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Environmental factors/pollen

V01

Air pollution and climate change effects on allergies in the anthropocene: abundance, interaction, and modification of allergens and adjuvants

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This presentation provides an overview of physical, chemical and biological interactions between air pollution, climate

change, allergens, adjuvants and the immune system, addressing how these interactions may promote the development of allergies. Air pollution and climate change can be potential drivers for the increasing burden of allergic diseases. The molecular mechanisms by which air pollutants and climate parameters may influence allergic diseases, however, are complex and elusive. We reviewed and synthesized key findings from atmospheric, climate, and biomedical research. The current state of knowledge, open questions, and future research perspectives will be outlined and discussed. The Anthropocene, as the present era of globally pervasive anthropogenic influence on planet Earth and thus on the human environment, is characterized by a strong increase of carbon dioxide, ozone, nitrogen oxides, and combustion- or traffic-related particulate matter in the atmosphere. These environmental factors can enhance the abundance and induce chemical modifications of allergens, increase oxidative stress in the human body, and skew the immune system towards allergic reactions. In particular, air pollutants can act as adjuvants and alter the immunogenicity of allergenic proteins, while climate change affects the atmospheric abundance and human exposure to bioaerosols and aeroallergens. To fully understand and effectively mitigate the adverse effects of air pollution and climate change on allergic diseases, several challenges remain to be resolved. Among these are the identification and quantification of immunochemical reaction pathways involving allergens and adjuvants under relevant environmental and physiological conditions.

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V02

Are modern big cities in Germany worse for allergic sensitization?

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The incidence of allergic diseases is increasing worldwide. However, the factors controlling allergenicity are still not well understood, making life hard for physicians in their diagnosis and treatment. The influence of lifestyle and Climate Change were reported as factors that drive allergic sensitization. Nonetheless, studies are lacking that correlate these possible causes with the incidence of allergic diseases.

We used two studies ongoing in two different cities—SEAL Climate (Günzburg), in cooperation with the Government of Bavaria, and Ae2R Kids (Munich) to understand environmental drives of allergic sensitization in children aged 5–6 years old.

In total 409 children (312 in Günzburg and 97 in Munich) were recruited, capillary blood was collected and the parents were asked to fill in a questionnaire. The sensitization levels were measured with the ImmunoCAP[®] sIgE. Pollen and traffic-related air pollution data from both cities were used to analyse possible correlation with the sIgE levels.

Regarding grass pollen sensitization levels, 10.9% in Günzburg showed sensitization to Phl p 1 and 7.69% to Phl p 5, contrasting with Munich, which showed 20% and 4.12% ($P < 0.05$), respectively. For HDM, Munich children showed higher sensitization rates (11.34% for Der p 1, 12.37% for Der p 2) comparing to Günzburg (6.73% for Der p 1, 9.29% for Der p 2). In Günzburg 66% children had no sensitization at all compared to 58% in Munich (city). Pollution data from the two cities showed that Munich background pollution data had higher NO, NO₂ and PM_{2.5} levels. Grass pollen levels were higher in Günzburg.

Phl p 1 sensitization was significantly higher ($P < 0.05$) in Munich children and Phl p 5 showed no significant differences between the two groups. HDM allergens showed no significant differences between the two cities. The analysis of the questionnaires will be helpful to understand the symptomatic factors.

The lifestyle in modern big German cities (more pollution, less pollen) is detrimental for allergic sensitization.

V03

Atmospheric protein chemistry influenced by anthropogenic air pollutants: nitration and oligomerization upon exposure to ozone and nitrogen dioxide

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The allergenic potential of airborne proteins may be enhanced via post-translational modification induced by air pollutants

like ozone (O₃) and nitrogen dioxide (NO₂). The molecular mechanisms and kinetics of the chemical modifications that enhance the allergenicity of proteins, however, are still not fully understood. Here, protein tyrosine nitration and oligomerization upon simultaneous exposure of O₃ and NO₂ were studied in coated-wall flow-tube and bulk solution experiments under varying atmospherically relevant conditions (5–200 ppb O₃, 5–200 ppb NO₂, 45–96% RH), using bovine serum albumin as a model protein. Generally, more tyrosine residues were found to react via the nitration pathway than via the oligomerization pathway. Depending on reaction conditions, oligomer mass fractions and nitration degrees were in the ranges of 2.5–25% and 0.5–7%, respectively. The experimental results were well reproduced by the kinetic multilayer model of aerosol surface and bulk chemistry (KM-SUB). The extent of nitration and oligomerization strongly depends on relative humidity (RH) due to moisture-induced phase transition of proteins, highlighting the importance of cloud processing conditions for accelerated protein chemistry. Dimeric and nitrated species were major products in the liquid phase, while protein oligomerization was observed to a greater extent for the solid and semi-solid phase states of proteins. Our results show that the rate of both processes was sensitive towards ambient ozone concentration, but rather insensitive towards different NO₂ levels. An increase of tropospheric ozone concentrations in the Anthropocene may thus promote pro-allergic protein modifications and contribute to the observed increase of allergies over the past decades.

V04

LPS on Artemisia pollen is critical for allergic sensitization

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Endotoxin is one of the strongest stimulants of human immune system. We discovered that airborne Artemisia pollen is carrying significant high amount of LPS in comparison with other pollen. The aim of this work was to investigate the effect of LPS (naturally carried by Artemisia pollen) on allergic sensitization against this pollen type.

We isolated the bacterial communities from Artemisia pollen and used these bacteria in the challenging phase of a mouse model of allergy. This is an animal model for allergic sensitization, symptoms and inflammation of the lung. Six groups of animals were used: 1. Receiving Artemisia pollen low in LPS in PBS; 2. Receiving Artemisia pollen high in LPS; 3. Receiving only PBS; 4. Receiving only the same amount of LPS as the pollen with low LPS; 5. the same with only high LPS. The LPS was isolated from the bacteria from this pollen. and 6. Receiving the Artemisia pollen low in LPS with only the LPS from the pollen high in LPS.

The groups high in LPS (2, 5 and 6) resulted in an enhanced inflammatory cell infiltration in the BALF. A. vulgaris pollen with high LPS (group 2) and the group 6 (pollen low LPS with added only LPS) evoked the strongest influx of eosinophils, neutrophils and lymphocytes into the BALF, increased lung hyper-reactivity upon challenge and increased Artemisia spe-

cific IgG1. The latter two effects were absent in *A. vulgaris* pollen with low LPS, PBS group and the two only LPS groups.

Only the combination of LPS + Artemisia pollen showed health effects in lung hyper-reactivity and specific IgG1 production. We also observed that Artemisia pollen with LPS make mice more prone to develop asthma attacks. This could be one of the reasons why Artemisia pollen is highly related with asthma attacks and being highly allergenic. Our mouse model shows that the presence of LPS was essential in combination with the allergen to produce allergic sensitization. This phenomenon could also drive allergic diseases against other allergens in the world.

Diagnosics

V05

Identification and immunological characterization of *Polistes* venom hyaluronidase Pol d 2

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Background: Venom specific immunotherapy is highly effective in patients at high risk of severe or fatal anaphylactic reactions to insect stings, but selection of the right venom for therapy is a prerequisite. In regions where *Vespula spp.* and *Polistes spp.* coexist, discrimination between allergy to one or both insects is difficult due to the limited information about *Polistes* venom components and the high degree of cross-reactivity between the venom allergens. Prominent candidate allergens, leading to cross-reactivity between the venoms of *Hymenoptera* species, are the hyaluronidases. The hyaluronidases of honeybee and *Vespula* venom are well characterized, but no information about the hyaluronidase of *Polistes dominula* venom and its cross-reactivity were available.

Methods: *Polistes dominula* venom hyaluronidase was identified by mass spectrometry, cloned from venom gland

cDNA, recombinantly produced in insect cells, characterized by immunoblotting and assessed for IgE reactivity with sera of venom-allergic patients.

Results: *Polistes dominula* hyaluronidase was successfully identified in the venom, and produced recombinantly in insect cells together with its homologues from honeybee and *Vespula* venom. The analysis of specific IgE in sera from honeybee, *Vespula* and *Polistes* venom-allergic patients showed IgE reactivity of all allergens with diverse cross-reactivity patterns.

Conclusion: The *Polistes* venom hyaluronidase proved to be IgE reactive with sera of venom allergic patients, independent of cross-reactive carbohydrate determinants. Hence, it might be able to complete the panel of *Polistes* venom allergens for improved molecular diagnostics in the future. Due to its allergenic properties, the new *Polistes* venom allergen was designated as Pol d 2.

V06

Evaluation of a modified skin prick test for diagnosis of Hymenoptera venom allergy

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Introduction: Diagnostic evaluation for Hymenoptera venom (HV) allergy usually includes skin prick tests (SPT) and intradermal tests (IDT) with HV extracts of a maximum concentration of 100 µg/ml and 1 µg/ml, respectively. Here, we studied the value of a SPT with 300 µg/ml HV (SPT300) as a substitute for IDT.

Methods: 75 patients with a history of anaphylaxis to HV were evaluated by SPT and IDT, including SPT300. Additionally, HV-specific IgE antibodies were measured by ImmunoCAP. In a subgroup of monosensitized patients ($n=37$) with IgE antibodies to only either yellow jacket (YJV) or honey bee venom (HBV) the specificity and sensitivity of SPT300 was assessed.

Results: While 64% and 68% of patients showed positive test results in SPT with 100 µg/ml (SPT100) and IDT with 1 µg/ml (IDT1) of HV, respectively, 82.7% reacted in the SPT300. Analysis of individuals monosensitized to only YJV ($n=27$) or HBV ($n=10$) by IgE measurement revealed an almost equally satisfactory sensitivity of more than 80% for all SPT100, IDT1 and SPT300 with both allergens. However, when evaluating the specificity, it differed substantially between both HV for the SPT300, but not SPT100 or IDT1. While it exceeded 90% for SPT with YJV, it was only 51.9% for HBV. Other factors like atopy, grade of anaphylaxis, and the time lag between index sting and performing the skin tests were without impact.

Conclusions: SPT300 shows a high but not substantially superior sensitivity utilizing both YJV and HBV extract for diagnosis of HV allergy, when compared to SPT100 and IDT1. However, since SPT300 with BV extract demonstrated a very low specificity, our results suggest that the SPT300 has only very limited added value in the diagnosis of HV allergy.

V07

Skin Prick Test (SPT) and specific IgE may measure different IgE reactivities and be complementary in allergy diagnosis

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Skin Prick Test (SPT) and serum specific IgE (sIgE) are supposed to be qualitatively concordant. Allergen immunotherapy (AIT) trials require both tests to assess patient eligibility. This analysis evaluates the quantitative relationships between the tests from large adult grass-pollen (GRP) and dust mite (HDM) trials.

Material and Methods: SLIT tablet trials included SPT and sIgE testing with non-identical extracts. Positive SPT (ALK-Abelló) wheal diameters and sIgE levels (ImmunoCAP Singleplex, ThermoFisher) from GRP ($n=226$) and HDM-allergic subjects ($n=424$) were compared retrospectively. Further analysis included an adult cohort from Copenhagen (grass $n=72$, birch $n=74$), tested by SPT and sIgE (ADVIA Centaur, Siemens) with identical birch pollen (Bet v) and timothy grass pollen (Phl p) extracts.

Results: Across trials there was a lack of quantitative correlation between SPT and sIgE with $r=0.21$ between log mm Phl p SPT and log Phl p sIgE (US-GPR study P05238) and $r=0.08$ between log mm Der p SPT and log Der p sIgE (EU-HDM studies MT-02, MT-03 pooled). Except, in the Copenhagen Allergy Study, a correlation of $r=0.7$ and $r=0.48$ between log mm Phl p SPT and log Phl p sIgE and between log mm Bet v SPT and log Bet v sIgE were noted.

Discussion: Additional variables on top of sIgE may shape final biological responses (i. e. SPT) to allergens:

- total IgE concentration, regulation of high affinity IgE receptors, and sIgE/total IgE ratio
- individual mediator response (intrinsic sensitivity),
- cellular pre-activation (i. e. Syk levels),
- inhibitory effects by competing („blocking“) antibodies
- number/tissue distribution of effector cells,
- end organ responses.

SPT and sIgE may show acceptable qualitative concordance, but poor quantitative relation. Using identical allergen extracts and single center data sets may improve the quantitative correlation. Noteworthy, each test measures different allergen-specific IgE reactivity responses and hence may complement each other when selecting patients for AIT.

V08

Molecular phenotyping of house dust mite allergic patients—allergy diagnostic beyond extracts

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Background: The DZL-flag ship project “basic science” focuses on the effect of allergenic structure on allergenicity with particular emphasis on molecular phenotyping to investigate the evolution of sensitization. Studies on inhalant and food allergens have recently associated lipophilic allergens with more severe allergic reactions and respiratory symptoms such as asthma. Therefore, we aimed to investigate the impact of house dust mite allergens, in particular lipid-associated allergens, on sensitization and symptom phenotype development to identify suitable biomarkers for diagnosis, prognosis and therapeutic monitoring.

Methods: House dust mite (HDM) allergens Der p 2, 4, 5, 7, 10, 15, 18, 20, 21 and 23 were produced recombinantly in *E. coli* and purified by chromatography steps in downstream processing. Der p 1 was obtained commercially. Individual HDM sensitization patterns of patients from the KIRA cohort and further allergic patients were screened by use of the HDM allergens in a newly developed multiplex system.

Results: The established HDM allergen multiplex system allows for the simultaneous identification of specific IgE directed against various antigens and consequently the molecular phenotyping of patients with a broad spectrum of allergic symptoms. HDM-allergic patients often showed a sensitization to the major HDM allergens Der p 1 and Der p 2, and to a lesser degree to the lipophilic allergens Der p 5, 7 and 21. In comparison to routine diagnostic extracts, the use of our multiplex system increased the detection rate of HDM sensitized children of the KIRA cohort by almost 20%.

Conclusion: Molecular phenotyping of patients beyond standard clinical tests allows a better identification and characterization of patients and, thereby, guides clinical decision making towards the most effective treatment.

Therapy

V09

Adjuvant combinations in protein-based nanocapsules induce superadditive stimulation of dendritic cells, and highly effective T cell responses

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One aspect of vaccine development is to combine distinctly acting adjuvants to archive superadditive effects on immune cell activation. Moreover, a functional vaccine also requires an antigen source to enable the induction of T cell responses via antigen-presenting cells, in particular dendritic cells (DC).

A promising approach to meet this challenge is the application of nanoparticles as a drug-delivery-system. In a first initial step, we identified resiquimod (R848, specific for TLR7) and muramyl dipeptide (MDP, specific for NOD2) as a superadditive stimulatory adjuvant combination. Particulated in spermine-modified dextran-nanoparticles, this combination stimulates murine bone marrow-derived dendritic cells (BMDC) stronger than the soluble adjuvant equivalent. The second step was to combine adjuvants and antigen in one nanocarrier. For this purpose, we encapsulated the experimentally evaluated adjuvant combination (R848+MDP) in well-characterized polymeric nanocapsules, whose shell consists of cross-linked ovalbumin (OVA) protein, a commonly used model antigen in immunology. To assess the capsules' immunostimulatory potential, we treated BMDC with the adjuvant-loaded nanocarriers. The expression of the surface activation markers CD80/CD86 and the secretion of proinflammatory cytokines were measured by flow cytometry and cytometric bead array, respectively. The capability of the adjuvant-loaded OVA-nanocapsules to mediate OVA-specific T cell responses was assessed by performing T cell proliferation assays with transgenic OT-I (CD8+) and OT-II (CD4+) T cells that recognize OVA-derived antigens. Our data showed that particulated co-delivery of R848 and MDP activates murine BMDC in a superadditive manner as compared with single-delivery of the adjuvants. Additionally, the application of R848+MDP via OVA-nanocapsules evoked a strong antigen-specific T cell proliferation. These results show that R848/MDP-loaded OVA-nanocapsules are highly active to induce superior antigen-specific T cell responses.

V10

Epidermal allergen-specific immunotherapy in an Api m 1-allergic mouse model

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Introduction: Allergy to Hymenoptera venom (HV) is the second most common cause of IgE-mediated anaphylaxis. While subcutaneous HV-specific immunotherapy (HV-IT) shows high efficiency in inducing allergen tolerance, it is associated with potential severe systemic reactions. Utilizing a HV-allergic mouse model, we wanted to assess whether epidermal HV-IT represents a safe and effective therapeutic alternative. Thus, mice sensitized to the major allergen of honeybee venom, Api m 1, were treated with different, topically applied concentrations of Api m 1 and both clinical outcome and immunological changes were assessed.

Methods: Balb/c mice were treated for 4 weeks with different concentrations of Api m 1 (0/0.25/0.625/1 mg/ml), solved in either PBS or microemulsion. Subsequently, mice were sensitized intraperitoneally (i.p.) by 3 injections of 5 µg Api m 1 adsorbed to Al(OH)₃ followed by an i.p. challenge with 100 µg of Api m 1. Blood samples were collected at different time points for determination of IgE and IgG levels by ELISA and flow cytometric analysis of regulatory T cells. In addition, immune cells were isolated from lymph nodes and spleen after challenging for analysis by ELISpot and flow cytometry.

Results: Sensitized mice treated with PBS only showed a maximum rectal temperature drop of around 5°C, measured over 6 h after challenge. In contrast, prophylactic treatment with increasing doses of Api m 1 led to a marked reduction in temperature drop with a decrease of only 2°C and a substantially faster recovery at the highest HV-IT dose. This was associated with increased production of Api m 1-specific IgG antibodies, whereas no changes were observed in the frequencies of IL-5-, IL-10- and IFN-γ-secreting T cells as well as Foxp3+ regulatory T cells in peripheral blood, lymph nodes and spleen.

Conclusion: Prophylactic epidermal HV-IT with the major honeybee venom Api m 1 represents a safe and effective treatment preventing anaphylaxis, most likely mediated by the synthesis of allergen-specific IgG antibodies.

V11

Immunisation of mice with protein antigen and microcrystalline tyrosine (MCT) depot adjuvants stimulate strong antibody and Th1-like immune responses in mice

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Introduction: Microcrystalline Tyrosine (MCT) represents a patented depot adjuvant formulation and features in licensed allergoid vaccines and various other (pre-)clinical developments. The aim of the study was to analyse innate and adaptive immunogenicity of MCT-based vaccines in murine models in vivo and in vitro, compared to aluminium-based vaccines.

Objectives: After subcutaneous immunization of BALB/c or C57BL/6 with ovalbumin (OVA) combined with MCT or aluminium hydroxide (alum) OVA-specific antibody responses in blood and CD4 and CD8 T-cell responses in cultures of spleen cells restimulated in vitro with OVA were tested. For T-cell response, C57BL/6 mice were adoptively transferred with lymphocytes from transgenic T-cell receptor OT-I (CD8) and OT-II (CD4) mice. Serum antibody responses and cytokine secretion in culture supernatants or spleen cells were measured.

Results: The onset and the peak of the antibody IgG1 and IgG2 responses in mice immunised with alum were typically faster than with MCT, but no significant difference in the endpoint titres were observed between groups. A significant secretion of the T-helper type 1 cytokine IFN- γ from spleen cells was observed both in MCT- and alum-immunised mice measured. In the C57BL/6 model, MCT but not alum mediated IFN- γ secretion. In the OT-I/OT-II cell transfer model, Th1 cytokine secretion with OVA-MCT was comparable or even higher than cells with OVA-alum. This confirmed in flow cytometry after staining of spleen cells with fluorescent antibodies against CD4, CD8, activation molecules and intracellular cytokines.

Conclusions: Comparative potential of MCT and aluminium-based adjuvants with regards to antibody responses and T-cell responses were demonstrated. The favourable Th1-like immune responses produced by MCT together with the previously demonstrated favorable safety profile of MCT-based vaccines, suggest that MCT may meet the requirement for a wide range of future vaccines and immunotherapies.

V12

Impact of vitamin D on subcutaneous grass pollen-specific immunotherapy—first immunology data from a controlled clinical pilot trial

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Vitamin D impacts the type I-allergic immune response. Activated human lymphocytes can produce the active vitamin D metabolite, calcitriol (1,25-dihydroxyvitamin D3) from its inert precursor. We have recently shown at the genetic level that lymphocyte-derived calcitriol inhibits the IgE response in mice deficient in the essential gene *cyp27b1*. In established allergy, allergen-specific immunotherapy is more potent together with endogenous calcitriol to control the IgE response and also allergic airway inflammation.

In this work, we analyzed patients with grass pollen-induced allergic rhinoconjunctivitis \pm allergic asthma over 3 consecutive years with specific immunotherapy in a pre-seasonal regimen. Concomitantly the participants received daily 5000 I.U. vitamin D orally or placebo in a controlled randomized fashion (NCT01466465). We analyzed over time the frequencies and activation of defined subsets of T and B lymphocytes and also the humoral specific immune response. The data show that after each vitamin D supplementation period both groups differ significantly regarding the vitamin D serum concentrations. At the cellular level, the frequencies, subset composition and cytokine pattern of specific T lymphocytes in the peripheral blood were altered over time by specific immunotherapy, but also vitamin D in a specific manner. The data suggest a regulation of the humoral specific immune response by vitamin D in the first treatment period by reducing specific reduced IgE serum concentrations compared to placebo along with intact IgG4 induction.

V13

Allergie Immuntherapie kann das Fortschreiten von bereits bestehendem Asthma verhindern

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Einleitung: Die Allergie-Immuntherapie (AIT) gilt als die einzige Behandlungsmethode, die den natürlichen Verlauf einer allergischen Erkrankung zu modifizieren erlaubt. Langzeiteffekte nach Beenden einer Behandlung und präventive Effekte auf die Entwicklung von Asthmasymptomen und Asth-

mamedikamenten wurden für einige Präparate dokumentiert. Es wurde bislang noch nicht untersucht, ob die AIT die Progression von einem bereits bestehenden Asthma verhindern kann.

Methoden: Anhand einer Krankenkassendatenbank wurde eine Kohortenstudie durchgeführt, bei der ICD-10-Diagnosen und Verschreibungsdaten von 1,74 Mio Versicherten über einen Zeitraum von 10 Jahren ausgewertet wurden. Die Anforderungen an eine Asthmad diagnose waren: mind. 2 ICD-10-Codes J45 (Asthma) und mind. 2 Verordnungen von Asthmedikamenten (SABA, ICS, ICS+LABA). Die Asthmedikamente wurden nach den von GINA 2006 empfohlenen Behandlungsstufen eingeordnet. Verschiedene Altersgruppen im Jahr 2005 (B: 12–17 Jahre, C: 18–50 Jahre, D: 50+ Jahre) wurden ausgewertet. Übergänge zwischen den GINA-Stufen wurden für Alter sowie für Geschlecht analysiert.

Ergebnis: Im Vergleich zur Versichertengruppe ohne AIT zeigte die Durchführung einer AIT ein signifikant reduziertes Risiko von GINA-Stufe 1 hin zur GINA-Stufe 3 zu wechseln. Dieser Effekt war bei den jüngeren Patienten am stärksten ausgeprägt (Hazard ratio (95% CI): gesamt 0.87 (0.80–0.95%), Gruppe B: 0.72 (0.58–0.88), Gruppe C: 0.89 (0.80–0.98), Gruppe D: 1.09 (0.87–1.38)). Die AIT verringerte zudem das Risiko von GINA-Stufe 3 zu GINA-Stufe 4 zu wechseln. Die Anzahl der zu behandelnden Patienten, die benötigt wurde, um zu verhindern, dass 1 Patient innerhalb von 5 Jahren von GINA-Stufe 3 auf GINA-Stufe 4 aufstieg, betrug 10,9 (8,2–16,2).

Schlussfolgerung: Dies ist die erste große Kohortenstudie die zeigt, dass AIT die Progression von Asthma effektiv verhindern kann. Die Ergebnisse sollten unterstützend zu der in randomisierten klinischen Studien dokumentierten produktspezifischen Wirksamkeit betrachtet werden.

V14

Anti-IgE antibody treatment interferes with the frequencies of IFN-gamma- and IL-31-secreting T cells in patients with chronic urticaria

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Introduction: Patients with chronic urticaria (CU) suffer from recurrent itching wheals with or without angioedema. The anti-IgE antibody omalizumab has been approved for the treatment of CU patients. However, its mode of action is still not fully understood and immunological data comparing responder with non-responder patients are rare. To obtain deeper insights in the immunological changes induced by anti-IgE treatment, we analysed cell-bound IgE and frequencies of T cell subtypes during therapy in CU patients.

Methods: CU patients ($n=15$) received monthly injections of omalizumab for up to six months and symptoms were assessed by both the urticaria control test (UCT) and the CU quality of life score (CU-Q2oL). According to their clinical changes, patients were subdivided into full- ($n=7$; FR), partial- ($n=5$; PR) and non-responders ($n=3$; NR). Peripheral blood was drawn prior

to each injection determining the basophil reactivity to anti-FcεRI and fMLP stimulation by basophil activation test. The impact of anti-IgE treatment on cell-bound IgE was analysed on FcεRI+ and FcεRII+ cell subsets by flow cytometry. In addition, frequencies of IFN-γ-, IL-5-, IL-10- and IL-31-secreting T cells were measured by ELISpot.

Results: All patients showed a decrease in surface IgE and FcεRI expression on basophils after 6 months of treatment, while the responsiveness of basophils to anti-FcεRI-stimulation increased. Accordingly, cell-bound IgE on both FcεRI+ and FcεRII+ cells declined except for B cells. Interestingly, we could also detect reduced frequencies of IFN-γ-, IL-31- and by trend IL-10-secreting T cells after 6 months in FR and PR compared to NR. Correlating the clinical outcome with the immunological parameters revealed that an improvement in UCT was associated with a decrease in basophil-bound IgE, IL-10- and IL-31-secreting cells.

Conclusions: In addition to reduced cell-bound IgE and downregulation of FcεRI, our results show that treatment of CU patients with anti-IgE antibodies interferes with distinct T cell subsets.

V15

Influence of synbiotics on the T cell response of grass pollen-allergic individuals in vitro

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Background: Seasonal allergic reactions to airborne allergens such as grass pollen are triggered by Th2 cells that can differentiate from naive CD4+ T cells upon stimulation by allergens. As sensing of the intestinal microbiota by the host mucosal immune system is important to induce protective immune responses, modification of the gut microbiota might be able to support health and to prevent or treat allergies. Hence, we aimed to investigate the effects of synbiotics on the differentiation of human immune cells in the context of grass pollen allergy.

Methods: Peripheral blood mononuclear cells from grass-pollen allergic patients and healthy controls were stimulated *in vitro* with grass-pollen extract and supplemented with different synbiotics. T helper cell subset differentiation was examined by flow cytometry and the measurement of cytokines in culture supernatants.

Results: In contrast to non-allergic controls, in grass pollen-allergic individuals, stimulation with grass pollen extract induced proliferation of Th2 cells. The addition of synbiotics, containing different probiotic strains of *Lactobacillus*, *Bifidobacterium* and fructooligosaccharides as prebiotic supplement, regulated the immune response of allergic individuals on the T helper cell level. A shift from an allergic Th2-driven immune response towards a Th1 immune phenotype was observed. This effect was mediated directly by synbiotics as well as indirectly by "only" culture supernatants of the probiotic bacteria.

Conclusion: Taken together, the presence of synbiotics showed an immune-modulating capacity via suppression of Th2-type inflammation in PBMCs of grass pollen-allergic individuals. Hence, these effects of synbiotics on the host immune response might implicate a potential approach to modify allergic immune responses. Further studies might give an insight into the regulatory mechanisms of specific compounds derived from probiotics, which suppress the Th2-driven immune-phenotype.

V16

Herbal extracts and their active compounds attenuate toll-like receptor 2 and 4 pathways

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Inflammatory processes contribute to most severe diseases, such as inflammatory bowel disease (IBD), allergic asthma, autoimmune diseases and liver fibrosis, resulting in organ dysfunction. On the molecular level, inflammation is associated with a cyclic process, prominently involving Toll-like receptor (TLR) stimulation, the production of reactive oxygen and nitrogen species (ROS/RNS) and secretion of pro-inflammatory cytokines/chemokines, as well as host-derived damage associated molecular patterns (DAMPs). Our intention is the identification of compounds in herbal extracts, with the ability to interrupt the self-perpetuating process of inflammation. A screening of more than 100 herbal ethanolic extracts for their effects on LPS-induced TLR4 activity in HeLa-TLR4 cells and THP-1 monocytes resulted in 28 extracts showing moderate to high dose-dependent anti-inflammatory activity. Among them, an ethanolic extract of *Cinnamomum verum* bark exhibited very potent effects. It was fractionated using high-performance liquid chromatography that allowed the identification of 12 individual compounds. In a comparative TLR2/TLR4 assay system, cinnamon extract and its active compounds were shown to interfere with the signaling pathways of both, TLR2 and TLR4. In addition, synergistic effects between different *Cinnamomum verum* compounds, e.g. cinnamaldehyde and cinnamic acid were observed, which complicates the identification of the underlying molecular mechanisms.

Given that the *Cinnamomum verum* extract as well as its active compounds appear to exert no toxic effects, the results may contribute to the development of new oral treatment strategies for different inflammatory diseases.

V17

Analysis of a possible anti-inflammatory effect of cinnamon extract in allergic immune responses

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Recently, cinnamon extract has been shown to inhibit mast cell degranulation and de novo synthesis of mast cell-specific inflammatory mediators. The aim of this study was to analyze the effects of cinnamon extract on allergen-specific immune responses in vitro and in vivo. Therefore, BALB/c mice were immunized with ovalbumin (OVA)/alum and orally treated with cinnamon extract or the solvent ethanol as control. For the in vitro experiments, allergen-pulsed monocyte-derived mature dendritic cells (DC) from grass or birch pollen allergic donors were treated with cinnamon extract or ethanol and co-cultured with autologous CD4+ T cells, and/or cinnamon extract/ethanol was directly added to these co-cultures. Furthermore, leukotriene release of basophils was analyzed in the presence or absence of cinnamon extract. Treatment of OVA/alum-sensitized mice with cinnamon extract led to a shift from OVA-specific IgE towards OVA-specific IgG2a production together with a slight reduction in eosinophil numbers and airway hyperreactivity. In vitro, addition of cinnamon extract but not ethanol strongly reduced leukotriene release of basophils as well as allergen-specific T cell proliferation and cytokine production in selected donors, while some donors were not affected by this treatment. Taken together, our data indicate that cinnamon might be a candidate for treatment of allergic inflammation in special donors which needs to be further investigated.

Skin/contact dermatitis

V18

The complement component C3 and calcium binding proteins are differentially regulated via pro-inflammatory cytokines in normal human epidermal keratinocytes

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Pro-inflammatory mediators play a pivotal role in pathophysiology of chronic inflammatory skin diseases. In this context, IL-17 represents a key cytokine in psoriasis and is shown to be elevated in the skin of patients with acute atopic dermatitis (AD). Proteomic analyses in human psoriatic epidermis revealed the calcium binding proteins S100A8, S100A9 and the complement component C3 as being the most up-regulated

proteins. Increased serum levels of C3 may refer to the compromised host defense in AD patients.

In order to study a possible link between relevant pro-inflammatory cytokines and C3 as a crucial part of the complement system and innate immunity, undifferentiated and Ca²⁺-induced terminally differentiated normal human epidermal keratinocytes (NHEKs) were cultured and stimulated with IL-17, Oncostatin M (OSM) and TNF- α .

The complement component C3a receptor (C3aR) mRNA expression was increased via OSM and TNF- α and decreased via IL-17 in undifferentiated keratinocytes.

Contrary to this, the complement component C3 was up-regulated via TNF- α and mostly via IL-17 and down-regulated through OSM in undifferentiated keratinocytes.

In addition, we observed that S100A7, -A8 and -A9 mRNA expression could be enhanced by OSM and IL-17 in undifferentiated keratinocytes.

Remarkably, S100A8, -A9 and C3 were up-regulated during differentiation of keratinocytes and all three were additionally increased by IL-17.

Experiments with IL-17 stimulated undifferentiated keratinocytes and S100A9 siRNA suggest that S100A9 may be involved in this context as a regulator for C3 expression.

In summary, OSM, TNF- α and, in particular, IL-17 regulate the C3/C3aR axis as essential cytokines associated with inflammatory skin diseases such as psoriasis and AD. Importantly, IL-17-dependent up-regulation of S100A9 may mediate gene modulation of C3. Elevated levels of these mediators may impair inflammatory skin diseases which emphasizes their potential as being a target in therapy.

V19

Cytotoxic immune response of CD8+ T cells and NK cells in patients suffering from atopic dermatitis with a history of eczema herpeticum

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A subgroup of patients with Atopic dermatitis (AD) is suffering from recurring, generalized herpes simplex virus symptoms, known as eczema herpeticum. To date, genetic predispositions as well as reduced interferon gamma responses have been reported to underlie this increased susceptibility, and we and others recently showed that Th2/Tc2-polarized specific T cells play a role in these patients (Traidl et al. 2017). However, the ability of immune cells regarding a proper cytotoxic response to herpes simplex has not been addressed in detail.

This study aimed to assess the cytotoxic capacity of CD8⁺ T cells and NK cells in patients suffering from AD and especially

in the subgroup of AD patients with a history of eczema herpeticum (ADEH+).

HSV1-specific CD8⁺ T cells were identified by MHC-tetramers, stimulated and stained intracellularly for granzyme B. Further on, granzyme B secretion was assessed by means of ELISA in isolated NK cells and T cell lines grown in the presence of the HSV1 proteins UL25, ICP0 and 8, glycoprotein D, as well as crude extracts of HSV1-infected human cells, respectively. The NK cell degranulation was further investigated via the markers CD107a/b.

No differences in HSV1-specific T cell-derived granzyme B responses were detectable between ADEH+ and ADEH- patients. NK cells also showed no differences between the cohorts regarding CD107a/b expression. However, the secretion of granzyme B in K562-stimulated isolated NK cells was remarkably reduced in ADEH+ compared to healthy individuals. Further on, we detected significantly reduced frequencies of NK cells in the circulation of ADEH+ compared to ADEH- and healthy individuals.

While we were unable to detect a reduced cytotoxic capacity in CD8⁺ T cells, we report a combination of lower frequencies and lower granzyme B secretion of NK cells in patients suffering from AD and a history of eczema herpeticum. This problem may arise as a consequence of the chronic type 2-polarized inflammatory milieu present in AD.

V20

Skin microbiome in atopic dermatitis: opposing roles of *S. aureus* and *P. acnes*.

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Background: The skin microbiome in atopic dermatitis (AD) plays a role in disease severity and impacts local and systemic immunity. The overgrowth of *Staphylococcus aureus* correlates with AD flare-ups in AD patients. *S. epidermidis*, which is considered a harmless skin commensal, promotes an innate immune response and inhibits the growth of *S. aureus* and *Propionibacterium acnes*. Role of *P. acnes* in AD is less known.

Results: After sampling the skin of AD patients and healthy individuals in a standardized manner we analyzed the microbial composition by next-generation sequencing (Illumina platform). Lesional versus non-lesional skin from patients with atopic dermatitis exhibited a higher abundance of *S. aureus* and diminished quantities of *P. acnes* and *Lawsonella clevelandensis*. The abundance of *P. acnes* correlated negatively with *S. aureus* ($\rho = -0.6501$, $p < 0.0001$). Fermentation products of *P. acnes* inhibited the growth of *S. aureus* and *S. epidermidis* in vitro (measured using optical density). Serum from AD patients inhibited the growth of *S. aureus* to a greater extent than serum from healthy individuals.

Conclusions: Decreased *P. acnes* abundance might be one of the factors for an increased *S. aureus* colonization in AD. The reason for the lower *P. acnes* abundance may be due to diminished function of the sebaceous glands in AD patients. Our data suggest that a selective modification of the skin microbiome (e.g. by microenvironment modification or the direct microbial supplementation with commensal Propionibacteria) may potentially be used as a therapeutic strategy in atopic dermatitis leading to the stabilization of the skin microbiome and preventing flares.

V21

A case of hypersensitivity against suture material initially misdiagnosed as artificial dermatitis

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Skin and soft tissue reactions against suture material are rare and include non-specific inflammatory reactions, foreign body reactions as well as contact or granulomatous allergies. We report the case of a 49 year old female patient who has had a work accident years ago. Due to fractures in her thigh neck she underwent several surgical procedures and wound revisions including the implantation of a titanium-coated femoral head prosthesis. Every surgical procedure was followed by complications such as wound infections, swelling, pain, bad wound healing and hip head loosening. There was no evidence of bacterial or mycotic infection. A skin biopsy performed under the differential diagnosis of pyoderma gangraenoum showed acute toxic dermatitis with hydropic keratinocytes and was compatible with artificial dermatitis. To rule out any form of allergy we performed patch testing including baseline series, medicaments, leather and textile colours, resins and adhesives as well as a metal series that showed late type sensitizations against benzoyl peroxide and manganese(II)-chloride without clinical relevance. Patch testing with native suture material (Vicryl, coloured with D&C violet no. 2 and Prolene, coloured with phthalocyanine blue, colour index 74160). Both suture materials showed positive late type reactions. Further testing was performed by stitches with different sutures (Vicryl stained, Prolene stained, Monocryl unstained and Ethibond Excel stained). In readings after 20min, 24, 48 and 72 h Vicryl and Ethibond Excel stained showed positive reactions including, redness, swelling and itching. A skin biopsy from the reaction showed a deep dermal granulomatous infiltrate following the stitch without evidence of foreign bodies. Further surgical procedures in which unstained sutures materials were used were tolerated well and showed normal wound healing. Allergy against suture material should be taken into account in cases where prolonged and poor wound healing is present.

Respiratory tract/asthma

V22

Wnt signaling as potential modulator of allergic airway diseases—differences and similarities of Wnt1 and Wnt5A in immune regulation

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Recent findings have shown that canonical Wnt/ β -Catenin signaling is upregulated in asthma patients and that the activation of this pathway is able to suppress allergic airway disease in mice. The Wnt/ β -catenin pathway has the capacity to modulate the immunosystem in several ways. Yet, the precise suppressive mechanisms of the canonical pathway is largely unknown. Additionally, immunoregulatory functions of the non-canonical pathways are almost completely unrevealed in allergic airway disease.

The present study aimed to further characterize the immune regulatory mechanisms of canonical and non-canonical Wnt signaling and compare their therapeutic potential in a murine model for asthma.

C57BL/6 mice have been sensitized against Ovalbumin (OVA) or House Dust Mite Extract (HDM) with Aluminiumhydroxyd (D0/D14) and challenged via the airway with the appropriate allergen (D28-30) to induce an allergic airway disease. During the challenge phase mice were treated intranasally with canonical Wnt1 Ligand or non-canonical Wnt5A Ligand. On day 32 the allergic airway disease and the immune response has been analysed.

Especially Wnt1 was able to decrease all hallmarks of the allergic airway diseases, like airway hyperresponsiveness, goblet cell metaplasia and inflammation. Treatment affected DC response in the lung and reduced the secretion of IL-13 a key mediator of allergic airway disease. In comparison to Wnt1, Wnt5A has only attenuated the airway inflammation in the OVA model and failed completely to suppress the allergic airway disease in the HDM model.

Both signaling pathways were able to modulate immune responses *in vitro* and *in vivo*. However, due to its higher potential to attenuate the asthma phenotype *in vivo* Wnt1 induced canonical signaling seems to be the more promising target for development of new treatment options for asthma.

V23

CMV infection promotes sensitization towards harmless antigen and development of an allergic airway disease

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Worldwide a large number of people are infected with the lung targeting cytomegalovirus (CMV). While immunocompromised persons can develop an interstitial pneumonia, an infection of healthy people remains symptomless.

Airway infections with viruses such as rhinovirus and respiratory syncytial virus (RSV) are associated with development and exacerbation of bronchial asthma. Role and function of CMV infections on formation of an allergic airway disease are largely unknown. Aim of the current project was to characterize the impact of a CMV infection on the sensitization towards

a harmless antigen via the airways and the development of an allergic lung disease.

The non-immunogenic antigen Ovalbumin (OVA) was applied alone or in combination with murine CMV into the airways of C57BL/6 mice. Mice were challenged by OVA nebulization and the developing immune response was analyzed in the lungs. An antigen-specific inflammation in the lung and induction of an OVA specific-CD8 T cell and -immunoglobulin response was exclusively detectable in animals receiving OVA in combination with mCMV.

Analyzing the underlying mechanisms we were able to demonstrate that mCMV affect the migration, activation and sub-type composition of OVA positive dendritic cells.

We assume that CMV can modulate DC, change the allergenicity of normally harmless antigens and so facilitate the development of allergic airway diseases.

V24

Interleukin 6 has a key role during virus induced exacerbation of asthma

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Viral infections of the lung are the major cause of acute asthma exacerbations. Double-stranded RNA motifs, produced during replication of respiratory viruses, can trigger immune responses via activation of Toll-like receptor 3 or RIG-I. We have previously shown that local application of the synthetic TLR-3/RIG-I activator poly(I:C) alone is sufficient to trigger exacerbation of experimental allergic asthma in mice.

This study aimed at identifying early regulatory mechanisms leading to asthma exacerbation. In a mouse model of poly(I:C) triggered exacerbation of allergic asthma is characterized by acute worsening of airway inflammation, mucus production and airway hyperresponsiveness. This was associated with increased production of proinflammatory cytokines e.g. IL-4, IL-5, IL-6, and IL-13. Interestingly, among these cytokines IL-6 levels revealed by far the earliest and the highest increases not only in broncho-alveolar lavage (BAL) fluid but also in nasal lavage (NAL) and serum. We made similar observations in air-liquid interface (ALI) cultures of primary human bronchial epithelial (hBEC) cells, one of the main target cells of respiratory viruses. Primary hBECs incubated with poly(I:C) + IL-13 displayed increased expression of IL-6 and mucus secretion compared to controls incubated with either IL-13 or poly(I:C) alone. We therefore, investigated the role of IL-6 in the pathogenesis of an acute exacerbation of experimental asthma. While, application of recombinant IL-6 instead of poly(I:C) did not trigger an exacerbation, it was not possible to induce a poly(I:C) triggered exacerbation in animals deficient for IL-6. Thus, even if IL-6 alone is not sufficient to trigger an exacerbation these results indicate a critical role for IL-6 in poly(I:C) induced acute exacerbation of experimental asthma. This study suggests IL-6

potential target for therapy of virus induced acute asthma exacerbations.

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V25

Time-dependent analysis of lipid mediators in stimulated human blood cultures from the German Center for Lung Research (DZL) adult asthma cohort

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Background: Asthma is an obstructive chronic inflammatory disease, caused by overreaction of the airways in response to various stimuli. Eicosanoids, derived from polyunsaturated fatty acids contribute to the inflammatory process and some metabolites have been shown to play a key role in asthma development and acute exacerbations. However, a detailed understanding of the complex regulation of whole eicosanoid biosynthesis pathways in asthmatic subjects is still missing.

Objectives: Comprehensive analysis of eicosanoid profiles from stimulated blood cultures of asthmatic subjects compared to healthy controls.

Methods: Blood was collected from 173 asthmatic adults and 33 healthy controls and after stimulation with zymosan for either 4 or 48 h, eicosanoids were extracted and analyzed using a targeted LC/MS² approach.

Results: We found significant differences with distinct time kinetics in the activity of the five main eicosanoid biosynthesis pathways. After 4 h of stimulation metabolites belonging to the 5-Lipoxygenase (5-LOX) pathway show increased median levels within the group of asthmatic patients. In contrast, metabolites belonging to the cyclooxygenase (COX), 12- or 15-LOX and to the cytochrome P450 monooxygenase (CYP) pathway were produced at lower concentrations compared to the healthy controls. An opposite pattern was observed after prolonged stimulation. Whereas the concentration of most metabolites did not change between 4 h and 48 h of stimulation within the control group, we observed a decrease in the amount of eicosanoids of the 5-LOX pathway in the asthmatics after 48 h of stimulation. In contrast, these patients exhibit an increased production of metabolites belonging to the COX, 12- and 15-LOX pathway. Metabolites of the CYP pathway tend to increase after 48 hours of stimulation, reaching a median level comparable to those of the control group.

Conclusion: Asthmatic adults show a time-dependent pattern of eicosanoid production which may contribute to the pathogenesis of airway inflammation.

V26

Acute exacerbation of experimental asthma in mice is preceded by an early production of IL-6, KC, and TNF**R. Bodenstern-Sgro, S. Webering, M. Wegmann**

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Epidemiologic studies show that respiratory infections trigger acute asthma exacerbations as distinct clinical manifestations during the course of chronic disease. These acute episodes of progressive worsening of the disease symptoms account for unscheduled visits of physicians, emergency departments or hospitalization and requirement of increased and/or systemic corticosteroids and therefore have a major impact on public healthcare. Hence, early detection of developing asthma exacerbations is needed for early intervention strategies.

An established mouse model of experimental asthma exacerbation was used to analyse the early virus-induced specific cytokine immune response in asthmatic mice. For this purpose, we induced experimental allergic asthma in female C57BL/6 mice by systemic sensitization to and challenge with ovalbumin (OVA). Intra-nasal application of the TLR-3/RIG-I ligand poly(I:C) induced an acute exacerbation of the established disease. We analysed leukocyte numbers in the BAL as well as the expression and production of various cytokines, chemokines and immuno-modulatory factors in different compartments 2, 4, 8, and 12 hours after poly(I:C) application.

An early influx of eosinophils and neutrophils increasing over time and reaching highest numbers after 12 hours was detected. Foregone was an increased expression and release of chemokines like eotaxin and especially KC. Though T helper 2 (TH2) cell cytokine increase was only moderate, type I as well as type III interferons and proinflammatory cytokines like interleukin (IL) 1 and tumor necrosis factor were produced early and increased steadily. Most interestingly, IL-6 levels in bronchoalveolar lavage, nasal lavage, and serum were already high 2 hours after poly(I:C) application and remained at even more increased levels until 12 hours.

Based on these data we suggest IL-6, TNF and KC as early markers of a developing exacerbation of experimental allergic asthma.

V27

IL-37 ameliorates experimental asthma by interfering with the proinflammatory IL-1 signaling**A. Schröder¹, L. Lunding¹, C. Vock¹, B. Schaub², U. Zissler³, H. Fehrenbach¹, M. Wegmann¹**¹Forschungszentrum Borstel, Borstel, Germany²LMU Munich, Munich, Germany³Center of Allergy and Environment—ZAUM, Munich, Germany

We have previously demonstrated that the production of the antiinflammatory cytokine interleukin (IL) 37 is diminished in allergic asthmatics and that local treatment with IL 37 reduces all pathological signs of experimental asthma in mice. Based

on these findings, we hypothesize that an impaired production of IL-37 could lead to a reduced capacity to counterbalance an ongoing inflammation and thus could favour the development of chronic inflammatory diseases. Therefore, supplementation of IL-37 deficiency could represent a novel therapeutic intervention in asthma.

However, the mechanism through which IL-37 ameliorates experimental asthma is not completely understood.

By using different knock-out mouse strains we could demonstrate that IL-37 functions via the cell surface receptors IL-18R α and SIGIRR, which are expressed on all cells playing a role in asthma pathogenesis including T helper 2 (TH2) cells, dendritic cells (DCs), and airway epithelial cells (AECs). In vivo studies revealed that IL-37 significantly reduced the production of TH2-type cytokines and expression of the transcription factor GATA-3. Therefore, we further investigated the effects of IL-37 on TH2 cells. IL-37 did not influence cytokine production of TH2 cells activated with antibodies against CD3 and CD28. However, IL-37 significantly reduced the production of IL-4, IL-5 and IL-13 as well as of IL-1 β in allergen-specifically activated mononuclear cell cultures. Since gene array analysis of lung tissues revealed that IL-37 treatment of animals with experimental asthma lead to differential expression of more than 90 genes induced by IL-1 signaling, we analysed if IL-37 interferes with IL-1 signaling. And, in fact, IL-37 markedly reduced IL-1 β -induced expression of proinflammatory cytokines and chemokines in AECs. Furthermore, IL-37 failed to ameliorate experimental asthma in mice lacking IL-1R1.

In conclusion, these findings indicate that IL-37 down-regulates allergic airway inflammation by inhibiting the proinflammatory activity of IL-1.

Food allergy/enteritis

V28

Investigation of reduced ELISA recovery of almond and hazelnut traces from roasted nut samples by SDS-PAGE and mass spectrometry**S. Perner¹, L. Heupel¹, K. Vongehr¹, H. Elbedewy¹, L. Zimmermann², Y. Peters², S. Loos-Theisen², B. Lindemann², S. Siebeneicher³, T. Weiss³, T. Hektor³, K. Schneider¹**¹Institute for Biomolecular Research, Hochschule Fresenius, Idstein, Germany²Institut für Lebensmittelsicherheit, Hochschule Geisenheim University, Geisenheim, Germany³R-Biopharm AG, Darmstadt, Germany

Background: Unintended traces of tree nut allergens in food pose a high risk factor for allergic consumers. Almonds (*Prunus dulcis*) and European hazelnut (*Corylus avellana*) are among the most commonly used tree nuts and pose a strong allergenic potential. The most commonly used method to check for potential cross contamination is an Enzyme-linked Immunosorbent Assay (ELISA). Unfortunately, available tests have limited sensitivity in detecting traces of processed nuts (e.g. roasted or baked). Nonetheless, these processed samples retain their allergenic potential.

Methods: Almonds and hazelnuts were roasted under controlled conditions in a drum roaster at varying temperature and

time profiles. Cookie dough was spiked with known amount of nut samples obtained from roasting experiments prior to baking. Proteins from raw nuts, roasted nuts and baked cookies were extracted and analysed with SDS-PAGE, MALDI-TOF-MS and LC-MS/MS to identify the extracted proteins.

Results: Analysis of the nut extracts using SDS-PAGE showed a broad range of protein bands for both nut species. In accordance to the ELISA results, the bands became less prominent with rising roasting times and temperatures. This was a first indicator, that the thermal processing has either a detrimental effect on the extractability or leads to the degradation of the proteins. All bands were subjected to a tryptic digest followed by MALDI-TOF MS analysis, which resulted in identification of Cor a 9 as main allergen for hazelnut and of prunin for almond. The LC-MS/MS analysis confirmed these results. Peptides which were specific for the respective tree nut and were present in all samples were identified.

Conclusion: The study showed that the major part of the proteins extracted from hazelnuts and almonds consists of mainly allergenic proteins (Cor a 9 and prunin). Additionally, the results indicate that the loss of ELISA recovery is probably accounted by inefficiencies in extraction of the samples or the degradation of the proteins during processing.

V29

Allergens from the lipophilic peanut extract: Important pieces of the diagnostic puzzle

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Background: Peanut allergy is the most common cause for life-threatening anaphylaxis among children and adolescents and its prevalence is on the rise. The identification of patients at risk of having clinical reactions to peanuts is of major interest for clinicians. However, routine diagnostic test rely on aqueous peanut extracts and water-soluble single allergens, and are devoid of lipophilic allergens which is why oral provocation tests are still considered as diagnostic gold standard. Therefore, we aimed to analyze so far unknown components of peanut and their relevance for the diagnosis of peanut allergy.

Methods: Peanut defensins and oleosins were isolated from the lipophilic fraction of peanuts. Following purification, molecules were identified by means of mass spectrometry and N-terminal sequencing. Sera from peanut-allergic patients with different symptom phenotypes were screened for allergen-specific IgE in immunoblot experiments. The ability of defensins and oleosins to trigger allergic reactions was assessed by basophil activation test (BAT).

Results: Defensins (Ara h 12 and 13) and oleosins (Ara h 10, 11, 14, 15) were obtained as highly purified proteins after chromatographic or electrophoretic separation. IgE-binding to both molecule classes was exclusively observed in peanut-allergic patients suffering from severe reactions, however, binding to oleosins was more frequent. BAT showed the ability of defensins

and oleosins to stimulate basophils and thus demonstrated their relevance for a comprehensive peanut allergy diagnostic. A statistical evaluation of the diagnostic performance of the BAT with oleosins revealed a high sensitivity but also a high specificity of the test.

Conclusion: Defensins and oleosins are clinically relevant allergens that should be included in routine diagnostic tests to determine the full sensitization pattern of peanut-allergic patients, especially as oleosins seem to be biomarkers for symptom severity.

V30

Identification and molecular characterization of allergenic non-specific Lipid-Transfer Protein (nsLTP) from durum wheat (*Triticum durum*)

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Over-expression of nsLTPs in durum wheat is considered to increase the resistance against plant pathogens. Although both, common wheat (*Triticum aestivum*, bread) and durum wheat (*Triticum durum*, pasta) are involved in food allergy (FA)/WDEIA or baker's asthma (BA), *T. durum* allergens are unknown.

Aim of the study was the recombinant expression, molecular characterization and allergenicity assessment of nsLTP from *T. durum* (Tri d LTP) in comparison to Tri a 14 (*T. aestivum*) and Pru p 3 (peach).

Recombinant (r) Tri d LTP was over-expressed in yeast and purified via two step chromatography. Secondary structure and purity were assessed by CD spectroscopy and SDS-PAGE. 32 wheat allergic patients were enrolled: 20 Spanish patients with BA and positive bronchial challenge to wheat flour and 12 Italian patients with wheat FA/WDEIA and positive DBPCFC/OFC to pasta. Specific IgE values to wheat, Tri d LTP, Tri a 14 and Pru p 3 were determined by ImmunoCAP testing. Allergenic potency and cross-reactivity of Tri d LTP, Tri a 14 and Pru p 3 was investigated by in vitro mediator release and IgE competition assays, respectively.

Tri d LTP shares 48% aa-id with Tri a 14, but 52% aa-id with Pru p 3. rTri d LTP displayed conserved secondary structure comparable to Pru p 3. Among 25 Tri a 14 CAP positive sera, 92% were reactive to wheat extract, 88% to Tri d LTP and 80% to Pru p 3. The correlation between Tri a 14 and Tri d LTP specific IgE levels was $r=0.78$ (BA) and $r=0.93$ (FA/WDEIA). The subgroup of FA/WDEIA patients showed high specific IgE values to Tri d

LTP and Pru p 3, whereas nsLTP-specific IgE values were lower in patients with BA. Tri d LTP displayed allergenic potency and IgE cross-reactivity with Pru p 3. IgE cross-reactivity between both wheat LTPs varied between individual patients.

The first time an allergen in *T. durum* was identified. Sensitization to Tri d LTP is closely associated to Pru p 3-mediated food allergy and appears to be more important in wheat food allergy than in baker's asthma.

V31

Determination of the epitope preference of profilin-specific IgEs derived from individuals living in different geographic locations

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Epitope mapping of different profilins revealed a large number of various IgE-binding sites, with little overlap between different species. Since cross-reactivity should be mediated by specific IgEs, this observation was surprising. We wanted to examine whether the geographic location contributes to an epitope preference of profilin-specific IgE-antibodies in patients from different regions. The protein profilin is a small cross-reactive allergen in pollen and vegetable foods, which is known to be responsible for cross-reactivity in allergic patients. Epitope mapping of profilin revealed a large number of different IgE-epitopes with little overlap between different species. To determine the suspected epitope preference of profilin-specific IgEs, nine different profilins, which were known to be relevant for a profilin sensitization in Northern and Southern Europe, were selected for an epitope scanning approach (Api g 4, Bet v 2, Ole e 2, Phl p 12, Pho d 2, Cuc m 2, Cit s 2 and Sola l 1, respectively). Microarrays were designed with the selected profilin sequences (233 peptides, 15mer offset of four amino acids) and used for IgE-binding assays with sera of tomato allergic patients from Northern and Southern Europe. The microarray analysis revealed that patient cohorts with mild symptoms (Oral Allergy Syndrome) from different geographical locations (Germany and Spain) dispose of profilin-specific IgE to identical epitopes close to the actin-binding site. In contrast, in profilin-allergic patients with systemic reactions from Spain further epitopes beyond the actin binding site are recognized.

V32

Wheat amylase/trypsin inhibitors aggravate oral antigen induced food allergy

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We have identified nutritional amylase/trypsin inhibitors (ATI) of wheat as potent stimulators of intestinal dendritic (DC) and other myeloid cells via activation of toll-like receptor 4 (Junker Y

et al. JEM 2012; Zevallos VF et al., Gastroenterology 2017). ATIs represent 3% of wheat protein, are highly resistant to intestinal digestion and fuel not only intestinal but also extraintestinal inflammation and autoimmunity. Mice fed an ATI containing diet comparable to human daily wheat consumption demonstrate an enhanced activation of myeloid cells not only in the intestine but also in draining lymph nodes. To investigate the influence of ATI on the development of food allergy we used a model of oral antigen induced food allergy in mice.

Mice were fed a carbohydrate, protein and lipid defined diet with or without 0.7% of protein as ATI comparable to human daily wheat consumption for 6 weeks before immunization and during the experiment. ATI-fed mice developed a more severe oral antigen induced food allergy compared to the control mice. All animals on the ATI containing diet developed diarrhea after 7 antigen administrations whereas 20% of animals on an otherwise identical ATI-free diet displayed only minor symptoms. ATI-fed mice also showed increased mast cell numbers in the jejunum and enhanced mast cell activation indicated by increased mouse mast cells protease-1 (mMCP-1) release after antigen challenge.

From these results we conclude that nutritional ATI promote experimental food allergy by activating monocytes-macrophages/DC to migrate to mesenteric lymph nodes where they serve as coactivators of the allergic immune response. A direct effect on mast cells is currently under investigation. An ATI-free or ATI-reduced diet may prevent or ameliorate food allergies and therefore might be an additional therapeutic aspect of food allergy treatment.

V33

Nitration of wheat derived alpha amylase trypsin inhibitors (ATIs) increases their immunostimulatory capacity

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Nitration of proteins is often found in inflammatory processes and can cause elevated immune reactions. Protein nitration can occur in the body and by environmental factors. For example, summer smog conditions can generate nitrogen dioxide and ozone concentrations which are capable to modify airborne allergens. This mechanism was already demonstrated for the major birch pollen allergen Bet v 1 and led to an increased allergic potential.

To elucidate the effect of different *in-* and *ex vivo* nitration mechanisms, we chemically modified wheat derived alpha amylase trypsin inhibitors (ATIs), major inhalative allergens in Baker's asthma. Moreover, ATIs have been shown to serve as adjuvants of pre-existing allergic sensitization through activation of the TLR4 pathway.

Our results demonstrate that nitrated ATIs led to an increased pro-inflammatory cytokine release by primary human dendritic cells as well as an increased T cell proliferation. On the single cell level, an enhanced NF- κ B translocation from cytoplasm to the cell nucleus was observed using high content screening fluorescence microscopy. Furthermore, we investigated whether

chemically nitrating agents affect the protein structure of ATIs, essential for TLR4 binding and stimulation. Therefore, we established a novel HeLa TLR4 dual luciferase reporter cell line and found that both, modified and unmodified ATIs, induced full TLR4 reporter signal.

Taken together, these findings indicate that nitration of ATIs leads to an enhanced immunogenicity but does not affect the TLR4 agonistic capacity.

V34

Mast cells are involved in an axis CCR8-CCL1 leading to eosinophil migration in experimental allergic enteritis

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Background: The pathological mechanism of Allergic enteritis (AE) is not fully characterized in comparison to other clinical phenotypes in food allergy. Our aim is to elucidate cellular and molecular mechanism of AE using a murine model. Our previous study showed that gene expressions of CC chemokine receptor 8 (CCR8) and its ligand, CC chemokine ligand 1 (CCL1, I-309) were up-regulated in inflamed intestinal tissues of AE mice. Mast cells have been suggested to produce CCL1 in respiratory tissues, but their role in AE remained unclear. In the present study, we investigated the role of mast cells and CCR8 in induction of AE.

Methods and Results: BALB/c wild type (WT), mast cell-deficient (Cpa3(Cre/+)) mice, or CCR8 knock out (KO) mice were sensitized by i.p. injection with ovalbumin (OVA, a major egg white allergen) plus ALUM, and fed egg white (EW) diet. Histological analysis showed induction of AE, i.e. morphological changes, goblet cell hyperplasia and granulocyte accumulation, in WT mice. However, Cpa3(Cre/+) and CCR8 KO mice exhibited less eosinophil accumulation in their inflamed tissues. FACS analysis showed reduction in the frequencies of eosinophils (Siglec F+ cells), but not of neutrophils (Ly6G+ CD11b+ Siglec F- cells) in lamina propria leukocytes (CD45+ cells) of Cpa3(Cre/+) and CCR8 KO mice. The concentrations of CCL1 and CCL11 (eosinophil-attracting chemokine eotaxin-1) were reduced in intestinal tissue homogenates of Cpa3(Cre/+) mice, whereas the concentrations of CCL11, but not of CCL1, were reduced in those of CCR8 KO mice. Serum levels of Ova-specific IgE antibodies were comparable among the three types of mice.

Conclusions: Our results suggest that (i) mast cells are a source of CCL1, and (ii) the axis of CCR8 and CCL1 is involved in CCL11 expression and subsequent eosinophil migration in AE. Through a better understanding of the AE mechanism, this study will provide a basis to establish a novel anti-inflammatory strategy for treatment of food allergy.