanisms and clinical characteristics of drug allergy

175
Copy number variations in PTGS1, PTGS2, LTC4S, ALOX5 and PTGER1-4 genes associated with AERD and MNSAID-UA

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Background: Non-steroid antiinflammatory drugs (NSAIDs) are the compounds more frequently involved in hypersensitivity drugs reactions. Aspirin-exacerbated respiratory disease (AERD) has been the most studied model; although Multiple NSAID-triggered urticaria and/or angioedema and anaphylaxis in patients without pre-existing chronic urticaria (MNSAID-UA) is considered the most frequent entity. A non-specific immunological mechanism based on pharmacological properties of the NSAIDs and its capacity to unbalance the metabolic pathway of arachidonic acid was proposed for AERD and suggested for MNSAID-UA. Copy number variantions (CNVs) are DNA segments >1 kb in length that are present at a variable copy number. CNVs may affect the expression of genes and be associated with susceptibility to diseases. The aim of this study was to analyze clinical association of CNV in PTGS1, PTGS2, LTC4S, ALOX5 and PTGER1-4 genes with MNSAID-UA and AERD.
Method: All the participants were recruited from Allergy Services integrated into Spanish Network for allergic diseases (RIRAAF). We studied a total of 150 patients with AERD, 310 MNSAIDS-UA and 315 healthy controls. CNVs of these genes were analyzed using TaqMan copy number assays designed to hybridize within the open reading frame into the each gene. The results were analyzed by means of the Copy Caller Software. Statistical analysis was done using SPSS 11.5 program.
Results: All control individuals included had two copies of each gene analyzed. In case of patients, there were no CNVs in genes PTGS1, PTGS2, LTC4S, PTGER2, PTGER3, PTGER4. Nevertheless, we found some differences in CNVs of genes ALOX5 and PTGER1. Concerning to ALOX5, we identified seven AERD patients (5.0%; AERD vs Controls $P < 0.001$) and 13 MNSAIDS-UA patients (4.17%; MNSAID-UA vs Controls $P < 0.001$) with a single copy of the gene. There are not significant differences between AERD and MNSAIDs patients in CNVs of ALOX5. Concerning to PTGER1, we identified 19 MNSAIDS-UA patients (6.11%; MNSAID-UA vs Controls $P < 0.0001$) with a single copy of the gene. All the AERD patients showed two copies of PTGER1.
Conclusion: We analyzed for the first time the association between CNVs in PTGS1, PTGS2, LTC4S, ALOX5 and PTGER1-4 genes and MNSAID-UA and AERD. We found statistically significant differences in CNVs of genes ALOX5 and PTGER1 between healthy controls and MNSAID-UA and AERD. Whether these variations imply a dysfunctional gene expression required further studies.

176
Allergic reactions to infliximab: a pilot study to identify IgE-binding epitopes

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Background: Hypersensitivity reactions to biologicals are increasing. Whereas Cetuximab, a chimeric mouse-human IgG1 monoclonal antibody approved for oncological target therapy, has been shown to elicit anaphylaxis via pre-existing IgE to the disaccharide galactose-[alpha]-1,3-galactose (alpha-GAL), reactions to Infliximab, a chimeric antibody as well and grown in the same cell system, have not been observed during treatment initiation, suggesting different epitopes or a different epitope presentation. The aim was to identify IgE-binding epitopes on biologicals as a prerequisite for a specific assay for IgE-detection as a measure of risk management.
Method: Among 19 patients in the project, seven with rheumatic diseases, aged 40–76, three male, four female, had infusion reactions to Infliximab which occurred after inter-individually different intervals (4th to 9th Infliximab application plus 2 exceptions). 4/7 patients reacted immediately up to 30 min, 2/7 after 24 h, and 1/7 after 48 h. A standardised questionnaire was developed to evaluate their allergy status and the details of the drug reaction. Forty sera of healthy donors were included. Anti-Infliximab IgE were analyzed via immunoblots with different target antigens. Anti-alpha-GAL-IgE were detected via HSA-alpha-GAL (Dextra Laboratories, Reading) and alpha-GAL ImmunoCAP (Platts-Mills, Virginia). The serum of a Cetuximab allergic patient was used as positive control, because of its strong reaction with Infliximab on immunoblot.
Results: 2/7 patients were classified as type alpha-reaction. 5/7 showed typical immediate type reactions (type beta reactions) (facial swelling, dizziness, collapse, hypertension, hypotonia, flush, and rash). 6/7 sera had anti-Infliximab IgE in the respective immunoblot (two patients with type α-reaction and 4/5 with a typical immediate infusion reaction). With regard to anti-alpha-GAL-IgE, 2/7 infliximab sera were negative in the HSA-alpha-GAL blot, 3/7 were positive, and 2/7 only showed a weak positive reaction. 18/40 healthy individuals were IgE-positive in the Infliximab blot. All seven sera were negative in alpha-GAL-CAP analysis.
Conclusion: 2/7 patients showed IgE to Infliximab, but not to alpha-GAL, neither in HSA-alpha-GAL blot, nor in alpha-GAL-CAP, although Infliximab is a chimeric antibody like Cetuximab. The results indicate the presence of a different IgE-binding epitope on Infliximab.

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177
Development of methods for the detection and identification of candidate target proteins for haptenation by amoxicillin in human serum

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Background: In the development of hypersensitivity reactions to beta-lactam antibiotics, protein haptenation is a critical process. In the case of amoxicillin (AX), the chemical characteristics of the haptenation process and the identity of the target proteins have not been completely elucidated. Here we have combined immunological and mass spectrometry approaches in order to get insight into the nature of AX targets and of the haptenation process.
Method: HSA and serum proteins were incubated with increasing concentrations of AX at different pH conditions. AX-modified proteins were separated by SDS-PAGE and the ability of various monoclonal anti-AX antibodies to detect AX-protein adducts by Western blot was evaluated. Serum proteins modified by AX were then identified by 2D-electrophoresis and immunological detection followed by peptide mass fingerprint analysis using MALDI-TOF/TOF MS/MS. Samples of HSA modified by increasing AX concentrations were analyzed by MALDI-TOF MS in order to estimate the stoichiometry of the modification.
Results: The formation of HSA-AX adducts is dependent on the concentration of AX and the pH of the incubation, with the extent of modification being higher under basic conditions compared with physiological conditions. HSA modified by AX *in vitro* can be detected by immunological and proteomic methods. Moreover, HSA, transferrin and heavy and light chains of immunoglobulins were identified as prominent AX-adducted proteins in AX-treated human serum. Transferrin appears to be modified by AX in a high proportion taking into account the plasma concentration of this protein whereas other abundant proteins like apolipoprotein or haptoglobin were negative in this assay.
Conclusion: This study sets the basis for the sensitive detection and identification of AX-protein adducts. Although the best detection results have been obtained under non physiological conditions, this is a first approximation to characterise the modification of serum proteins by AX and to identify some candidate target proteins in human serum. Our results show that, in addition to HSA, other serum proteins are adducted by AX and the potential involvement of these newly identified adducts in the immune response to AX deserves further study.

178
Non-steroidal antiinflamatory drug hypersensitivity in childhood

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Introduction: Non-steroidal antiinflamatuary drugs (NSAIDs) have been used frequently in children for analgesic and antipyretic purposes. The aim of this study is to develop a diagnostic approach to children with a history of NSAID hypersensitivity and to determine the clinical and epidemiological features of NSAID allergy in children.
Method: The children with a history of suspected allergic reaction to any of the NSAIDs who admitted to our department between January and September 2012 were included in the study. After filling ENDA questionnaire, oral provocation tests were performed initially with the offending drug. If the initial test was positive, the other provocation tests were done with acetylsalicylic asid (ASA) and/or other NSAIDs to find out the cross reactive agent or safe alternative drug. A positive oral provocation test was defined as the occurence of an objective physical finding related with hypersensitivity or a 15% decrease in FEV1 in lung function test during oral provocation test.
Results: Thirtyone patients [8.5 (6–10.8) years, median (interquartile range), 17 male) with a history of 47 NSAID hypersensitivity reactions were enrolled. We performed 53 oral provocation tests in study population with 18 positive results in 11 children. Five patients exhibited positive oral provocation tests with ≥2 NSAIDs. Angioedema, urticaria, macular rash, conjunctivitis and 15% decrease in FEV1 were the positive findings in 10 (55.6%), 4 (22.2%), 3 (16.6%), 3 (16.6%), 5 (27.8%) of the positive reactions respectively. None of the patiens with a decrease in FEV1 was diagnosed as asthma before. Children with or without NSAID hypersensitivity did not differ in terms age, gender, atopy, atopic disease and chronic illness. The prevalence of history of anaphylaxis during NSAID induced reaction was higher in NSAID allergic group compared to non allergic group ($P = 0.04$). Occurrence of angioedema after offending drug intake in the history of the patient was found to increase the risk for NSAID allergy in logistic regression analysis (OR: 7.6, %95 CI: 1.5–37.9, $P = 0.014$).
Conclusion: Actual drug allergy is frequent (35%) among children with a suspected history of hypersensitivity reaction to NSAIDs. Angioedema history increases the risk for true NSAID allergy and it's the most frequent positive physical finding during oral provocation tests.

179
Non-steroidal antiinflammatory drug-induced urticaria/angioedema does not contraindicate non-steroidal antiinflammatory drug treatment when necessary

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Background: Urticaria and/or angioedema (U/AE) are the most frequent and less severe forms of non-allergic hypersensitivity reactions to non-steroidal antiinflammatory drugs (NSAID). Management of NSAID-induced U/AE includes (i) the avoidance of the culprit drug and of cyclooxygenase (COX)-1 inhibitors; (ii) the use of weak COX-2 inhibitors; and/or (iii) desensitisation to aspirin. Since these possibilities may have drawbacks, we tested the possibility of preventing NSAID-induced U/AE by the administration of antihistamines and/or a combination of antihistamines and leukotriene antagonists.
Objective: To test the preventive effect of antihistamines and/or leukotriene antagonists on the development of U/AE in patients with a history of NSAID hypersensitivity confirmed by a positive challenge.
Methods: A single, placebo-controlled, oral challenge using the culprit NSAID was applied to 65 patients with a history of NSAID-induced U/AE. In the case of recurrence of the symptoms, another oral challenge was performed under premedication with antihistamines alone or combined antihistamines/leukotriene antagonists.
Results: 59/65 patients (90%) tolerated a normal dose of NSAID, confirming previous data on the poor reproducibility of non-allergic hypersensitivity reactions to NSAIDs upon challenge. Of the 6/65 patients who experienced recurrence of the U/AE upon NSAID challenge, antihistamines and combined antihistamines/leukotriene antagonists prevented the hypersensitivity reactions in two and three of them, respectively. Only one patient/65 still developed a moderate NSAID-induced urticaria despite the double premedication.

Conclusion: Treatment by NSAIDs at normal doses is possible and well-tolerated in patients who have experienced NSAID-induced U/AE, which could be prevented by the concomitant use of antihistamines and leukotriene antagonists.

180
Toxic epidermal necrolysis in northeastern China: a hostpital-based study

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Background: Toxic epidermal necrolysis (TEN) has been reported to be associated with considerable morbidities and mortality rate. We evaluated the outcomes of patients treated in a university hospital for toxic epidermal necrolysis over a 13-year period and compared the outcomes of a subset of patients treated with intravenous immunoglobulin (IVIG) plus systemic corticosteroid with those managed by conventional systemic corticosteroid monotherapy.
Method: We retrospectively studied 47 patients with TEN in a university hospital in northeastern China between 2000 and 2012 to evaluate the treatment of TEN and prognostic factors for the mortality of TEN. The severity of illness of the patients was evaluated by independent risk factors including body surface area involved at day 1, age, temperature, heart rate, blood pressure, serum urea level, serum glucose level, serum albumin level, serum alanine aminotransferase level, aterial oxygen pressure, serum total IgE level, ESR level.
Results: Forty-seven patients were included in this study. The average age was 51.7 years; the male/female ratio was 24/23. Antibiotics were implicated in causation in 21 patients (45%). Other causative medications included analgesics (7, 14.9%), antiinflammatory agents (5, 10.6%), Chinese traditional medications (5, 10.6%), antiepileptic drugs (2, 2.1%), allopurinol (2, 2.1%). The underlying disorders included hypertension (15), diabetes mellitus (13), cardiovascular diseases or cerebrovascular diseases (11). Twenty-two were treated with systemic corticosteroid alone. Twenty-five were treated with IVIG plus systemic corticosteroid. Antibiotics were used in all 47 patients. Antifungal drugs were used in 17 patients. Albumin was administered in 30 patients. The average length of stay in all 47 patients was 20 days. Three patients (6.38%) died of pulmonary infection (two cases) or disseminated intravascular coagulation (one cases). All of the three patients were treated with IVIG plus systemic corticosteroid.
Conclusion: IVIG plus systemic corticosteroid and systemic corticosteroid monontheray are both effective therapies in TEN. It seems that the prognosis of TEN depends mostly on the severity of the disease, but not merely on the choice of treatment.

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Oral Abstract Session 31

Clinical issues addressed by molecular allergology

181

The additional values of microarray allergen assay in the management of polysensitised patients with respiratory allergy

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Background: The IgE response in atopic patients is directed against specific components from an allergenic source. The traditional diagnostic methods use whole extracts, containing allergenic, non allergenic, and cross-reactive molecules. This may pose diagnostic challenges in polysensitised patients. Microarray techniques can detect protein-specific IgE against multiple molecules at the same time, but their value in term of additional information and economic saving has not been yet defined.
Objective: We assessed the additional diagnostic information provided by an allergen microarray in a large population of polysensitised subjects in real life.
Methods: In this multicentre study, allergists were required to carefully record diagnosis and treatment of consecutive patients referred for asthma/rhinitis, using the standard methodology (history, skin prick test, IgE assay). Then, a microarray allergen assay. Was carried out. Clinicians were required to review their diagnosis/treatment according on microarray results. An economical analysis was also performed.
Results: Three hundred and eighteen allergic patients (30% reporting also non-respiratory symptoms) and 91 controls were enrolled. The clinicians reported at least one additional information from the microarray in about 60% of patients, this resulting in therapeutic adjustments. In 66% of patients IgE to pan-allergens were detectable, being this clinically relevant in 38% of patients with polysensitisation to pollens. The microarray assay resulted to be economically advantageous when more than 10 recombinant/purified molecules would be required for a satisfactory diagnosis.
Conclusion: Microarray IgE assay represents an advancement in allergy diagnosis, as a third-level approach in polysensitised subjects, when the traditional diagnosis may be problematic. When 10 or more single recombinant allergens are required for diagnosis, microarray is economically superior.

182

Component-resolved molecular diagnosis of house dust mite allergy using a comprehensive repertoire of recombinant allergens

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Background: Allergen-specific immunotherapy (SIT) is the only radical treatment of allergic disorders. However, current SIT with crude allergen extract has limitations including a risk of systemic anaphylaxis and a need for long-term medication. Defined SIT vaccine only composed of sensitised allergen molecules would be preferable to avoid fatal side effects as well as to improve its efficacy. Objective of this study is to develop the component-resolved diagnosis (CRD) of house dust mite allergy using a comprehensive repertoire of recombinant allergens.
Methods: We produced 23 major house dust mite allergens as soluble recombinant proteins using the *Escherichia coli* cold shock expression system. Individual IgE-binding profile against those allergen molecules was evaluated by immunoblotting and ELISA. We also tested whether this CRD system was also useful for detecting sensitised allergens in those who had been diagnosed as 'non-atopic' asthmatics with RAST score for dust mite was zero.
Results: We found that mite-allergic patients showed quite differential IgE-binding signatures against allergen molecules, which enabled us to easily determine the sensitised allergens in the individual patients. Moreover, we found that non-atopic type of asthmatic patients showed positive IgE-binding against recombinant mite allergens, suggesting that this CRD system was also beneficial in the detection of sensitised allergens in the 'mite-negative' asthmatics diagnosed by conventional CAP-RAST test.
Conclusion: The recombinant allergens are applicable for the development of CRD of house dust mite allergy.

183

IgE vs IgG4 epitopes of the peanut allergen Ara h 1 in patients with severe allergy

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Background: Development and maintenance of tolerance to food allergens may be associated with increased levels of specific IgG4. It has been suggested that co-localisation of IgG4 and IgE binding epitopes may be of great significance for the tolerance, where IgG4 may act by blocking IgE binding to the allergen. However, recent studies have demonstrated the very importance of the IgG4-epitope affinity for the blocking ability. Studies comparing IgE and IgG4 binding epitopes mainly focus on the identification of linear epitopes. Peanut allergy is one of the most severe and persistent forms of food allergy. The importance of conformational epitopes, of the major peanut allergen Ara h 1, has been demonstrated. The aim of this study was to compare Ara h 1-specific epitope patterns for IgE and IgG4 in patients with severe peanut allergy applying a method suitable to identify both linear and conformational epitopes.
Method: Ara h 1-specific IgE and IgG4 epitope patterns were examined by competitive

immunoscreening of a phage-displayed random 7-mer peptide library using polyclonal IgE and IgG4 from three individual patients suffering from severe peanut allergy. The resulting peptide sequences were mapped on the surface of a 3D model of the Ara h 1 molecule to mimic epitopes by the use of a computer-based algorithm.

Results: All identified epitope mimics corresponded to conformational epitopes. Each individual peanut allergic patient had his/her own distinct IgE as well as IgG4 epitope binding profile. Although three motifs were identified for all three patients and accounted for half of all identified IgE epitope mimics, no consensus motifs were identified for IgG4. Even though the epitopes overlapped, the IgG4 binding epitope mimics were more heterogeneous than the IgE binding epitope mimics. In addition a higher epitope binding affinity for IgE than IgG4 was indicated.

Conclusion: This preliminary study using competitive immunoscreening of a phage-displayed peptide library successfully distinguished IgE binding patterns from IgG4 binding patterns. The method allows an identification of both, linear and conformational epitopes and gives information on the specificity, diversity and affinity of the identified epitope mimics. This could be a valuable tool to study the balance and dynamics of the antibody IgE- and IgG4-repertoire, and increase the knowledge and understanding of the mechanisms involved in the development of allergy and tolerance.

184

Prevalence estimation of IgE, IgG and IgG4 recognition of a single epitope of grass pollen allergen Phl p 5 in an Italian allergic population by a mimotope approach

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Background: Sensitisation to grass pollen is one of the most common findings in human patients suffering of allergic rhinitis or asthma. A majority of grass pollen specific IgE is directed against the highly cross-reactive group 1 and 5 allergens. A recent study from our group could show that a single epitope mimic of Phl p 5, designated H32, exhibited strong antiinflammatory effects in a mouse asthma model. In order to estimate the potential impact of H32 for treating allergic patients, we aimed to investigate the prevalence of specific IgE, IgG and IgG4 antibodies to H32 in a large population.

Method: A synthetic dimer of H32 was spotted in addition to the standard array of allergens found on the ImmunoCAP ISAC® 112 microarray. Nine hundred and ninety-two randomly incoming patients to an allergy outpatient clinic in Rome were assessed for H32 specific IgE during their standard allergy diagnosis using the experimental ImmunoCAP ISAC chip. In 678 of these subjects also specific IgG4 were measured. The outpatient cohort analyzed here is thus not representative for a whole unselected population.

Results: Phl p 5 specific IgE was found in 28% of the patient population, from which 11% reacted to the H32 mimotope. Of the 678 subject being additionally screened for specific IgG4 antibodies, 45.9% showed specific IgG4 reactivity to Phl p 5 and 28% towards H32, with the binding activity to mimotope and allergen being significantly correlated.

Conclusion: Within the population randomly visiting an outpatient clinic for suspected allergy, the IgE epitope mimicked by mimotope H32 represents i) a IgE reactive epitope in 39% of Phl p 5 allergic patients of the IgE-reactive part of Phl p 5 and ii) an important part (being recognised by about 60% of Phl p 5 reactive patients) of IgG4 reactivity to major grass pollen allergen Phl p 5. H32 may therefore be suitable for development of novel mimotope-based immunotherapy strategies in patients allergic to Phl p 5.

185

In-depth characterisation of the recombinant Bet v 1 allergen established as a chemical reference substance by the European Pharmacopoeia Commission

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Background: Virtually all birch pollen allergic patients exhibit IgE against Bet v 1, and a majority of those patients react exclusively to this relevant allergen. In this context, we developed a recombinant Bet v 1.0101 allergen (rBet v 1) to be used for specific immunotherapy. Because such a pharmaceutical grade recombinant allergen is thoroughly characterised and controlled, it was further evaluated by the European Pharmacopoeia as a possible Chemical Reference Substance. Herein, we present data assessing rBet v 1 structure, as well as the epitopes captured by specific monoclonal antibodies (mAbs) used to perform Bet v 1 quantification by ELISA.

Method: Current good manufacturing practices (cGMP) grade rBet v 1 was expressed in *Escherichia coli* and purified to homogeneity by chromatography. Primary, secondary, tertiary and quaternary structures were evaluated using electrophoresis, liquid chromatography (LC), circular dichroism, fluorescence, mass spectrometry (MS) and X-ray crystallography.

Results: The 159-amino-acid sequence of rBet v 1 was fully covered using MS/MS and low levels of both deamidation (of asparagine 47) and oxidation (of methionine 139) were confirmed. Secondary and tertiary structures of rBet v 1 were confirmed to be highly similar to the ones determined from natural Bet v 1 or from the literature. The high purity of rBet v 1 bulk product was also demonstrated using different methods including LC and MS. Moreover, the 2 mAbs used for ELISA quantification (5B4 and 6H4) were shown to react with distinct, either linear discontinuous or conformational Bet v 1 epitopes respectively, as characterised by hydrogen/deuterium exchange MS studies.

Conclusion: Overall, cGMP grade rBet v 1 is highly homogeneous and structurally similar to natural Bet v 1. The precise epitopes recognised by mAbs used for ELISA quantification were identified. Based on those properties, rBet v 1 was recently adopted by the European Pharmacopoeia Commission as Chemical Reference Substance Y0001565, and is currently used as a reference standard for the determination of Bet v 1 content by ELISA.

186

The allergome, proteome and transcriptome of pollen from the prominent subtropical Johnson grass (*Sorghum halepense*)

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Background: The world's highest population densities reside in subtropical regions

where pollen from subtropical grasses including Panicoideae; Johnson grass (*Sorghum halepense*) are clinically important for allergic rhinitis and asthma. We aimed to characterise the molecular allergenic components of pollen from *S. halepense*.

Methods: The total pollen transcriptome, proteome and allergome were examined. Skin prick tests (SPT) and serum IgE reactivity were assessed in 64 patients with grass pollen allergy from a subtropical region and control subjects with other allergies ($n = 24$) and no atopy ($n = 19$).

Results: Serum IgE of patients with allergic sensitivity to Johnson grass pollen reacted with two dominant allergenic components by immunoblotting; Sor h 1 and a 50 kDa allergen identified herein as Sor h 13. Five additional pollen components showed IgE-reactivity. Serum IgE with purified Sor h 1 was observed in 40 of 41 patients with IgE reactivity to JGP (97.5%) as well as nine additional grass pollen-allergic patients without IgE to JGP (76.5%). Serum IgE reactivity with JGP and Sor h 1 was highly correlated ($r = 0.969$, $P < 0.0001$). Of 48 grass pollen-allergic patients with serum IgE to JGP, 28 (58%) showed IgE reactivity with purified Sor h 13. cDNA transcripts and peptides determined by mass spectrometry corresponded to pollen allergen families 2, 3, 4, 11 and 12. The group 5 and 6 temperate grass allergens were not evident in the proteome, whereas novel allergens homologous to Chloridoideae allergens (groups 15, 22 and 23) and Birch pollen Bet v 6, were present in Johnson grass pollen.

Conclusion: Sor h 1 and Sor h 13 of Johnson grass pollen are major allergens for patients with grass pollen allergy in subtropical regions. This comprehensive molecular approach demonstrated qualitative differences between allergenic composition of Johnson grass and temperate grass pollens with relevance for care of patients with allergic respiratory diseases in subtropical regions of the world where subtropical grasses predominate.

Oral Abstract Session 32

Immunodeficiencies and immunogenetics

187
A novel c-kit mutation in exon 18 in familial mastocytosis

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Background: Mastocytosis is a benign tumor with tumor cells usually located in the skin and/or bone marrow. It is often caused by a spontaneous mutation in the exon 17 (D816V) of the c-kit gene, which has been included in the 2007 WHO classification system of mastocytosis (Valent *et al.*, 2007). Inherited forms of mastocytosis are rare. We describe eight out of 17 family members of a family of four generations suffering from flushing and vertigo due to unspecific triggers such as physical exercise or showering suspicious of a mast cell activation syndrome [sMCAS, (Hamilton *et al.*, 2011; Valent *et al.*, 2012)].
Method: Four of the four members additionally suffered from cutaneous mastocytosis. Skin biopsies were collected from 2, bone marrow biopsies from another 2, serum tryptase, whole blood and mucosal mouth swabs were collected from all for DNA extraction. The study has been approved by the institutional ethics committee (Ethics committee of the Medical University of Vienna, approval number 901/2009).
Results: Sequencing the c-KIT gene by PCR in the skin biopsies from the two patients showed an up to now unreported mutation in exon 18 on position 849 (S849I). A second mutation in exon 18 on position 835 (c-Kit M835K) was found exclusively in the most severely affected patient. The mutations could be identified in material derived from skin biopsies but not in blood samples or buccal mouth swabs from our patients and all other family members. This could be explained by somatic mutations of the c-Kit gene indicating mosaicism.
Conclusion: We present a previously unreported mutation in the exon 18 of the c-KIT gene, contributing to a phenotype of cutaneous mastocytosis with sMCAS and a tendency to incomplete resolution in adulthood. We also identified an additional mutation in exon 18 of the c-KIT gene possibly associated with a more severe phenotype.

190
Clinical gene therapy for X-linked chronic granulomatous disease

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Background: X-linked chronic granulomatous disease (X-CGD) is a primary immunodeficiency caused by mutations in the CYBB gene encoding the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalytic subunit gp91phox. Patients suffer from recurrent life threatening infections with bacteria and fungi, often requiring bone marrow transplantation. In case no matched bone marrow donor is available, the only alternative treatment option is gene therapy of autologous hematopoietic stem cells.
Method: A recent Swiss-German clinical trial for X-CGD using a gamma-retroviral vector has demonstrated clear therapeutic benefits in four patients although complicated by enhancer-mediated mutagenesis and diminution of effectiveness over time due to silencing of the viral long terminal repeat. In collaboration with other centers in Europe a new lentiviral SIN (self-inactivated) gene transfer vector for X-CGD has therefore been developed to improve efficacy and safety. In this vector expression of the therapeutic transgene gp91phox is mediated by a chimeric promoter – a synthetic fusion of two myeloid promoter elements derived from the CathepsinG and the cFes gene regulatory regions.
Results: This vector results in high levels of gp91phox expression and normal NADPH oxidase activity in committed myeloid cells and granulocytes from transduced human X-CGD CD34+ cells.
Conclusion: Based on these results the chimeric vector was selected for large scale GMP-production in a joint effort between labs in Zürich, Frankfurt, London and Paris aiming at a multicenter clinical gene therapy trial phase I/II. First patients (pediatric and adult) are planned to be treated by 2013 in Zürich in this EU-FP7 funded trial.

191
Immunophenotypes and thymic/bone marrow output assessment are useful tools to identify underlying pathophysiology in common variable immunodeficiency

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Background: Patients with Common variable immunodeficiency disorders (CVID) have distinct immunological defects. Using B-cell replication history and somatic hypermutation status, we previously identified five pathophysiological B-cell patterns (Driessen et al. Blood 2011); (i) a B-cell production defect, (ii) an early peripheral B-cell maturation or survival defect, (iii) a B-cell activation and proliferation defect, (iv) a germinal center defect and (v) a post-germinal center defect. Dysfunctions of T-cells also contributed to mechanisms of diseases. T cell receptor excision circles (TRECs) and kappa-deleting recombination circles (KRECs) are the assays for determine the newly produced T and B lymphocytes. We aimed to explore T-cell defects and the TREC/KREC quantification in patients with different B-cell patterns in order to provide more clear pictures on the immune homeostasis in CVID.
Method: Eight-color flow cytometric immunophenotyping of peripheral blood was performed to categorise B and T cell phenotypes. TREC/KREC amounts were determined with real-time quantitative PCR assay.

Results: Thirty-seven CVID patients were included. The age range was 6–76 years. Twelve patients (54.5%) with early stages of B-cell production and maturation defects (pattern 1&2) had significantly reduced numbers of naïve CD4+ and CD8+ T cells compared to controls. KRECs were significantly decreased in both groups. TRECs were also reduced in pattern 1 & 2 (pattern 1; $P < 0.05$). The patients with B-cell pattern 1, which defects are in the early stages, had the lowest KREC and TREC levels. Our approach clearly demonstrated that the defects in B-cell pattern 1 and 2 result from thymic and bone marrow impairment rather than from survival defects of peripheral cells. Naïve T-cell numbers of patients in pattern 3–5 were not reduced compared to controls. Major defects of the later groups were B-cell activation and germinal center dysfunction which were confirmed by the normal results of KREC/TREC assay.
Conclusion: Evaluation in both immunophenotypes and thymic/bone marrow output provides new information regarding pathophysiology of the disease. Subpopulations of CVID with early B and T cell defect were identified. Treatment strategies for close monitoring and early detection of the complications should be considered in these populations.

192
IL-17F mutation in recurrent aphthous stomatitis

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Background: Recurrent Aphthous Stomatitis (RAS) is a common oral inflammatory disease with unknown pathogenesis. Although the immune system alterations could be involved in predisposition of individuals to oral candidiasis, precise ethiologies of RAS have not been understood yet. Considering the inflammatory nature of interleukin (IL)-17F and RAS, we aimed to sequence the gene in a number of patients with RAS to identify any disease-associated mutation.
Method: Sixty-two Iranian patients with RAS and fifty healthy subjects enrolled in this study. After DNA extraction from the whole blood, amplification was accomplished by polymerase chain reaction for IL-17F.
Results: The results of sequencing revealed a missense, heterozygous mutation, converting a threonine to proline in a patient with RAS. The Poly-phen software suggested a damaging probability predicting this substitution to have a harmful effect on IL-17F protein function. Nevertheless, this substitution was predicted not to change the β-aggregation propensity using TANGO software. Such mutation was not detected in any control subject.
Conclusion: This is a first study showing a mutation that seems to be associated with susceptibility to RAS. Further studies on more patients with RAS are required to confirm this finding.

Oral Abstract Session 33

Diagnosing food allergy

193

Are house dust mite or shellfish allergic patients at risk when consuming food containing mealworm proteins

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Background: Due to the imminent growth of the world population, shortage of protein sources for human consumption will arise in the near future. Alternative and sustainable protein sources (e.g. insects and algae) are now being explored for the production of food and feed. One candidate for consumption is the mealworm. Mealworm (*Tenebriomolitor* L.) as a new protein source is of great interest since the production of mealworm is durable; less energy and agricultural land is needed and less greenhouse gasses are produced. In this project the safety of mealworm proteins for human consumption was tested. This in accordance to the European Food Safety Authority (EFSA) guidelines for allergenicity risk assessment of genetically modified organisms (GMO).
Method: According to the phylogenetic tree, mealworms are closely related to house dust mite and crustaceans, which are also common causes of allergy. Different mealworm protein fractions (soluble and insoluble) and extracts from mealworm faeces were prepared, and tested for cross-reactivity using IgE from patients with an inhalant allergy to house dust mite or food allergy to crustaceans, using immunoblotting and indirect basophil activation.
Results: The following patient groups were identified:

1 Crustacean allergic patients with house dust mite allergy.
2 Crustacean allergic patients without house dust mite allergy.
3 Crustacean tolerant patients who were only sensitised to crustacean.
4 House dust mite allergic patients without crustacean allergy or -sensitisation.

IgE from both house dust mite and crustacean allergic patients cross-reacted with several different proteins in mealworm, however different binding efficiencies were found. Some patient sera reacted strongly with proteins in the soluble fraction while others reacted with proteins in the insoluble fraction. The cross-reactivity was found to be functional, as shown by the induction of basophil activation. So far two cross-reactive proteins were identified. Arginine kinase, which is a well known allergen in shrimp, lobster, crab, and tropomyosin which is a well known allergen in these crustaceans as well as in house dust mite, cockroach and herring worm (Anisakis simplex).
Conclusion: Based on these cross-reactivity studies, house dust mite and crustacean allergic patients may be at risk when consuming food containing mealworm proteins.

194

Lipid peanut fraction contains important allergens that should be included in peanut diagnosis commercial extracts

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Background: We found patients with severe reactions upon peanut ingestion with negative skin test with commercial extracts.
Method: We studied seven patients with anaphylaxis upon peanut ingestion and negative skin prick test (SPT) with commercial peanut extract. We performed to each patient specific IgE (sIgE) against whole peanut and peanut components Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Ara h 9. We also performed basophil activation test against Ara h 1, Ara h 2 and Ara h 9. We performed the same tests to 15 patients with anaphylaxis and positive SPT to peanut as controls. We separated peanut lipophilic and hydrophilic fraction and performed immunoblotting with each fraction with all patients' sera.
Results: One negative SPT patient to peanut and 12 out of 15 SPT positive patients had sIgE against peanut. From the SPT negative group, one had sIgE against Ara h 1 and Ara h 9 whereas in the SPT positive group, 11 patients had sIgE against Ara h 9 and 1 to Ara h 1, 2 and 3. Those patients with negative SPT and sIgE to peanut only recognised the lipophilic fraction. However, controls with positive SPT recognised both hydrophilic and lipophilic peanut fraction.
Conclusion: Lipid fraction contains relevant peanut allergens that might be overlooked. In the preparation of commercial extracts usually lipid fraction is discharged removing relevant allergens. There is a need to characterise proteins embedded in oil bodies that are unrepresented or denatured in most diagnostic extracts of nuts and seeds that are usually extensively defatted.

195

Identification of an antimicrobial peptide, snakin, as a novel peach allergen, which relates to systemic reactions

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Background: Peach is one of the most important causes of food allergy in adults in Japan as well as in Europe. To date, peach allergy has been mostly reported in three distinct settings.

1 In subjects primarily sensitised to birch pollen as the result of cross-reactivity between the major birch pollen allergen, Bet v 1, and a homologous protein in the fruit, named Pru p 1.
2 In subjects characterised by IgE reactivity to multiple pollen extracts both in-vivo and in-vitro that co-recognise the plant pan-allergen profilin, which is called Pru p 4 in the peach.
3 Particularly in Mediterranean countries, in patients allergic to nonspecific lipid

transfer proteins, a family of heat-and pepsin-resistant plant food pan-allergen, named Pru p 3. Pru p 3, peach LTP, has been considered as a marker for severe allergic reactions due to peach in Europe, whereas Pru p 3 has been reported to be rarely reacted in Japanese patients with peach allergy, including patients developing systemic reactions.

Objective: We investigated the allergenicity of antimicrobial peptide, snakin, which has a similar molecular weight with approximately 9 kDa in peach allergy.

Methods: Thirty patients (M: F = 11:19, mean age 32.2 years) with peach allergy were divided into two groups, one group of 14 patients accompanied with systemic reactions (systemic group) and another group of 16 patients with oral symptoms alone (oral group). Using ImmunoCAP (Phadia), specific IgE levels against Pru p 1, Pru p 3, and Pru p 4 were measured. To evaluate the allergenicity of the purified peach snakin, ELISA and IgE-immunoblotting (IB) using patients' sera, and skin prick test (SPT) with the purified peach snakin were performed.

Results: Positivity for specific IgE against Pru p 1, Pru p 3 and Pru p 4 was 28.6%, 7.1% and 0% in systemic group and 68.7%, 0%, and 56.2% in oral group, respectively. ELISA, IgE-IB and SPT using peach snakin showed positive reactions in 50%, 50% and 80% of patients belonging to systemic group and in 12.5%, 6.3% and 0% of patients belonging to oral group, respectively.

Discussion: These results indicated that snakin is a novel peach allergen and, further, could be a marker of severe systemic reactions due to peach allergy.

196
Alternaria (Alt a 1) and kiwi thaumatin sensitisation. A new cross-reactivity profile?

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Background: A potential cross-reactivity between aerial molds and food allergens (spinaches and mushrooms) has been reported in a previous study.

Objective: To investigate cross-reactivity patterns between aerial molds and food allergens, using component resolved diagnosis and specific IgE inhibition test, in patients who had suffered from food anaphylaxis.

Method: One hundred fifty-three patients, older than 5 years, both genders, living in the area of Valencia, were evaluated because of anaphylaxis. Component resolved diagnosis was performed by InmunoCAP-ISAC 112 (ThermoFisher Scientific-Phadia), LuxScan scanner (CapitalBio Corporation) and software (MIA v.3.1.2.). Values $\geq$0.35 ISU were considered positive.

Specific IgE was evaluated with ImmunoCAP 250 (ThermoFisher Scientific-Phadia). First, specific IgE to complete alternaria extract and Alt a 1 was measured in a pool of six patient's sera (showing the highest specific Alt a 1 reactivity). Afterwards the Alternaria and Alt a 1 IgE reactivity was inhibited after 2 h of incubation with a commercial kiwi extract.

Results: Forty-nine patients (32%) were positive to Alt a 1 and 22 (14.37%) to kiwi thaumatin (Act d 2). In this last group, twenty patients (90.91%) were positive to Alt a 1 and all of them showed respiratory symptoms (asthma/rhinitis) related to alternaria exposure and twelve patients (60%) showed symptoms (OAS/urticaria) with kiwi consumption. Alternaria and Alt a 1 specific IgE reactivity was inhibited by increasing amounts of kiwi, showing a dose-response relationship: kiwi 10^{-3} inhibited 48.35% of specific IgE to Alt a 1 and 44.04% to whole alternaria, while kiwi 10^{1} inhibited 65.27% of specific IgE to Alt a 1 and 84.09% to whole alternaria.

Conclusion: Kiwi allergic patients are frequently sensitised to Alternaria. In our experience this sensitisation is clinically relevant, as all patients with allergy to kiwi have respiratory symptoms related to Alternaria exposure. We describe a new cross-reactivity profile between alternaria and kiwi as it has been shown by the specific IgE inhibition tests.

197
Dietary intervention with specific non-digestible oligosaccharides and *Bifidobacterium breve* M-16V support the development of tolerogenic DC

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Background: Dietary intervention using a combination of a specific non-digestible oligosaccharide mixture – containing short-chain galacto- and long-chain fructo-oligosaccharides (GF) in a 9:1 ratio – and *Bifidobacterium breve* M-16V (*Bb*; GF/*Bb*) is known to prevent the development of food allergic symptoms. We have indicated a potential protective role for intestinal epithelium-derived galectin-9 in suppressing mast cell degranulation in the gut mucosa. However, it is not known whether GF/*Bb* can modulate DC and T cell responses in the intestine.

Method: Using an ovalbumin (OVA)-induced murine model for food allergy, we evaluated whether dietary intervention with GF/*Bb* during allergic sensitisation results in changes in DC and T cell subsets in the gut-associated lymphoid tissue. Supporting *in vitro* experiments were performed to evaluate the involvement of galectin-9 in DC conditioning and T cell polarisation.

Results: Upon OVA challenge, an increase in $CD11c^{+}MHC\text{-}II^{mid}$ cells was observed in the small intestinal lamina propria (SI-LP). In allergic mice, $CD11c^{+}MHC\text{-}II^{mid}$ cells expressed lower levels of CD103, which was partially restored when mice were fed GF/*Bb*. Furthermore, $CD11c^{+}$ cells in the SI-LP of allergic mice produce more IL-4, which was paralleled with an increase in $CD69^{+}GATA\text{-}3^{+}$ activated Th2 cells and reduced $Foxp3^{+}$ Treg cells in the SI-LP. Dietary intervention with GF/*Bb* suppressed IL-4 production by SI-LP $CD11c^{+}$ cells and restored the frequency of Th2 and Treg cells to the level of sham sensitised mice. Furthermore, OVA re-stimulation of splenocytes from allergic mice showed increased production of IL-4, IL-5 and IL-13. The presence of recombinant galectin-9 during OVA re-stimulation partially reduced the secretion of IL-4 and IL-5, but not IFN-γ. Supporting *in vitro* experiments show that galectin-9 secreted by IEC induces RALDH activity in human monocyte-derived DC. Furthermore, monocyte-derived DC conditioned with galectin-9 had increased capacity to induce functional $Foxp3^{+}$ Treg cells.

Conclusion: Dietary intervention using GF/*Bb* supports the generation of tolerogenic DC, resulting in reduced Th2-associated cytokine production and enhanced Treg polarisation. Galectin-9 secreted by IEC may be involved in conditioning DC to induce Treg cell differentiation.

198
Fish allergy across Europe: results of a multicentre study within the FAST project

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Background: FAST project aims at the development of immunotherapy to fish allergy with hypoallergenic recombinant parvalbumin. In order to characterise the candidate molecules, patients sensitised to parvalbumin and with a challenged confirmed fish allergy were recruited in a multicentre study.

Method: Patients 12–65 years-old (y) reporting immediate reactions to fish were selected in Madrid (Spain), Odense (Denmark), Rome (Italy), Lódz (Poland), Athens (Greece), and Reykjavik (Iceland). A panel of fish was tested by skin prick tests (SPT) and serum IgE (sIgE) determinations (ImmunoCAP). sIgE to recombinant parvalbumins (rCyp c1 and rGad c1) was measured by ImmunoCAP. Patients with sIgE to rCyp c1 and rGad c1 $\geq$0.7 kU/l, and SPT to fish $\geq$5 mm (inclusion criteria) were invited to a double-blind placebo-controlled food challenge (DBPCFC).

Results: Sixty-six patients were screened and 43 fulfilled the inclusion criteria; 6/43 refused the DBPCFC, 1/43 was excluded for chicken allergy, and 3/43 for severe anaphylaxis. The remaining 33/43 patients underwent a DBPCFC: 24 had positive DBPCFC, and four had a negative DBPCFC but reacted with objective symptoms in an open challenge with cod. Finally 31 cases (28 challenge confirmed and three anaphylaxis) were included; 58.1% were male, mean age at study 29.4 year, median age at first reaction 4 year. Fifty-three fish adverse reactions were recorded. Reactions appeared in 92% in $\leq$30 min, comprised oral symptoms (OAS) in 66%, skin reactions in 68%, bronchospasm in 57%, digestive symptoms in 49%, upper airway involvement in 43%, anaphylaxis in 13% and two anaphylactic shocks (4%). Fish contact urticaria (CU) was found in 54%, and reactions through inhalation in 70%. Medication was administered in 58% of reactions, adrenaline in 16% and emergency room assistance was needed in 26%. Median values of SPT (ratio to histamine) and sIgE (kU/l) were: SPT to cod 1.6; sIgE to cod 5.45; sIgE to rCyp c1 5.2; sIgE to rGad c 1 5.0.

The clinical presentation was similar across centres with exceptions ($P < 0.01$) in the frequency of anaphylaxis, OAS, CU and reactions through fish inhalation. No differences were found in the treatment of reactions, nor in SPT/sIgE to fish and rCyp c1 and rGad c1.

Conclusion: Fish allergy in patients sensitised to parvalbumin is a persistent severe food allergy without geographical variations. This finding supports the application of parvalbumin in specific immunotherapy in fish allergy.

Oral Abstract Session 34

Immunology of the nose and the eyes

199

Deficient glucocorticoid induction of antiinflammatory genes in asthmatic nasal polyp fibroblasts

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Background: Patients with nasal polyposis and aspirin-intolerant asthma have the most severe forms of airway diseases and the poorest response to glucocorticoid treatment. Glucocorticoids, via the glucocorticoid receptor (GR), reduce inflammation by inhibiting pro-inflammatory gene expression and transactivating antiinflammatory genes.
Objective: To elucidate whether nasal polyp fibroblasts from aspirin-tolerant and aspirin-intolerant asthma patients have altered GR expression and signaling, compared to control nasal mucosa fibroblasts.
Methods: Nasal polyp fibroblasts from aspirin-tolerant ($n = 5$) and aspirin-intolerant ($n = 7$) asthmatics and control nasal mucosa fibroblasts ($n = 10$) were isolated and stimulated *in vitro* with dexamethasone (10^{-7} M). Expression of GRa, GRb, and the GR-induced genes mitogen-activated protein-kinase phosphatase-1 (MKP-1), glucocorticoid-induced leucine zipper (GILZ), and tristetraprolin (TTP) was analyzed by RT-PCR and immunoblotting, GR nuclear translocation by immunocytochemistry, histone H4 K5 acetylation by immunoblotting, GRa binding to MKP-1 and GILZ promoters by chromatin immunoprecipitation, and IL-8 release by ELISA.
Results: GRa and GRb mRNA and protein levels, and dexamethasone-induced GRa nuclear translocation did not differ between control nasal mucosa and nasal polyp fibroblasts. Nasal polyp fibroblasts, especially those from aspirin-intolerant asthmatics, had a significantly lower induction of MKP-1, GILZ and TTP mRNAs by dexamethasone than nasal mucosa fibroblasts. Nasal polyp fibroblasts also showed lower induction by dexamethasone of MKP-1 and GILZ proteins, histone H4 K5 acetylation, and GRa binding to MKP-1 and GILZ promoters, compared with nasal mucosa fibroblasts. Dexamethasone-induced expression of MKP-1 and GILZ mRNA positively correlated with dexamethasone-induced histone H4 K5 acetylation (MKP-1: $r = 0.68$, $P < 0.01$; GILZ: $r = 0.76$, $P < 0.01$) and negatively correlated with the concentration of dexamethasone that provoked 40% inhibition (IC_{40}) of IL-8 release (MKP-1: $r = -0.67$, $P < 0.01$; GILZ: $r = -0.48$, $P = 0.05$).
Conclusion: Nasal fibroblasts from patients with nasal polyposis and asthma have defective GRa transactivation of antiinflammatory genes that correlates with an impaired capacity of glucocorticoids to inhibit IL-8 in these cells. This reduced transactivation could be a mechanism by which glucocorticoid resistance occurs in these patients.

200

Down-regulation of EMP1 is associated with epithelial hyperplasia and metaplasia in nasal polyposis

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Background: Epithelial remodeling is one major pathological change in nasal polyposis (NP). Epithelial membrane protein 1 (EMP1) plays important roles in epithelial development. We aimed to study the expression of EMP1 in NP and its role in alteration of NP epithelium.
Method: NP tissues were obtained from 55 NP patients, while biopsies of inferior turbinate mucosa from 30 healthy subjects were used as controls. Quantitative PCR and immunohistochemistry were performed to determine the expression levels of EMP1 in tissues from the NP patients and healthy controls.
Results: EMP1 mRNA expression was significantly lower (2.77-fold) in samples from NP patients than those from control subjects. EMP1 was stained in nasal epithelium and was majorly co-localised with both basal (p63+) and differentiated (CK18+) epithelial cells. The EMP1 immunoreactivity was greater in tissues from healthy controls, while weak staining of EMP1 was found in the remodeling epithelium from NP patients. EMP1 mRNA levels were further down-regulated in those severe hyperplastic (1.79-fold) or metaplastic (1.85-fold) NP epithelium compared with those epithelium without remodeling. Positive correlations between EMP1 and other epithelial cell related genes (JUN, PTGS2, EGR1, AREG, HBEGF, CXCL12, CXCR4, and CDKN1A) mRNAs were found in all nasal tissues.
Conclusion: EMP1 could be a specific biomarker for aberrant epithelial remodeling in chronic inflammatory upper airway mucosa (e.g. NPs).

201

Invariant natural killer T cells in nasal polyp

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Background: Nasal polyps (NPs) are a common problem in adults especially in asthmatics but the exact etiology is not clear. Some studies showed that Invariant natural killer T cells (iNKT cells) may skew the immune response towards a Th2 profile in the lung of asthmatic patients. There are some interesting similarities between NP and asthma therefore, iNKT cells may play a role in pathogenesis of nasal polyps. The aim of this study was to compare frequency of iNKT cells in peripheral blood and nasal polyp tissue among some patients suffer from nasal polyposis.
Method: Sixteen patients with nasal polyposis (M/F ratio 9/7; mean age:35 years) participated in this study and nasal polyp specimens as well as peripheral blood samples were taken during polypectomy. Blood and polyp cell suspension were immunostained for CD3, CD4 and iNKT cells. The iNKT cells were identified by immunostaining with CD3 and 6B11 antibodies.
Results: The mean percentage of T cells was approximately 6% of total cells in tissue suspension. Among the CD3+ T cells, percentage of iNKT cells varied from 0.5%

to 11% (mean 4.7%) in Polyp. In the peripheral blood samples, The average number of CD3+, CD4+, iNKT cells and iNKTCD4+ cells, were 59%, 56%,0.52% and 43% respectively. The number of iNKT cells in nasal polyp was significantly higher than blood (mean 4.7 vs 0.52, $P < 0.001$).

Conclusion: This study shows that invariant natural killer T (iNKT) cells are fairly abundant in the nasal polyp and likely to play a role in pathogenesis of nasal polyps.

202
Effect of simvastatin on transforming growth factor beta-1-induced myofibroblast differentiation and collagen production in nasal polyp-derived fibroblasts

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Background: Statins are the most commonly prescribed drugs for the treatment of hypercholesterolemia. Statins exert not only lipid-lowering but also other cellular effects, including anti-fibrotic properties. The purposes of this study were to determine the effect of simvastatin on Transforming growth factor (TGF)-β1-induced myofibroblast differentiation and collagen production in nasal polyp-derived fibroblasts (NPDFs) and to verify the mechanism of the effect of simvastatin in TGF-β1-induced myofibroblast differentiation in NPDFs.

Methods: NPDFs were pre-treated with simvastatin with or without mevalonate or Y-27643 for 2 h prior to induction by TGF-β1. The expression of *α-smooth muscle actin (SMA)* and *collagen type IV* mRNA was determined by a reverse transcription-polymerase chain reaction, and the expression of α-SMA protein was determined by immunofluoescent cytochemical staining. Total soluble collagen production was analyzed by the SirCol collagen dye-binding assay. Phosphorylation of Smad 2/3 was evaluated by Western blot analysis.

Results: In TGF-β1-induced NPDFs, simvastatin significantly inhibited the expressions of *α-SMA* and *collagen type IV* mRNA and reduced α-SMA and collagen protein levels. Pre-treatment with mevalonate reversed the effect of simvastatin. The expression of α-SMA mRNA and protein was significantly decreased by pre-treatment with Y-27632. The TGF-β1-induced expression of pSmad 2/3 protein was notably decreased by pre-treatment with simvastatin.

Conclusions: We showed that simvastatin inhibits TGF-β1-induced myofibroblast differentiation (expression of α-SMA) and collagen production in NPDFs and Rho/Rock and TGF-β/Smad signaling is involved as an underlying mechanism. The results of our study suggest that simvastatin is a possible candidate for the suppression of nasal polyp formation.

203
Conjunctival expression of IL-9: a role for IL-9 in ocular allergy?

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Background: IL-9 is a pro-inflammatory cytokine that is associated with the immunopathogenesis of allergic diseases including asthma. The role of IL-9 in allergic conjunctivitis is unknown. The aim of this study was to investigate IL-9 expression in conjunctival biopsy specimens, and which cells secrete IL-9 during seasonal allergic conjunctivitis (SAC).

Method: IL-9 expression was investigated in conjunctival biopsies (3 μm) from SAC donors ($n = 7$, all males; age 18–65 years) at 8 h post allergen challenge, and normal, non-inflamed, conjunctival tissues from anonymised donors ($n = 5$ from three males; age 31–57 years). Donor tissues were collected after obtaining informed consent and Local Research Ethics approval in accordance with the Declaration of Helsinki. GMA-embedded sequential tissue sections from each biopsy specimen were stained with anti-human IL-9 (Abcam) or anti-human MC tryptase (Santa Cruz), and primary antibody was omitted as a negative control. Positively stained cells were visualised with a DAB staining kit (Vectastain). Two independent, masked observers enumerated positively stained cells per biopsy area (at least three fields). Human $CD4^+$ T cells, derived from human cord blood were stimulated with anti-human CD3/28 ± TGFb (0–10 ng/ml) and IL-9 levels assayed using Multiplex Bead Arrays (Luminex, Millipore, UK). Primary cultures of bone marrow-derived murine mast cells (MMCs) were stimulated by FceR1 cross-linking and IL-9 production assayed as above.

Results: IL-9 expressing cells were detected mainly within the subepithelial and stromal areas of conjunctival tissues. There was a significant increase in numbers of IL-9+ cells in SAC tissues (mean = 25.29 ± 4.7) as compared to controls (mean = 10.6 ± 1.1; $P = 0.014$). MC numbers were increased in SAC tissues and co-localised with IL-9. Production of IL-9 by activated CD4+T cells (70.69 ± 3.75 pg/ml) was TGFb-dependent ($P < 0.001$ relative to controls). IL-9 secretion of MMCs was also significantly increased upon activation (53.4 pg/ml; $P < 0.01$ relative to unstimulated cells).

Conclusion: IL-9 expression within the conjunctival tissues was upregulated during acute SAC. Human CD4+T cells and mouse mast cells secreted IL-9 upon stimulation *in vitro*. These models will be used to further investigate the role of this cytokine in ocular allergy.

204
Probiotic *B. lactis* NCC 2818 mitigates symptoms and Th-2 cytokine levels in individuals suffering from seasonal allergic rhinitis during the pollen season

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Background: The purpose of the current study was to evaluate the effect of oral administration of a candidate probiotic strain NCC 2818 (*Bifidobacterium lactis*) on allergic symptoms and immune parameters such as Th-2 cytokines (IL-5, IL-13) and basophil activation (CD63, CD203c) in seasonal allergic rhinitis subjects during seasonal exposure to grass pollen (May-July 2011).

Method: The protocol was accepted by the Ethics Commission of the Hospital of Lausanne, Switzerland. The study design was a double blinded, single center, randomised placebo controlled trial performed at Nestlé Research Center between March and August 2011. Twenty adult subjects aged 20–65 years who gave informed consent were recruited based on a clinical history to seasonal allergic rhinitis and positive skin prick test to grass pollen. The subjects (10 per arm) received one of the two treatments: *B. lactis* NCC 2818 or Placebo (maltodextrin) for 8 weeks. At the first visit (V1), the subjects were given the product and symptoms questionnaires to be filled in every week of the study. In addition, 10 ml whole blood was drawn in heparin coated tubes. At visit V2 (4 weeks after visit V1) and at visit V3 (8 weeks after visit V1) the corresponding questionnaires of the four preceeding weeks were collected and 10 ml of whole blood was collected. Basophil activation test and whole blood stimulation were performed on all subjects at the different visits (V1, V2, and V3).

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Results: The concentrations of Th-2 cytokines under anti CD2/CD28 stimulation were statistically significant lower in the *B. lactis* NCC 2818 group as compared to placebo group at the last visit of the study (8 weeks after the start of treatment administration). In terms of basophil activation, no significant reduction in the percentage of activated CD203 cells was observed, however the percentages of activated CD63 cells were statistically significantly lower in the *B. lactis* NCC 2818 group compared to placebo at V2. The total nasal symptom scores (TNSS) were significantly lower in the second month of the study (week 5–8) in the probiotic group compared with the placebo group.

Conclusion: The promising results obtained with 8 week administration of the probiotic on both allergic symptoms and immune parameters warrants that *B. lactis* NCC 2818 be further investigated in large scale trials to help allergic subjects better manage their symptoms.

Oral Abstract Session 35

Impact of workplace exposure on the outcome of allergic airway diseases

205
Identification of a novel allergen from the pine processionary moth *Thaumetopoea pityocampa*

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Background: The larvae of the pine processionary moth (Lepidoptera Notodontidae) are a medical problem in Europe, the Middle East, and northern Africa because of the reactions induced by microscopic urticating hairs (setae). Shed to protect larvae from predators, they can become airborne and are able to penetrate both the epidermis and mucous membranes. Skin reactions on the exposed areas are the most common clinical manifestation, although ocular and respiratory symptoms are also frequent, while anaphylactic reactions have been seldom reported. In addition to the irritant effect, an IgE-mediated mechanism has been demonstrated. The most studied allergenic protein in crude larval or setal extracts is Tha p 1 (15 kDa). Forestry workers are at major risk of accidental contact with setae. The aim of this work is to confirm, in patients with reactions due to suspected exposure, the presence of IgE antibodies against setal protein extracts.
Method: An '*in vitro*' clinical relevance assay was performed with a pool of sera from five forestry workers suffering from pruritis, especially between February and April, and were working in areas with infested pine trees. Setae from mature larvae were collected and, after total protein determination, SDS-PAGE and immunoblotting were performed. Blots were incubated with both the pool of sera and individual sera and then probed with anti-Human IgE. Chemiluminescence was developed and detected with the ChemiDoc imaging system. The most reactive band (75 kDa) was excised from the gel and digested with trypsin. Peptides were analyzed using a Nano HPLC system coupled with a LTQ Orbitrap-mass spectrometer. The data obtained were confronted with the database.
Results: Several bands reacted with the pool of the five allergic sera tested. In all the patients, a band of molecular weight of about 75 kDa, extrapolated from the SDS-PAGE results, was detected. The mass spectrometry analysis of the 75 kDa protein showed no similarity with the 15 kDa protein Tha p 1, thus excluding that the 75kDA protein might be a pentameric form of Tha p 1.
Conclusion: The results confirm the role of specific IgE in skin reactions due to processionary exposure. The immunoblot reaction of setal protein extracts shows the presence of a novel specific allergen that is located in the setae. These results highlight the importance of processionary risk management in forestry workers and the need for tools that can assess allergic sensitisation.

206
Ocular and nasal symptoms in compost workers: irritative or allergic background?

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Background: Compost workers are exposed to high concentrations of microorganisms and pollen. It was a purpose of the study to clarify if in currently exposed compost workers ($n = 190$) the prevalence of allergic symptoms and sensitisation is higher than in former compost workers (dropouts, $n = 59$), or in non-exposed control subjects ($n = 38$).
Method: In a cross-sectional study participants were asked for work-related symptoms, atopic diseases, and smoking habits. They underwent physical examination, lung function and exhaled nitric oxide (eNO) measurements. Additionally, total IgE, the concentration of specific IgE (sIgE) to environmental allergens (sx1) and to a mould mixture (mx1), as well as specific IgG (sIgG) to moulds (*Aspergillus fumigatus*, *Penicillium* spp.) and bacteria (*Thermoactinomyces vulgaris*, *Saccharopolyspora rectivirgula*) were measured in sera of all subjects (ImmunoCAP, Phadia, Sweden).
Results: About 20% of currently exposed compost workers suffered from conjunctivitis and/or rhinitis which may indicate an allergic process. Compared to the dropouts and the control subjects, especially symptoms of watering eyes were significantly more frequent ($P < 0.01$). However, while 22.6% of currently exposed compost workers reported to be allergic, this was the case for 40.7% of dropouts and 28.9% of controls. Smilar eNO-values (median 15 ppb, range: 5–66 ppb) and a lower percentage of obstructive symptoms in lung function (FEV_1 ≤70% predicted: 4.7%) than dropouts and controls (FEV_1 ≤70% predicted: 13.6% and 7.9%) were found in currently exposed compost workers. No significant differences in the number of positive results or in the concentrations of total IgE, sIgE, and sIgG were determined between compost workers and both other groups.

Asking dropouts about work-related symptoms during their active employment period in composting plants, they also reported ocular and nasal symptoms, but in 75% of them these symptoms improved or disappeared after termination of exposure.
Conclusion: Currently exposed compost workers often complain of conjunctivitis and/or rhinitis at work. However, in comparison to former compost workers and non-exposed controls, no higher prevalence of sensitisation, eNO values or reduced FEV_1 were observed. Considering the fact that ocular and nasal symptoms declined after termination of exposure it can be assumed that these symptoms are predominantly caused by irritative effects in terms of mucosal membrane irritation.

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207
Asthma associated with pesticide exposure among women in the rural Western Cape of South Africa

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Background: Pesticide exposure has been increasingly associated with adverse respiratory health effects in domestic and occupational settings. Few studies have investigated asthma associated with pesticides among women and farm workers in developing countries. South Africa is one of the largest users of pesticides on the African continent. The aim of the study was to investigate the association between pesticide exposure (primarily organophosphates and carbamates) and respiratory allergy and asthma.
Methods: A cross-sectional study of 211 women comprising those working and living on farms (farm dwellers, $n = 121$) and those residing in neighbouring farm areas (town dwellers, $n = 90$) was conducted using abbreviated ECRHS questionnaires, Phadiatop, specific IgE to mite allergens (HDM, storage mite, spidermite) (Phadia, ImmunoCAP) and FE_{NO} as per ATS/ERS criteria (2005). Outcomes included doctor diagnosed asthma (DA), current asthma (CA and attack in previous year), ocular nasal symptoms (ONS) and an asthma symptom score (ASS) (based on four symptoms in the previous year including wheeze with breathlessness, woken up with chest tightness, attack of shortness of breath at rest and woken by attack of coughing). Exposure variables included self-reported exposure to pesticides (household and occupational) and biomarkers as proxy for exposure as measured by whole blood cholinesterase (ChE).
Results: The median age was 37 years (interquartile range: 28–45 years). At least 9% had low ChE (below laboratory reference standard) of whom 78% were farm dwellers. The prevalence of DA, CA and ONS was 11%, 6% and 24% respectively. Adjusted models (age, smoking, years of schooling, atopy) demonstrated that ONS was associated with immediate re-entry in the pesticide sprayed field (RR = 2.97; CI: 0.93–9.50). ASS was also associated with farm dweller status (RR = 2.25; CI: 1.45–3.48) and low ChE (RR = 1.93; CI: 1.09–3.44). Subjects with a low ChE had a 5-fold increased odds of high FeNO (>50 ppb) (CI: 0.80–28.00; $P = 0.08$) suggestive of probable allergic asthma.
Conclusion: Pesticide exposure among women farm workers is associated with increased risk of ocular nasal symptoms and asthma. This study was limited by a cross sectional study design, small sample size and lack of information on specific pesticides used. These associations need further exploration in a larger longitudinal study.

208
Work disability in patients with hymenoptera venom allergy

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Background: Allergic sting reactions were largely investigated in selected occupational groups like gardeners and beekeepers but little is known about hymenoptera venom allergy impact on work ability. Despite the presence of VIT (venom-immunotherapy), for most patients an anaphylactic reaction after a hymenoptera sting is a very traumatic event affecting emotional, social and occupational behaviour. The main objective of this study is to evaluate prevalence and predictors of work disability in a group of patients treated with VIT.
Method: One hundred and eighty-one patients, aged 18–71, treated with VIT while working, were investigated by a questionnaire. Subjects were classified by employed and self-employed and by modality of work exposure to hymenoptera in three categories, high risk, occasionally at high risk and at low risk. Work disability was defined as to have changed job/task and have economic loss because of hymenoptera sting reaction. Predictors of work disability were assessed in logistic regression models.
Results: Thirty-one patients reported work disability. Nobody reported complete work change, 10 subjects reported job/task change and 25 an economic loss because of hymenoptera venom allergy. High risk job for sting (OR 2.81 95% CI 1.09–7.29) and being self-employed (OR 2.64 95% CI 1.06–6.56) were predictors of work disability. Type of stinging insect, intensity of the allergic reaction were not associated with work disability.
Conclusions: Hymenoptera venom allergy has an important impact on work, also through work disability. Self-employed workers and those at high risk of sting seem to be at higher risk of work disability related to hymenoptera venom allergy.

209
IgG4, IgE and allergy symptoms among employees working with microbial enzymes

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Background: Allergen specific IgE (sensitisation) indicates previous exposure to an allergen and is a precondition for development of respiratory allergy, but the role of IgG4 is more unclear. Studies in occupational medicine indicate that IgG4 is protective for development of allergy. In clinical allergology successful immunotherapy has been linked with increased IgG4 levels. Our aim was to assess the occurrence of enzyme specific IgG4 among employees in relation to exposure and to IgE sensitisation and allergy.
Method: Employees producing and handling microbial enzymes at Novozymes have through decades been monitored for sensitisation and respiratory symptoms in order to prevent occupational allergy. Sensitisation is assessed by custom made IgE-ImmunoCAPs to which industrial enzymes have been coupled. Serum is kept in a bio bank for validation and calibration.

Cases of allergy as well as asymptomatic sensitised and non-sensitised *controls* reflecting cases' employment year and exposure profile (department, job) were included in the study if serum was available for further analyses and if the person was still employed and gave consent.

Information on clinical status and enzyme specific IgE was picked from the surveillance database. IgG4 to enzymes [to which cases were sensitised (A)] and *control enzymes* (B and C to which they had been exposed) were measured by CAP.
Results: For enzyme A 31/37 (84%) of the exposed (allergic or sensitised) persons had an IgG4 response. IgG4 status was neither related to the quantitative IgE-response nor whether or not the person had a clinical allergy.

Sixty-five percent were IgG4 immunised to control enzyme B but surprisingly the prevalence was significantly related to disease status (related to enzyme A) as only 27% of the allergic persons had IgG4 against this enzymes vs 80% of the controls ($P = 0.008$ Pearson Chi-Square). This significant deviation was not reproduced for enzyme C; but taken together the added occurrence of IgG4 to B and C there was a trend ($P = 0.05$) for a correlation between clinical allergy and lack of IgG4 response to other occupational antigens.

Conclusion: IgE and allergy status seem not to be related to IgG4 if the same enzyme (or allergen) is tested for, and most persons with a sensitisation have IgG4. Choosing an alternative still relevant enzyme seems to differentiate between allergic and not allergic persons to the primary allergen.

210

Obeche wood allergen quantification: development of a sensitive sandwich-ELISA using for exposure assessment in three Spanish schools of carpentry

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Background: Wood dust is known to cause allergic occupational asthma and obeche (*Triplochiton scleroxylon*) is a prominent exponent in this field. To reduce the risk of IgE-mediated sensitisation it is essential to assess airborne obeche wood allergen concentration at exposed workplaces. Therefore, we developed an obeche wood sandwich-ELISA test sensitive enough to quantify obeche allergen in airborne samples. To prove the utilisability of the assay at workplaces, in a pilot study electrostatic dust collectors (EDCs) for passive airborne dust sampling were used in Spanish apprenticeship wood workshops.

Method: Obeche wood specific polyclonal antibodies produced in rabbit were used to develop a sandwich enzyme immunoassay (ELISA). The specificity of the ELISA was verified by different hard and soft wood and by mould extracts. To monitor obeche wood allergen exposure, EDCs were located in three Spanish apprenticeship wood workshops for 14 days. During this 14 days different kind of woods were processed (workshop1: pine and oak wood, obeche wood on 1 day for 2 h; workshop2: red pine, pine and oak wood, obeche wood on one day for 4–6 h; workshop3: pine, oak, olive, beech, sapelly and iroko wood, obeche wood on three days for 4–5 h).

Results: The detection limit of the obeche wood sandwich ELISA was 36 pg/ml and 100 times more sensitive than the formerly developed inhibition ELISA test system. The sandwich ELISA was highly specifical to obeche wood with only marginal reactivity to other hard wood and without reactivity to soft wood and mould. In all extracts from 27 EDCs loaded with wood dust obeche allergen was measurable (range 0.27–639 ng/ml). The highest obeche allergen concentration (mean value: 128 ng/ml; range: 2.3–639 ng/ml was measured in workshop3 where the period of obeche wood exposure was the longest (in total 12–15 h during the 14 days of sampling). Postulating that obeche wood was processed on the drilling and milling machine in workshop3 the allergen concentration reduces with increasing distance from the machines. The mean allergen concentration of workshop1 and 2 were remarkably lower and related to the time of obeche wood processing.

Conclusion: The obeche wood sandwich-ELISA is a valid tool to quantify obeche allergens exposure. Most likely it will be possible to monitor obeche allergen exposure during different processes as well as carry-over of obeche wood allergens in non-exposed areas.

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Oral Abstract Session 36

Mapping pollen all over the world

211

Grass pollen count and grass group 5-allergen release across eight European countries: results from HIALINE

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Background: Grass pollen is considered to be the most important outdoor aeroallergen in Europe. The grass 'pollen count' is usually used as a proxy for exposure. However, HIALINE has shown that the birch and olive pollen count is not always congruent with allergen concentrations. We therefore simultaneously measured daily exposure to grass pollen and the concentration of group 5 major allergens across eight countries in Europe during 2009–2011.
Method: Airborne allergens were collected using a high-volume cascade impactor for particulate matter (PM) >10 μm and 10 μm > PM > 2.5 μm. Grass group 5 allergens (Phl p 5) were determined by ELISA. Airborne pollen was collected using Hirst-type volumetric pollen traps. The System for Integrated modeLing of Atmospheric coMposition (SILAM) was used to compute the origin of the collected pollen.
Results: Allergen was recovered for >85% from the PM > 10 μm fraction of ambient air, showing that airborne allergen stems solely from pollen. On average pollen released 2.64 pg Phl p 5/pollen, comparable to birch and olive pollen. However, there was considerable variation between countries and between years. For instance in Evora, Portugal and Parma, Italy grass pollen released on average 40% less allergen per pollen than the European mean. When comparing individual years, the differences were up to 700% (Italy 2011 vs UK, 2009). This analysis concerns the comparison of yearly averages, which in themselves are already the average of approximately 60 days. Between days, between countries, the differences were even more extreme.
Conclusion: Grass pollen released different amounts of group 5-allergens depending on the country, year and day. We believe that allergen release per pollen is determined by different grass species in different countries with different environmental conditions determines Phl p 5 release per pollen. It is unknown how well the surrogate marker pollen count represents allergy relevant exposure. Our data shows that in addition to variations in pollen exposures, there are also substantial allergen release variations on top.

212

The Italian map of exposure to airborne molecular allergens

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Background: Pollen count has been used for over 50 years for the assessment of allergen exposure both in clinical practice and experimental studies. Allergy diagnostic has changed in the last 10–15 years, moving from the use of extracts for *in vivo* and *in vitro* diagnostic to Component Resolved Diagnosis, based on purified or recombinant allergens. As expected, aerobiology developed same way. Measurement of the concentration of single allergenic components in the air showed that allergen count deviates from pollen count. Earlier results have been recently confirmed in a multicentric European study (www.hialine.com).
Method: On the basis of the geo-climatic classification proposed by the Italian Association of Aerobiology (AIA, www.ilpolline.it), the maps of pollen distribution (www.polleninfo.org), the classification of allergens based on homology as defined by Lorenz in 2009 and the scientific literature on both allergen count measurements and molecular sensitisation profiles of allergic patients, we drawn a map of exposure to molecular aeroallergens in Italy.
Results: Given the latitudinal extension of Italy, the profile of exposure varies greatly from North to South. This is particularly evident for 'Birch Group' (Bet v 1-like family) which is supposed to be different in the North (prevalence of exposure to Bet v 1 and Aln g 1), the Centre (Cor a 1 and Aln g 1), the South and Islands (Cor a 1 and Que a 1). In the '*Oleaceae* group', exposure is prevalent to Fra a 1 (*Fraxinus*) in the North, to Ole e 1 (Olive tree) and Fra a 1 in the Centre and to Ole e 1 only in the South. Exposure to allergens from grasses is similar throughout the country, while that of weed allergens (mainly from *Urticaceae* and *Compositae*) is dramatically different because of the distribution of *Parietaria* (Centre, South and Islands) and *Ambrosia* (North).
Conclusion: Both allergy diagnosis and aerobiology are moving toward a 'molecular era'. This map can contribute to a global molecular vision of allergology, helping clinicians to look at exposure to pollen in a new way. Exposure profile of the area where patients live can indicate the correct choice of molecular diagnostics and, therefore, of the appropriate allergen immunotherapy.

213

Plantago lanceolata, a relevant trigger for weed pollinosis shows limited IgE cross-reactivity to grass pollen

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Background: In the temperate climate zone 30% of pollen allergic patients are sensitised to *Plantago lanceolata* (plantain) pollen. The concomitant appearance with other pollen makes it difficult to distinguish between IgE cross-reactivity and co-recognition. The only identified allergen is Pla l 1, an Ole e 1 homologue, which is commonly believed to cross-react with other Ole e 1-like allergens.
Method: IgE sensitisation profiles against proteins from plantain pollen were identified with immunoblot using sera of 27 patients from Austria allergic to plantain. Plantain pollen extract was separated by 2D-gel electrophoresis and tryptic peptides of the IgE reactive spots were analyzed with mass-spectrometry. Sensitisation frequencies of

identified IgE reactive proteins were determined. In addition, patients' sera were tested for IgE levels against pollen extracts from plantain, ryegrass, timothy-grass, olive, ash, ragweed, mugwort and birch in ELISA. IgE cross-inhibition assays to solid-phase coated plantain were performed using the above mentioned extracts.

Results: Besides Pla l 1, various other IgE reactive proteins in the molecular weight range from 10 - 70 kDa were observed. Mass spectrometry based analysis was used for identification of these proteins and highest sensitisation frequencies were found for the malate dehydrogenase (51.8%), fructose-bisphosphate aldolase (48.2%), profilin (48.2%), ATP synthase subunit beta (44.4%), and calmodulin (37.0%). *In vitro* reactivity to plantain extract was confirmed for all patients, 92.6% and 74.1% were additionally reacting to grass and olive/ash pollen, respectively. Two patients were mono-sensitised to plantain, while 14 reacted to all tested extracts. Notably, a low IgE cross-inhibition was detected for all extracts (mean 13.9–25.5%). Solely five patients showed IgE cross-reactivity with grass-pollen and three of those individuals demonstrated extensive cross-inhibition with all tested pollen. Profilin was identified as main elicitor of the observed IgE cross-reactivity whereas very low reactions to Pla l 1 were detected in immunoblot.

Conclusion: Although previous studies suggest an association between plantain and other Ole e 1-like containing allergen sources, we found only limited IgE cross-reactivity which was mainly due to profilin sensitisation. Therefore, pollen of *Plantago lanceolata* should be considered a primary sensitising weed for summer pollinosis.

214
Ambrosia airborne pollen migration seen in Vinnitsa, Ukraine during 2012

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Background: Ragweed (Ambrosia) is an important allergen seen currently in all 26 regions of Ukraine. Ambrosia control can reduce the number of pollen grains in the atmosphere, but as a recent study done in Vinnitsa shows, preventive measures must occur over wide territories due to long-distance pollen movement.

Method: Pollen counts were obtained at Vinnitsa National Pirogov Memorial Medical University (VNMU) in 2009–2011 on daily basis and in 2012 on a bi-hourly mode employing volumetric methods using a Burkard trap on the roof at 25 m height above ground.

Results: Years 2009 through 2011 showed stable Ambrosia pollen seasons in Vinnitsa onset in the first 10-day period of August and a peak August 25–27. The second maximum is usually seen in the second 10 day period of September.

Pollen count using a bi-hourly mode in Vinnitsa in 2012 showed constant increase of airborne ragweed pollen concentrations during the day at 1PM and at night at 1 AM. While Ambrosia pollen release occurs at mid-day, another peak may occur due to pollen influx from other areas. Analysis of weather conditions determined that the ragweed peak (100 p.g/m^3) on August 25 was due to pollen migrating to Vinnitsa at around 1 a.m. from areas 117 km south of Vinnitsa. The seasonal peak for 200 p.g./m^3 due to warm autumn was shifted to September 18, 2012 with the local pollen peak collected at 1 p.m. and a night pollen fraction coming from 75 km southeast of Vinnitsa. The efficacy of ragweed prevention control measures taken by the Vinnitsa State Quarantine Inspection around the city before the Ambrosia season-2012 was likely decreased due to long distance migration of pollen.

Conclusion: Ambrosia pollen season in Vinnitsa occurs during the first 10 day period of August with peaks in the third 10 day period of August and second 10 day period of September. Airborne ragweed pollen grains collected in Vinnitsa can be come from both local sources and from airborne pollen originating over 100 km south of Vinnitsa. Efficacy of local ragweed eradication projects may be adversely impacted by airborne migration of ragweed pollen. To determine the exact sources of ragweed pollen meteorological models are required.

215
Threshold computation: first results from the patient's hayfever diary

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Background: The Patient's Hayfever Diary (PHD – www.pollendiary.com) is a free web-based service for people suffering from pollen allergy that is currently used in 13 European countries and exists in 10 languages. PHD users report their pollen-induced symptoms and medication use on a daily basis. PHD users also enter their geographical location so that their symptoms can be compared with pollen levels recorded at the nearest pollen-monitoring station in the European Aeroallergen Network (https://ean.polleninfo.eu/Ean/). This allows users to identify aeroallergens that they are likely to have been exposed to, and for research into thresholds of aeroallergens (i.e. the number of pollen grains per cubic metre of air necessary for causing symptoms).

Method: Daily PHD symptom scores and corresponding pollen data for 2009–2012 were analysed. The number of registered PHD users was 26 002. The total number of points related to ocular, nasal and respiratory symptoms and medication use ranged from 0 (no symptoms) to 26. PHD users were said to be 'allergic' if their symptom scores were correlated with levels of a particular pollen type. A symptom score of 15 was deemed to be 'high'.

Results: Analysis of symptom scores entered into PHD from different biogeographical areas confirms the hypothesis that sufferers inhabiting areas with higher atmospheric concentrations of certain pollen types have a higher tolerance. For example, when comparing symptom scores and atmospheric concentrations of ragweed pollen, it was found that Serbian users required a much higher pollen load to reach a 'high' symptom score than Austrian users.

Conclusion: The pollen count is considered to be a proxy for exposure to aeroallergens. Threshold values are important for sufferers because they can use them to manage symptoms and interpret pollen count information. This knowledge is also useful for general practitioners and allergists as it provides corroborative evidence for diagnosis. PHD is the key to solving the perpetual discussion about thresholds for aeroallergens. We emphasize that the thresholds for alerts vary from region to region. Our goal is for pan-European coverage of PHD. The data from which will be used to compile European threshold maps for allergy-relevant airborne particles. On this premise a smartphone application that can provide 'personalised pollen information' will be published in March 2013.

216
Evaluation by scanning microscopy of the adhesion of particulate matter on athmospheric pollen

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Background: In western countries, the atmospheric urban pollution originated by increased number of transport vehicles, has adverse effects in human health in other hand pollens is one of the most important cause of respiratory allergy[1]. Several studies suggest that a high prevalence of allergic respiratory diseases could be due to the interaction between pollutants and pollens in cities[2,3]. Particulate matter (PM) and Diesel exhaust particles (DEP) act synergistically with allergens, increasing the production of proinflammatory cytokines and its combination with antigens trigger allergic symptoms[2]. The PM from pollution of the surface of the pollen in the atmosphere may trigger the pollinosis, because the concomitance of particles promotes an inflammatory reaction[4].

Objective: This scanning electron microscope study on pollen, evaluates the presence and chemical composition of particles on its surface.

Method: Samples were prepared from atmospheric PM collected in 2012 from an air intake filtration system (Andersen high volume samplers) in Mexico city. The samples was removed from the reusable surface filters by in the polluted environment. After the pollen was collected and processed according to the conventional technique for Scanning Electron Microscope (SEM), and observed in a SEM JEOL35CF of the Chemistry Fac.of UNAM. In addition, chemical composition of particles adhering to pollen was analyzed by X-ray microanalysis (EDS).

Results: The pollen grains shows in SEM have size ~20 mm. The ultrafine PM to submicro order are found highly adsorbed on the surface of the pollen. These particles have sizes ranged from a few micrometers to >100 nm. The pollen grains not exposed to PM (control), present a clean surface in contrast the pollens exposed to contaminant presents a large number of PM adhered to their surface.

Several particle were found on surface of pollen: P, Pb, Si. Al, Fe, C, Zr, S, K, Mg

Conclusion: Particulate matter adsorbed on the surface pollen may affect the development of respiratory allergy, because the grain size of pollen is sufficient to deposit there, and its sculpture can help to get hung up, and then the grain can act as a vector for them. The PM are too small (submicro order) to deposit in upper respiratory airway or the eyes where the pollen grains can fall. These particles contain particles as C, Si, S, Zr, P, Al, Pb, Fe and others, that can interact with pollen grains, leading to an increase release of antigens.