Allergens

V01
Molecular characterization of the allergenic non-specific Lipid Transfer protein Pla a 3 from plane tree pollen
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Lipid transfer proteins (LTP) are considered to provoke plane pollen allergy, which frequently is associated with peach allergy. Aim of the study was the cDNA cloning of plane LTP Pla a 3, and to apply recombinant (r) Pla a 3 for molecular diagnosis in comparison to its natural (n) counterpart and peach LTP Pru p 3.

Two isoforms of Pla a 3 were identified, at which Pla a 3 (AM286249) was over-expressed and purified. nPla a 3 was purified from plane pollen extract and verified by MS. Secondary structure of the proteins were analyzed by CD spectroscopy. Specific IgE levels to plane pollen, rPla a 3, nPla a 3 and rPru p 3 were measured by ImmunoCAPTM in serum of (I) plane pollen allergic patients without peach allergy (n=10), (II) peach allergics without plane pollen allergy (n=15) and (III) plane and peach allergics (n=15). Allergenic potency of rPla a 3, nPla a 3 and rPru p 3 was investigated by in vitro mediator release assays. IgE cross-reactivity was assessed by ELISA.

Expression of both Pla a 3 isoforms was confirmed in plane tree pollen. rPla a 3 and rPru p 3 showed reasonable purity and structural integrity. Recombinant and nPla a 3 displayed same IgE binding capacities. 24/25 plane pollen allergic patients were sensitized to plane extract. Frequency of sensitization to Pla a 3 was 10% in plane pollen allergies and 53% in peach allergic patients. In plane pollen and peach allergies 87% of the patients were co-sensitized to rPla a 3 and rPru p 3. All proteins displayed allergenic potency and showed strong IgE cross-reactivity, at which Pru p 3 displayed highest IgE-binding.

rPla a 3 is a suitable tool for component-resolved allergy diagnosis. Pla a 3 does not act as primary sensitizer for plane pollen allergy and does not serve as diagnostic marker in plane pollen allergies without peach allergy. In patients with allergy to plane and peach, IgE reactivity to Pla a 3 is likely due to primary sensitization to Pru p 3 and subsequent cross-reactivity to plane LTP.

V02
Minor Allergens in Birch Allergen Products – Is Standardization Possible?
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Birch (Betula) pollen is a major cause of allergy in northern and central Europe. The allergenic potency of products for diagnosis and therapy of birch pollen allergy is adjusted nearly exclusively to the major birch pollen allergen Bet v 1. However, many patients have also specific IgE-antibodies against minor birch pollen allergens like Bet v 4, Bet v 6 and Bet v 7. The content of these minor allergens is currently not controlled in birch allergen products and their clinical relevance remains undetermined.

We developed and validated ELISAs for quantification of Bet v 4, Bet v 6, and Bet v 7 and determined these allergens in birch pollen allergen products from different manufacturers (prick test solutions [PTS], products for sublingual [SLIT] and subcutaneous immunotherapy [SCIT]). The results were also analysed in relation to allergenic activity, Bet v 1 and total protein content as laid down in the Monograph on Allergen Products in the European Pharmacopoeia.

All assessed products complied with their respective specifications for these parameters. Moreover, we could detect all three minor allergens in each of the assessed products. However, their concentration was highly variable when comparing different products and manufacturers and even in several batches of the same product (PTS, SLIT and SCIT products alike). While allergenic activity, Bet v 1 and total protein content were clearly interdependent, none of the minor allergens showed a similarly strong correlation with these factors. Without adaptations to the current manufacturing processes, it therefore seems unfeasible to standardize birch allergen products also to minor allergens. Nevertheless, a future combination of data on minor allergen content in different birch allergen products on the one hand and clinical observations in birch pollen allergic subjects on the other hand could clarify the clinical relevance of minor allergens.
**Antigens 5 of different hymenoptera species are highly cross-reactive and do not allow the clear identification of the culprit venom**

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**Background:** Allergies due to the venoms of hymenoptera can cause severe systemic reactions. In spite of the progress of component-resolution in the last years, diagnosis as well as therapy of venom allergy is still challenging. Amongst others this is due to extensive cross-reactivity between different venoms. In this study the antigens 5 of 7 hymenoptera species were recombinantly produced, characterized in detail and their cross-reactivity analyzed.

**Methods:** The antigens 5 Ves v 5, Vesp c 5, Pol d 5, Pol a 5, Dol m 5, Sol i 3 and the potentially hypoallergenic Poly s 5 were recombinantly produced in insect cells. The resulting purified proteins were characterized by immunoblotting and structural models were generated. Moreover, sera of venom allergic patients were assessed for IgE cross-reactivity.

**Results:** All antigens 5 were successfully produced in Sf9 insect cells. As expected from sequence alignments structural models reveal identical folding, although surface charges differ between the different molecules. Immunoblot analyses showed the presence of N-linked glycan structures only for Sol i 3. However, due to the use of Sf9 cells as expression host all antigens 5 were devoid of carbohydrate-based cross-reactivity. The analysis of sera from antigen 5-reactive patients revealed extensive cross-reactivity of all antigens 5, independent of glycosylation. Some sera showed distinct reactivity profiles with the diverse antigens 5 and others reacted with all of them, indicating the presence of shared as well as of individual IgE epitopes.

**Conclusion:** The comparative analysis of antigens 5 from 7 different hymenoptera species revealed extensive cross-reactivity between all allergens on protein level. This implicates that antigens 5 are inappropriate marker allergens for the diagnostic discrimination between antigen 5-containing venoms. Moreover, detailed analyses on a molecular level can contribute to elucidate the clinical impact in the observed cross-reactivity.

**ImmunoCAP cellulose displays cross-reactive carbohydrate epitopes and can cause false-positive test results in patients with anti-CCD IgE antibodies**

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**Rationale:** CCDs in plant and insect venom extracts may cause false-positive results in vitro test results. We noticed that some CCD-positive sera show multiple positive ImmunoCAP results even with CCD-free recombinant allergens.

**Methods:** IgE-binding to recombinant allergens and to allergen-free blank ImmunoCAPs (BIC) was compared in CCD-positive sera before and after CCD inhibition.

**Results:** 35/52 (67%) CCD-positive sera (bromelain 1.01–59.6 kU/l) reacted with BIC ≥0.35 kU/l (0.35–4.22 kU/l). IgE-binding to BIC correlated with binding to bromelain (r=0.80) and was completely abolished by a CCD inhibitor. Binding to another five lots of BIC was lower but correlated strongly with the first lot (r=0.94). Of 10 CCD-reactive sera (14.0–52.5 kU/l) tested on recombinant Phl p12, Fel d 1, Ara h 2, and Pru p 3, 8 were positive to all components (0.36–1.6 kU/l), 2/10 showed borderline results. Binding to components correlated with binding to bromelain (r=0.61) and BIC (r=0.97) and was completely blocked by CCDs. MS confirmed the presence of MMXF glycans in unprocessed and processed cellulose.

**Conclusions:** The ImmunoCAP cellulose allergen carrier contains varying traces of CCDs and can cause false-positive results to non-glycosylated allergens in patients with high levels of anti-CCD IgE antibodies.

**Anti- and pro-inflammatory properties of Aspergillus fumigatus cell wall chitin**

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Chitin is an important cell wall component of Aspergillus fumigatus spores of which hundreds are inhaled on a daily basis. Chitin is not a structure of human cells, but humans have chitinases. Elevated chitinase levels in serum were found in asthma patients, and chitin induces accumulation of immune cells associated with allergy. Previous studies have shown that chitin has both anti- and pro-inflammatory properties; however the exact mechanisms determining the inflammatory signature of chitin are poorly understood. We investigated the stimulation signatures induced in human peripheral blood mononuclear cells (PBMCs) by chitin in the presence or absence of human serum. Cytokines were measured in the cell culture supernatant by ELISA. Chitin showed an anti-inflammatory signature characterized by the production of IL-1Ra, which was dependent on opsonisation by immunoglobulins, internalization, and PI3K/Akt activation. In contrast, proinflammatory cytokines and IL-10 were not induced by chitin. Depletion of immunoglobulins and blocking the phagocytosis with cytochalasin D resulted in decreased IL-1Ra induction, while IL-1β production was increased. Heat inactivation of Ig depleted serum reduced IL-1β production.
suggested a complement dependent pathway. Co-stimulation of chitin with non-fungal pattern recognition ligands (LPS, P3Cys or MDP) had synergistic effects on the induction of pro-inflammatory cytokines. We conclude that chitin can have pro- and anti-inflammatory properties, depending on the presence of PAMPs and immunoglobulins during the stimulation. We hypothesize that human chitinases degrade chitin into small oligosaccharides to prevent the host from exaggerated pro-inflammatory responses to inhaled A. fumigatus conidia.

V06
Glutaraldehyde-modified birch pollen allergoid reveals high stability to endolysosomal degradation by dendritic cells
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Background: Allergen-specific immunotherapy (AIT) is the only causative treatment for immediate type allergies. During AIT side effects like anaphylactic reactions can occur. To improve the safety of AIT, chemically-modified allergen extracts, so called allergoids, have been introduced. Although allergoids are used in clinical practice routinely, immunological alterations are poorly described. Still, there are first evidences that allergoid-induced tolerance might be mediated by different immunological mechanisms than AIT with native allergen extracts.

Aim: We sought to determine the stimulatory capacity of allergoids in allergen-specific T cell lines (TCL) and to assess their endolysosomal digestion by antigen presenting cells.

Methods: Birch pollen (BP) allergoid was synthesized by glutaraldehyde (GA) modification and characterized in terms of molecular composition. In addition, binding of human BP-specific IgE and a Bet v 1-specific monoclonal antibody was investigated. BP-specific TCL were generated from PBMC of BP-allergic patients and stimulated with GA-modified and non-modified BP extract as well as Bet v 1. Endolysosomal degradation of allergoid by antigen-presenting cells was analyzed in vitro using endolysosomal proteases isolated from the murine dendritic cell line JAWS II.

Results: GA-modification of BP extract led to a diverse high-molecular weight allergen polymer with strongly reduced antibody binding. Proliferation assays with BP-specific TCL revealed decreased stimulatory capacity of the allergoid in comparison to native BP extract and Bet v 1. Of note, the allergoid was still stable after 24h of degradation with endolysosomal extracts while the majority of Bet v 1 was degraded.

Conclusion: Allergoids show reduced T cell stimulatory capacity compared to native allergen extract. While others have demonstrated retarded internalization of allergoids by dendritic cells, our data provide evidence that in addition their endolysosomal processing is delayed.

Immunology

V07
Dendritic cells are activated upon inhibition of tankyroses
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Constitutive activation of the canonical wingless (WNT) pathway that induces high-level expression of β-catenin target genes is a hallmark of many tumors. Several WNT inhibitors developed for cancer therapy are under clinical investigation. Immune cells are regulated in terms of differentiation and activation by the WNT pathway as well. Here we asked for potential effects of WNT inhibitors on the activity of dendritic cells (DCs) as the major regulators of peripheral tolerance and immunity. For this, we employed mouse bone marrow-derived DCs (BMDCs).

Treatment of unstimulated BMDCs with inhibitors that target-ed β-catenin resulted in moderate upregulation of MHCII and costimulators in a dose-dependent manner. However, their T cell stimulatory capacity remained unaffected. In contrast, the inhibitor XAV-939, engineered to prevent cellular accumulation of β-catenin by tankyrase inhibition, yielded pronounced DC activation. Application of XAV-939 exerted no effect on BMDCs stimulated with LPS. Ovalbumin-loaded DCs treated with XAV-939 promoted enhanced proliferation of antigen-specific CD8+ (OT-I) and CD4+ (OT-II) T cells, and favoured production of Th1/Th17-associated cytokines. Structurally different tankyrase inhibitors yielded similar results as obtained when using XAV-939. These results suggest that tankyrase may serve to limit activation of DCs under basal conditions.

V08
KSRP constitutes an important posttranscriptional negative regulator of cytokine and chemokine production by activated dendritic cells
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Posttranscriptional gene regulation has been recognized as an important checkpoint of immune cell differentiation and activation. So far, the focus of research has been on miRNA species that inhibit the stability and/or translational efficiency of their cognate mRNA targets. However, RNA-binding proteins constitute important posttranscriptional regulators of gene expression as well. Many RNA-binding proteins bind AU-rich mRNA sequence stretches, and govern mRNA stability/translation. Here we asked for the role of the RNA-binding protein KSRP (KH-type splicing regulatory protein) known to promote mRNA decay in dendritic cells (DCs). Mouse bone-marrow de-
rived DCs (BMDCs) derived from wild type (WT) and KSRP-/−
progenitor cells displayed comparable uptake and processing of
the model antigen ovalbumin. Expression of costimulatory
receptors was similar in both types of BMDCs, both at unstim-
ulated state and after activation with LPS. However, activated
KSRP-/− BMDCs showed higher expression levels for a number
of cytokine and chemokine encoding mRNAs than stimulated
WT BMDCs. RNA decay analysis confirmed that KSRP
impaired the half life of these mRNA species in WT BMDCs.
Quantitative PCR analysis of immuno-precipitated KSRP-RNA
complexes identified a panel of genuine KSRP mRNA targets.
In line with elevated mRNA expression, stimulated KSRP-/−
BMDCs produced cytokines at higher level than WT BMDCs.
Ongoing work is focussed to elucidate the functional conse-
quences of enhanced cytokine production by stimulated KSRP-/−
BMDCs with regard to their T cell stimulatory and polarizing
capacity.

Our results indicate that KSRP constitutes a major negative
regulator of DC activation to limit the extent of adaptive
immune responses.

V09
Local inhibition of IL-4- and IL-13-mediated signaling as
adjuvant for tolerance induction during allergen-specific
immunotherapy
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Background: Although specific immunotherapy represents an
effective treatment in many cases, success rates vary and patient
compliance is low due to long treatment times. Our aim is to
use an IL4-/IL-13 antagonist (IL-4 mutein) in combination
with local application of adjuvants and allergens applying a bio-
degradable hydrogel to induce allergen-specific systemic toler-
ance via generation of a tolerogenic microenvironment.

Methods: The thermogelling PLGA-PEG-PLGA triblock co-
polymer-based hydrogel was generated by means of chemical
synthesis. The characteristics of the hydrogel were analyzed by
different methods. The IL-4 mutein was recombinantly pro-
duced and its functionality assessed by various T and B cell dif-
ferentiation assays.

Results: The synthesized hydrogel showed the desired charac-
teristics and was fluid at room temperature and in a gelation
state at body temperature. Additionally, the hydrogel was able
to build a depot that is stable over several days in vivo. More-
over, the degradation of the gel in mice and the release of sub-
stances were visualized by magnetic resonance imaging (MRI).
The mouse IL-4 mutein was successfully produced using dif-
ferent recombinant expression systems and it’s functionality
could be demonstrated by dose-dependent inhibition of
IL-4-driven proliferation of the established cell line CTLL-2.
Additionally, the IL-4 mutein was able to down regulate IL-4
induced proliferation and IgE class-switching of mouse spleen
B cells. Moreover, IL-4 driven in vitro differentiation of naive
CD4+ T cells into TH2 cells was completely inhibited by addi-
tion of IL-4 mutein as analyzed by FACS.

Conclusion: Taken together our results build the first steps
towards the combination of specific immunotherapy and local
T cell modulation applying adjuvant molecules to create a
tolerogenic microenvironment. Moreover, the hydrogel together
with different immune modulators builds a free combinable
modular system that might help to shift immune balance under
various pathological conditions.

V10
IPSE/alpha-1, a secreted glycoprotein from Schistosoma
mansoni eggs, may inhibit inflammation
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Chronic infection with the parasitic worm Schistosoma man-
soni is characterized by a strong anti-inflammatory immune
response caused by schistosome eggs. Importantly, schistosome
eggs secrete potent immunomodulatory molecules, including
the glycoprotein IPSE/alpha-1. Previously, we have shown that
IPSE/alpha-1 triggers basophils to release IL-4 and IL-13. These
cytokines are well known as key cytokines for Th2 induction
but also as inducers of wound-healing alternatively activated
macrophages (AAMs). Moreover, in schistosome infection,
IL-4 and IL-4 receptor signaling plays a crucial role in prevent-
ing excessive lethal intestinal inflammation in mice. This
prompted us to investigate the anti-inflammatory potential of
basophil-derived IL-4 following stimulation with IPSE/alpha-1.

When co-cultured with IPSE/alpha-1-stimulated basophils
LPS-activated monocytes acquired an AAM-like phenotype
with decreased production of pro-inflammatory cytokines IL-6,
IL-1β and TNFα. Since immunohistochemical staining of in-
fected murine gut reveals the presence of basophils in schisto-
some egg granulomas, we propose that IPSE/alpha-1-triggered
basophil IL-4 turns down and controls schistosome egg-in-
duced inflammatory processes. We expect that these findings
may be translated to new strategies for treating chronic inflam-
mations such as allergy and autoimmune diseases.
(Funded by DFG-SCHR608/4-1)
Infection with the parasitic trematode *Schistosoma mansoni* protects mice against allergic airway inflammation. It was reported that regulatory B cells (Bregs) are involved in this process. However, the mechanism of Breg induction is largely unknown. Previously, we have identified a glycoprotein secreted from *S. mansoni* eggs, IPSE/alpha-1, that triggers the release of IL-4 and IL-13 from basophils via interaction with surface IgE. Here we show that IPSE/alpha-1 is an immunoglobulin-binding factor that binds to isolated human CD19+ B cells presumably via the B-cell receptor (BCR) as the binding can be blocked by anti-IgG/M antibodies. Confocal microscopy revealed that IPSE/alpha-1 is taken up by the B cells and accumulates to a confined area near the nucleus. Preliminary determination of the cytokine production and surface marker expression did not show a characteristic profile described for Bregs. Nevertheless, its uptake and its circumscribed perinuclear location suggest that IPSE/alpha-1 has an impact on B cell function.

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**V11**

IPSE/alpha-1, an immunoglobulin-binding factor from the parasitic worm *Schistosoma mansoni*, binds to and is taken up by human B cells

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**V12**

Human IgE is efficiently produced in biologically active form in lepidopteran cells

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The role of specific and high affinity IgE in reacting against minute amounts of allergens and parasites and to provoke severe anaphylactic reactions renders IgE a mechanistically outstanding isotype. IgE represents the least abundant serum antibody isotype and exhibits a variety of peculiarities regarding structure and effector functions. Despite large progress in antibody technologies however the recombinant access to isotypes more complex than IgG still is scarce, a consequence of the demands of the immunoglobulins and the capacity of the expression systems used.

In order to overcome the limitations often posed by mammalian expression systems we established the recombinant production of IgE in insect cells. Recombinant human IgE (rIgE) was efficiently assembled and secreted into the supernatant in high yields of >30 mg/L. Purification from serum free medium using different means provided large amounts of rIgE. The rIgE exhibited a highly specific interaction with its antigen, therapeutic anti-IgE and its high affinity receptor, the FceRI. Lectins and glycoproteomic analysis revealed the presence of a prototypic N-glycosylation of the epsilon heavy chain. Mediator release assays demonstrated the biological activity of the IgE in activating effector cells in response to trace amounts of antigen. In summary the expression in lepidopteran cells provide molecular access to IgE of retained characteristics and biological activity. Our data also contribute to the understanding and potential use of this important antibody isotype.

**V13**

Generation of specific Fab antibodies for functional and structural analyses

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**Background:** Addressing the molecular basis of the interplay of the adaptive immune response remains difficult as the multivalent nature of antibodies and the heterogeneity of allergens often hamper a detailed molecular dissection. Functional and structural analyses however demand the availability of specific antibodies in well-defined form.

**Objective:** Recombinant access to monovalent Fab antibodies for analysis of functionality, affinity and structure.

**Methods:** Selected antibodies were converted to Fabs by recombinant fusion with human IgG1 CH1 and κCL. The Fabs then were produced by baculovirus-mediated infection of insect cells and purified by affinity chromatography. Functionality and affinity of resulting antibody fragments was shown by ELISA and SPR analyses.

**Results:** Fab antibodies could be efficiently expressed as properly assembled and secreted proteins in Sf9 insect cells with high yields. The proteins could be purified by affinity chromatography approaches. Resulting specific Fabs showed pronounced reactivities in ELISA with their particular antigens. The affinities of the monovalent Fab antibodies were assessed by SPR and compared to those of the full immunoglobulins.

**Conclusion:** Recombinant production in insect cells is a suitable strategy for high yield production of specific Fabs and will allow for detailed functional and structural analyses of the molecular recognition of antibodies and allergens.

**V14**

Cross-reactivity and Allergenicity Assessment of Therapeutic Monoclonal Antibodies against TNF-alpha

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**Background:** Therapeutic monoclonal antibodies are novel types of drugs with high benefit for patients but also new side
effects during therapy. There is increasing evidence that biologicals can induce IgE-mediated allergic reactions like exanthema, urticaria and anaphylaxis. So far, little is known about the allergenic/immunogenic epitopes on biologicals and their characteristics. There are clinical cases where fatal anaphylaxis was observed during the application of the biological cetuximab, associated with IgE to the disaccharide α-GAL. These severe side effects in general lead to a discontinuation of the treatment and/or the switch to another type of therapeutic antibody. However, the therapy switch is not always safe and successful.

**Methods:** In our study, sera from patients treated with TNF-α-binding biologicals were evaluated positive for anti-biological antibodies by ELISA in routine clinical laboratory tests (IPM Biotech). These anti-biological-Ig-containing sera were analysed in terms of anti-biological IgE via immunoblot with different biologicals such as infliximab, adalimumab and cetuximab as target antigens. To identify the epitopes on these biologicals, oligopeptide microarray analysis was performed with overlapping peptide epitopes from the biologicals and the corresponding ELISA-positive patient sera.

**Results:** ELISA analyses show that sera from patients treated with anti-TNF-α biologicals contain antibody levels against these biologicals between 2 and 80 µg/ml. Infliximab-positive sera tested in an adalimumab-ELISA were evaluated negative, showing that there is no cross-reactivity as determined by ELISA. In an oligopeptide microarray epitope mapping approach of infliximab-positive patient sera, distinct epitopes were identified in the variable, antigen-binding region of the biologicals.

**Conclusion:**Taken together, these analyses shall lead to a knowledge-based allergenicity and side effect risk assessment of each type of biological and to a safer recommendation of a therapy switch.

**V15**

**Effective preclinical testing of relevant biologicals in humanized mice**

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Our immunologic knowledge is mainly based on analyses of human immune cells in vitro or on in vivo investigations in mouse models. However, complex biological processes are often species-specific and differences between mice and man limit the prognostic value in preclinical testing of novel biologicals for treatment of allergy, psoriasis or autoimmune diseases. Humanized mice have been developed to overcome these limitations. They are immunodeficient and allow the transplant of human tissues, patient-derived immune cells or blood stem cells (HSC). Analysis of such humanized mouse models is used for preclinical testing of biological drugs leading to identification of functional mechanisms and important side effects [Kubach et al. Int J Cancer. 2014,doi: 10.1002/ijc.29037]. Here we demonstrate the preclinical analysis of the HIV-1 glycoprotein gp120 as an efficient polyclonal Treg stimulator that blocks overwhelming T cell activities in vitro and in vivo. Gp120 activates human Treg by binding and signaling through CD4, leading to accumulation of cyclic adenosine monophosphate (cAMP) in their cytosol and functional activation of suppressive capacity. Utilization of gp120 in a xenogenic humanized mouse model a single treatment with gp120 inhibits strong inflammation of skin, liver and colon after transfer of human immune cells. Therefore, we are currently developing novel humanized mouse systems for effective preclinical testing of immunotherapeutic concepts in order to evaluate the impact of novel biologicals on the restoration of the immunological homeostasis. These models allow analysis of patient-derived immune cells in vivo and can be a helpful tool to evaluate the reasons but also feasible therapies of dysregulated immune responses observed in asthma, allergy and autoimmune diseases.

**V16**

**The role of tonsillar epithelial E-Cadherin for the control of regulatory T cells in allergic children**

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All inhalative or oral allergens have to pass the tonsils in the pharynx. Interestingly, the role of tonsils in the development of allergies or in the tolerance of allergens is not well investigated, even if there is no question about the tonsils’ role as first-line defense barrier. In human tonsils but also in other organs Foxp3+ regulatory T cells (Treg) have been identified as significantly involved in the development of tolerance and in the formation of immune balance. Recently, there is increasing interest in the role of Treg subgroups for the control of different forms of inflammation and infection and former Th2-transcription factor GATA3 seems to play an important role also in Treg. Moreover, there is a rising focus on the crosstalk of non-immune cells with immune cells becomes. One molecule of special interest is the adhesion molecule E-Cadherin which is widespread in all mucosal tissues like the airways or the intestines, but can also be found in human tonsils and Allergens that can attack epithelial barriers by breaking homologous E-Cadherin structures. E-Cadherin has two signaling partners on immune cells, KLRG1 and CD103 which are also expressed on Treg. We could show in a mouse model where intestinal N-Cadherin substitutes for E-Cadherin that KLRG1-E-Cadherin signaling is decisive in two directions: both the lack of E-Cadherin on intestinal epithelial cells and the KLRG1-knockout on all cells lead to the accumulation of Treg. In the current project we now want to dissect the importance of epithelial E-Cadherin for tolerance induction by priming Treg in pediatric tonsils and compare the data of allergic and non-allergic children. Moreover, the role of Treg subgroups like GATA3+-Treg in the protection of allergy pathogenesis is a special focus of this project.
V17
Biobanking in Allergology—A new perspective?
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Initially developed to support translational tumor research biobanks are well-recognized platforms to accomplish scientific approaches in nearly all fields of medical research. Biobanks contribute to clinical and basic investigations by providing mainly human liquid and solid biospecimens and their derivates (nucleic acids, purified cells, supernatants) that were obtained, processed and stored under standardized and quality-controlled conditions. By offering well-protected donor-specific data sets, biobanks contribute to a better understanding of disease development and to personalized medicine approaches that aim to improve therapy on the patient level.

While well-advanced in tumor research biobanks are still in the early stage of development in allergy research. This is remarkable as translational research is an emerging field of research in allergology. One step forward, inclusion of biospecimens obtained from other chronic inflammatory diseases might pave the way towards a better understanding of basic mechanisms that contribute to an imbalanced immune response and following autoimmune or allergic conditions.

CBBM is a core facility of the medical faculty and all clinical settings of the Marburg University Hospital are enroled to recruit donors for the Marburg Biobank. The Marburg Biobank was established to support the local research focus “Tumor and Inflammation”. Based on SOP (standard operation procedure)-guided protocols and accomplished by a LIMS (Laboratory information management system)-based data center, CBBM has developed a work process to built up tissue-, liquid specimen- and cell banks to collect samples for the investigation of malignant as well as chronic inflammation conditions. Tissue samples as well as lavages are prepared to be processed and cryoconserved for further analyses on the candidate and the -omics-level. In addition, sets of body fluids and blood cell populations were employed to later determine immune, metabolic, hormonal and (epi)genetic parameters in these samples.

Respiratory tract

V18
Pollen-derived adenosine plays an important role in induction of ragweed allergy
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Background: Adenosine is an important metabolite, which plays a role in inflammation as well as in immune suppression and regulation. Furthermore it has recently been shown that adenosine is released from pollen grains, giving rise to speculations of its possible role in pollen-induced allergic reactions.

Aim of the study was to evaluate the role of pollen-derived adenosine in allergic airway inflammation.

Methods: Mice were instilled intranasally with ragweed extract or ragweed extract depleted of adenosine and allergic airway inflammation was evaluated. Furthermore, the effect of adenosine-depletion was assessed separately for the sensitization or the elicitation phase. In vitro, migration of human eosinophils and neutrophils towards supernatants of ragweed-stimulated bronchial epithelial cells was analyzed.

Results: Eleven instillations with the total ragweed extract or ragweed extract depleted of adenosine and allergic airway inflammation was evaluated. Furthermore, the effect of adenosine-depletion was assessed separately for the sensitization or the elicitation phase. In vitro, migration of human eosinophils and neutrophils towards supernatants of ragweed extract-stimulated bronchial epithelial cells only in the presence of adenosine.

Conclusion: Pollen-derived adenosine is a critical factor in ragweed-pollen induced allergic airway inflammation. Future studies aim at therapeutic strategies to control these allergen-independent pathways.

V19
Airway surface dehydration is a novel risk factor for allergic airway inflammation
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Introduction: We have previously demonstrated that mice with airway specific overexpression of the amiloride-sensitive epithelial Na+ channel (βENaC-Tg) and mice lacking the epithelial Cl– channel SLC26A9 suffer from airway surface dehydration and airway mucus obstruction, which might be implicated in the pathogenesis of allergic airway disease (Anagnostopoulou et al., JCI 2012 and Mall et al., AmJRespirCritCare Med 2008). Here, we hypothesized that airway surface dehydration
Aspergillus fumigatus extract (Af) was applied by intratracheal instillations into juvenile βENaC-Tg mice or WT mice (C57BL/6) and BAL cell counts, IL-13 levels and airway hyperresponsiveness (AHR, by Flexivent lung function) were determined. Genetic deletion of the IL-4/IL13 receptor signal transducer STAT6 was introduced to ENaC-Tg mice to analyze the contribution of type 2 inflammation to the Af immune response. Furthermore, preventive treatment of βENaC-Tg mice and WT mice with amiloride was performed and BAL and IL-13 levels were determined.

**Results:** Airway eosinophils and pulmonary IL-13 expression were significantly increased in Af-challenged compared to vehicle-treated βENaC-Tg mice. Airway hyperresponsiveness was elevated in βENaC-Tg mice and increased further in response to Af treatment. Genetic deletion of STAT6 abrogated Af-induced airway eosinophilia and IL-13 expression in ENaC-Tg mice. Preventive treatment with amiloride significantly reduced allergic inflammation in both βENaC-Tg and WT mice. **Conclusion:** Collectively, our results indicate that airway surface dehydration is a risk factor for key pathologies in allergic airway disease. Amelioration of airway surface dehydration (i.e. by amiloride) might constitute a novel target for prevention and treatment of allergic airway disease.

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**V20**

**Inflammatory effect of wheat alpha-amylase/trypsin inhibitors (ATIs) on murine allergic airway activation**

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**Introduction:** Wheat alpha-amylase/trypsin inhibitors (ATIs) are non-gluten proteins that activate innate immunity via the toll like receptor 4 (TLR4)-MD2-CD14 complex in cells of the mononuclear phagocyte system (Junker Y et al, J Exp Med 2012). ATIs are also present in gluten preparations and are implicated as additional pathogenic triggers of celiac disease (CD) and other autoimmune and inflammatory diseases.

**Aim and methods:** We investigated the effects of dietary ATIs on allergic airway inflammation. Female C57BL/6 mice on a gluten-free diet (GFD) were sensitised and challenged with ovalbumin (OVA). Animals were divided in 5 groups: 1. continued with the GFD and mock-sensitised with PBS, 2. continued with the GFD and sensitised with OVA, 3. changed to a diet containing 25% gluten (containing amounts of ATIs equivalent to the human wheat based diet), 4. changed to a diet containing purified ATIs, and 5. changed to a diet containing 25% gluten-de-enriched of ATIs. We measured invasive lung function, bronchoalveolar lavage (BAL), proliferation of OVA stimulated splenocytes and histological sections of lung with Hematoxylin and eosin (HE) and Periodic acid–Schiff (PAS).

**Results:** Mice on a GFD sensitized with PBS did not develop airway hyperreactivity (AHR) after local provocation with OVA. Interestingly, mice on a GFD or on a diet containing 25% gluten-de-enriched of ATIs and sensitized with OVA developed a reduced AHR compared to mice fed the pure ATIs rich diet or 25% gluten (plus ATI) diet. Similar results were observed for eosinophilic infiltration in BAL (HE) and mucus production (PAS) in the lung.

**Conclusions:** We demonstrate that dietary ATIs enhance allergic airway inflammation in OVA-challenged mice, GFD (ATI-free) diet appears to have a protective effect on AHR and gluten-depleted of ATIs has a reduced stimulatory effect compared with gluten containing ATIs or ATIs alone. Therefore, ATIs appear to be major and clinically relevant nutritional triggers of innate immunity fuelling allergic airway inflammation.

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**V21**

**Deletion of the α1 isoform of soluble Guanylyl Cyclase interferes with allergic response in a murine model of chronic asthma**

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**Background:** The soluble Guanylyl Cyclases (sGC) are widely distributed nitric oxide (NO)-dependent cyclic guanosine 5′-monophosphate (cGMP)-generating enzymes which beside others regulate smooth muscle tone and exert anti-inflammatory effects. sGCs are obligate heterodimers, composed of α and β subunits. The sGCα1β1 is the most prevailing form in the lung and its expression is downregulated in asthma. To date mainly local pharmacological intervention of the sGC/cGMP signaling was performed to address its impact on bronchial hyperreactivity and inflammation. In this study we use α1sGC knock out mice in a chronic model of asthma to illuminate the isoform-specific impact of sGCs on the pathology of asthma in the mouse.

**Methods and Results:** For the asthma model, wildtype (WT) and α1sGC knock out (KO) mice were sensitized and then challenged with ovalbumin for 8, 12 and 15 weeks, respectively. Asthmatic phenotype was determined by analysis of the bronchoalveolar lavage (BAL), proliferation of OVA stimulated splenocytes and histological sections of lung with Hematoxylin and eosin (HE) and Periodic acid–Schiff (PAS).

**Results:** Our results show that α1sGC-deficiency interferes with the allergic cascade. This might originate from a limited antigen presenting capacity of the antigen presenting cells (APCs) or impaired migration of eosinophils. However, thick-
ening of the reticular basement membrane is not altered. This indicates a α1sGC dependent and partially inflammation-independent profibrotic process. Furthermore the absence of α1sGC does not aggravate the AHR in either treated or untreated animals suggesting that other than α1sGC-dependent mechanisms regulate bronchial relaxation.

**V22**

Analysis of sensitization patterns against classical allergens and pathogen-specific IgE in patients with cystic fibrosis

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**Background:** IgE-production directed against facultative airway pathogens such as Staph. aureus, Pneumococci, H. influenza and Aspergillus species has been described in different chronic lung diseases. In the context of bronchial asthma, increases in pathogen-directed IgE titres accompany exacerbations of steady-state and have been associated with the development of IgE-production against classical allergens. Such associations are lacking for other chronic lung disease where similar studies have not yet been performed.

**Hypothesis:** Sensitization patterns against bacterial, fungal and classical allergens correlate in a disease-specific manner with the development, chronification and exacerbation of different chronic inflammatory lung diseases and influence the course of infections with facultative pathogenic organisms.

**Methods:** IgE against Staph. aureus and E. coli were detected by immunoblotting, IgE against H. influenza and Pneumococci were detected by DELFIA, IgE against 17classic allergens were assessed by an allergen chip. Sensitization (IgE and IgG) against Aspergillus were assessed via commercially available CAP-systems. Microbial colonization was assessed by standard microbiological culture techniques.

**Results:** We collected sera from 35 patients with CF and increased total IgE levels. Analysis of our patient samples revealed very distinct sensitization patterns. Correlations between sensitization against classical allergens and pathogen-specific IgE as well as microbial colonization status and clinical parameters were performed.

**Outlook:** These analyses will have to be extended to more patients, sequential measurements and other lung diseases. They will provide insight into the complex interplay between microbial colonization and allergic sensitization and promise to identify allergen-specific IgE production as easily accessible biomarkers for different chronic lung diseases.

**V23**

House dust mite-specific sublingual immunotherapy prevents allergic inflammation development in mice

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**Background:** Sublingual immunotherapy (SLIT) is a disease-modifying treatment for respiratory allergies. Evidence regarding SLIT efficacy and its good safety profile has made it an attractive treatment option also for allergic asthma. The majority of studies have focused on grass pollens; however, data regarding efficacy for other allergens, like house dust mite are now available. Nevertheless, the underlying immunological mechanisms still remain only partially understood.

**Objectives:** To establish a house dust mite (HDM) SLIT model and investigate local immunological changes.

**Methods:** Balb/c mice were i.n. sensitized/challenged with a HDM extract. HDM SLIT was performed in advance of (prophylactic) the induction of an allergic airway inflammation. Lung inflammation and airway hyperresponsiveness were assessed by bronchoalveolar lavage cell counts, lung histology and flexivent measurement, respectively. Humoral and cellular immune responses were monitored in serum, lung and BALF by ELISA and FACS-analysis.

**Results:** Prophylactic sublingual administration of HDM prevents the recruitment of leukocytes, (mainly eosinophils and neutrophils) and airway hyperresponsiveness (resistance) as well production of the TH2 cytokines IL-5 and IL-13 in re-stimulated lymph nodes. No effects on the immunoglobulin titer were observed. FACS-analysis of the lung T cell compartment revealed an involvement of CD4-CD8-γδ-T cells and CD8+CD25+IFN-γ+ cells in regard to the underlying mechanism.

**Conclusion:** A clinical relevant HDM animal model may be useful to further elucidate the efficacy and mechanisms of HDM SLIT against asthma.

**V24**

Functionalized nanoparticles target B cells in vivo due to their opsonization with complement and are suitable for allergy immunotherapy

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The vast potential of nanoparticles as carriers for antigen and immunomodulatory drugs for vaccination has been well acknowledged. Cell type specific delivery of nanomedicine constitutes a major aim of current research. For this, nanoparticles are conjugated with derivatives of natural ligands or cell receptor-spe-
cific antibodies. Many approaches focus on the targeting of so-called antigen presenting cells and among these dendritic cells (DCs) as potent inducers of primary T cell responses. In contrast, despite their important role as inducers of humoral immune responses, B cells have received little attention so far.

Here we show that dextran-coated ferrous oxide nanoparticles (Fe-NPs) are opsonized by complement, which in turn facilitates specific binding to B cells via complement receptors CR-1/2. In order to exploit their intrinsic B cell targeting property for the induction of an antigen-specific antibody response, Fe-NPs were coated with ovalbumine (OVA) as a model antigen and immunostimulatory CpG oligonucleotides (ODN). When injected into naive C57BL/6 mice, Fe-NPs elicited a robust Th1-biased antibody response as reflected by high IgG2a and concomitantly low IgG1 serum levels. In therapeutic OVA-dependent anaphylaxis and asthma models, only particles conjugated with OVA and CpG inhibited IgE production, attenuated bronchial hyper responsiveness and lung inflammation (asthma), and promoted survival (anaphylaxis).

To the best of our knowledge this report is the first to demonstrate B cell specific targeting of a nanoparticle in a serum-dependent manner and serve as nanomedicines for the treatment of infections and other diseases which require the induction of a strong humoral immune response.

V25

Potential anti-inflammatory and anti-viral effects of Petasites hybridus (butterbur extract) on primary human nasal epithelial cells in vitro

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Background: As integral part of the innate immune system, nasal epithelial cells are involved in early steps of allergic inflammation and defence against respiratory infections. A CO2 extract of butterbur leaves (Tesalin) has been clinically shown to improve congestion in allergic rhinitis and reduce IL8 secretion, however the mode of action remains unclear.

Aim of study: To evaluate the anti-inflammatory effect of butterbur leaf extract for potential topical application in allergic and non-allergic, acute rhinitis/rhinosinusitis and to elucidate its mechanism of action in nasal epithelial cells.

Methods: Human primary nasal epithelial cells were obtained from turbinoplasticsurgery. Cells were stimulated with the viral mimicks (PolyIC:TLR3, PolyIC-LyoVec:RIG-1/MDA5, CpG:TLR9), bacterial TLR ligands (Pam3CSK4:TLR1/2, Flagellin: TLR5). Stimulation occurred either alone or in combination with Tesalin. Readouts were production pro-inflammatory cytokines and chemokines (IL8, GCSF, CCL3, CCL4, CCL5, CXCL10, IL6, TNFa, IL1a) and neutrophil chemotaxis towards stimulated supernatants.

Results: Stimulation of human nasal epithelial cells with the TLR3 ligand PolyIC leads to the release of proinflammatory cytokines and chemokines and induced neutrophil chemotaxis towards cell supernatants. Tesalin reduced the PolyIC-induced production of proinflammatory cytokines and chemokines. Reduction of IL8 was paralleled by significantly decreased neutrophil chemotaxis towards supernatants of PolyIC/Tesalin-treated cells. In addition, Tesalin influences the regulation of RIG-1/MDA5, TLR9 and TLR3, but not TLR1/2 and TLR5, when stimulated with the different ligands of these receptors.

Conclusion: Besides its clinical benefit in allergic rhinitis, our data imply that ‘Tesalin might be an effective treatment for non-allergic rhinitis and rhinosinusitis. Of note, Tesalin seems to specifically target virally triggered pathways in human nasal epithelial cells, suggesting potent anti-viral potential of the herbal drug in topical application.

V26

Wnt-1 treatment prevents inflammation in the mouse model of allergic airway disease

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Introduction: Recent publications reveal an anti-inflammatory function of the Wnt-1-induced β-catenin signaling in allergic airway disease. Binding of secreted Wnt-1 to its receptor (Frizzeld) and co-receptor (e.g. LRPS) leads to an intracellular accumulation of β-catenin. Afterwards β-catenin translocates to the nucleus and activates specific gene expression via the TCF/LEF transcription factor.

We could previously show that overexpression of lung specific Wnt-1 attenuates experimental allergic airway disease (Reuter, JI, 2014). This effect seemed to be independent of regulatory T cells (Treg) or IL-10 but was linked to the suppression of DC migration and there capacity to activate T cells in vivo and in vitro. The aim of this study was to investigate Wnt-1 as a treatment option for allergic airway disease.

Method: C57BL/6 mice were sensitized intraperitoneally with Ovalbumin/Aluminium hydroxide on days 0 and 14. For induction of allergic airway disease mice were challenged via the airways by nebulization with a 1% OVA solution on days 28–30. Some mice received intranasal Wnt-1 treatment one hour prior to allergen challenge. At day 32 airway inflammation was quantified by measurement of lung function, lung histological staining, flow cytometry of lung and draining lymph nodes and analysis of cytokine secretion of restimulated cells.

Results: The application of Wnt-1 leads to a significant reduction of airway hyperresponsiveness, inflammation and goblet cell metaplasia. Changes of Treg cells were not detectable in the lung and lymph nodes. Furthermore, the number of DC remains equally, but the ratio of subtypes changed to a more Ly6C- CD11b-CD103+ phenotype. Hence, the activation of the β-catenin signaling pathway by intranasal application of Wnt-1 leads to a
Reduced allergic airway inflammation and offers new therapeutic options for the treatment of allergic diseases like asthma.

**V27**

**CD4-mediated activation of human regulatory T cells for treatment of allergic diseases**

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Regulatory T cells (Treg) play a critical role for maintenance of peripheral immunologic tolerance. Inefficient Treg function is associated with the development of allergic diseases. To restore the immunological balance in patients with allergy, we are developing biological drugs for functional activation of endogenous Treg. We have shown that human Treg can be activated by a polyclonal CD4-activated mechanism, without affecting the activity of CD4+ T helper cells. One candidate (BT-061/Tregalizumab) is now in clinical trials (psoriasis, rheumatoid arthritis). A second promising candidate is HIV-1 envelope protein gp120 which we are developing for clinical application. We have shown that gp120 activates polyclonally human Treg and efficiently inhibits the inflammatory symptoms of asthma in a humanized mouse model of allergic asthma (Martin et al. JACI). However, this effect was Treg-dependent as gp120 did not affect the immunological reaction in the absence of Treg. Treatment with gp120 prior to challenge reduced infiltration of human immune cell into the lung tissue and abrogated the development of airway hyperresponsiveness.

Since patients with allergic diseases are treated with standard medications, that have the risk of adverse reactions and sometimes show reduced efficacy, the use of biological drugs for induction of Treg-mediated tolerance is a more efficient approach. In addition, not only the successful treatment of established allergy but also the development of a prophylactic approaches is of great importance. Here we already could show that next to the therapeutic benefit, the application of gp120 represents a prophylactic approach as well.

Next to the treatment of allergic airway inflammation other autoimmune disorders like psoriasis and rheumatoid arthritis are potential indications. Furthermore, gp120 would encounter a great unmet medical need in the field of the prevention of transplant rejection.

**V28**

**GARP reduces allergic airway inflammation in a clinical relevant humanized mouse model**

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Glycoprotein A repetitions predominant (GARP) is an activation marker on the surface of human regulatory T cells (Treg). We have recently shown that a soluble derivate (sGARP) has strong anti-inflammatory and regulatory properties in vitro as well as in vivo (Hahn, Blood, 2013). sGARP represses proliferation and cytokine production of naïve and resting CD4+ T helper cells and induces CD4+ Treg. Furthermore, sGARP prevents inflammation in a preclinical in vivo humanized mouse model of graft versus host disease (GvHD).

Because modulation of Treg responses could also have therapeutic effects in other inflammatory diseases, aim of this study was to investigate the impact of sGARP in treatment of allergic airway diseases using a humanized mouse model (Martin, JACI, 2012). To address this question, adult NOD/Scid γc-/- mice received peripheral blood mononuclear cells (PBMC) from a birc pollen allergy sufferer.

To analyze the effects of sGARP, additionally Treg alone or in combination with sGARP were transferred into the animals. Twenty days following cell application the allergic airway disease was induced by a three-day intranasal challenge with birch pollen allergen. 48 hours after last challenge allergic inflammation was assessed by measurement of airway hyperresponsiveness (AHR), quantification of cells in the bronchoalveolar lavage (BAL) and analysis of human immune cells in different tissues by flow cytometric and histological staining.

In comparison to mice that received Treg alone, additional transfer with sGARP significantly reduced AHR, total human immune cell counts, neutrophils and macrophages in the BAL and human CD4+ T cells in the lungs. Furthermore, number of mucus producing goblet cells was reduced upon sGARP injection. Hence, amplifying of Treg cell function via sGARP seems to be a promising treatment option for allergic airway diseases.

**Skin**

**V29**

**Superior suppressive capacity of skin Tregs compared to lung Tregs in a murine model of epicutaneous priming**

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We have previously shown that a Th2-polarized airway inflammation can facilitate priming to new antigens in the lungs which we call “collateral priming”. To investigate whether an allergic skin inflammation can also facilitate priming towards new antigens, we established a murine allergic skin inflammation model in analogy to an allergic lung inflammation model.

Mice were sensitized intraperitoneally towards ovalbumin as primary antigen. Challenge was performed intranasally or
We present the case of a 10-year old boy who suffered a serious episode of an anaphylactic reaction after topical application of an herbal ointment (Kytta Salbe). This ointment was applied for aching muscles on the back. Only a few minutes after application he developed an urticarial skin reaction on the whole back attended and followed by abdominal pain, generalized urticaria, shivering, numbness, dyspnea and twitching of the eyelids. Emergency treatment with corticosteroids and antihistamines led to complete remission of the symptoms within two days. Skin prick testing with single ingredients of the ointment provided by the manufacturer showed urticarial reaction against the whole ointment and its ingredient phenonip. All other single ingredients were negative. Additional testing of parabens and further preservatives was completely negative. Skin prick testing with grass tree and herbal pollen and with house dust mites and cat epithelia was negative as well. Phenonip is a broad spectrum antimicrobial agent comprising a synergistic blend of esters of para-hydroxybenzoic acid (parabens) in phenoxyethanol designed for preservation of a wide range of cosmetics and toiletries. Furthermore, it can frequently be found in ink pens and in medical vaccines. Usually allergic reactions against phenonip or its components phenoxyethanol and parabens are late type allergies that results in allergic contact eczema. This is one of the rare cases in which phenoxyethanol led to the development of contact urticaria followed by a systemic anaphylactic reaction. To our knowledge only three cases have been published so far. It must remain unclear what product led to the acquisition of the sensitization against phenonip, as this ointment had been applied for the first time. Possibly, phenoxyethanol containing vaccines had been etiological.

V30

A case of severe anaphylactic reaction following topical application of an herbal ointment

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We present the case of a 10-year old boy who suffered a serious episode of an anaphylactic reaction after topical application of an herbal ointment (Kytta Salbe). This ointment was applied for aching muscles on the back. Only a few minutes after application he developed an urticarial skin reaction on the whole back attended and followed by abdominal pain, generalized urticaria, shivering, numbness, dyspnea and twitching of the eyelids. Emergency treatment with corticosteroids and antihistamines led to complete remission of the symptoms within two days. Skin prick testing with single ingredients of the ointment provided by the manufacturer showed urticarial reaction against the whole ointment and its ingredient phenonip. All other single ingredients were negative. Additional testing of parabens and further preservatives was completely negative. Skin prick testing with grass tree and herbal pollen and with house dust mites and cat epithelia was negative as well. Phenonip is a broad spectrum antimicrobial agent comprising a synergistic blend of esters of para-hydroxybenzoic acid (parabens) in phenoxyethanol designed for preservation of a wide range of cosmetics and toiletries. Furthermore, it can frequently be found in ink pens and in medical vaccines. Usually allergic reactions against phenonip or its components phenoxyethanol and parabens are late type allergies that results in allergic contact eczema. This is one of the rare cases in which phenoxyethanol led to the development of contact urticaria followed by a systemic anaphylactic reaction. To our knowledge only three cases have been published so far. It must remain unclear what product led to the acquisition of the sensitization against phenonip, as this ointment had been applied for the first time. Possibly, phenoxyethanol containing vaccines had been etiological.

V31

IgE-specific immunoadsorption as new treatment option for atopic dermatitis

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Immunoglobulin E (IgE) plays a major role in the pathophysiology of atopic predisposition, although its role in atopic dermatitis (AD) is not fully understood. Anti-IgE treatment with omalizumab has become available and was approved for treating asthma and urticaria. However, application of omalizumab in patients with asthma and concomitant AD led to varying results on AD disease activity. Immunoadsorption (IA) has been used in AD using either specific depletion of IgE and/or in combination with IgG adsorption. We now assessed the effects of IgE-specific IA (IgE-IA) on quality of life and disease activity in severe AD.

We analysed 4 patients that received IgE-IA. All of them suffered from severe AD with score-points ranging from 34 to 87 (SCORAD) and from 14 to 51 (EASI). Patients had received different treatments for AD before according to guideline recommendations without sufficient control of disease. As add on therapy, all patients received 5 2-day treatment cycles of IgE-IA within 10 weeks every 2 weeks. IgE levels, disease activity (SCORAD/EASI) and quality of life (DLQI, wellbeing five) were monitored before every IA.

All patients had highly elevated basal total serum IgE levels (range 1.352-23.553kU/l). The mean reduction of circulating IgE for all 5 cycles of all 4 patients was 85% (range 82-90%). However, within the treatment-free interval, IgE levels raised significantly again. Disease activity after all 5 cycles was strongly reduced in all 4 patients (mean 48%, range 27-66% as assessed by SCORAD, and mean 80%, range 69-89% in EASI). In line, the quality of life was improved by 55% (DLQI, range 21-71%) and by 81% (wellbeing five, range 17-200%).

In summary, our preliminary results in a small patient collective prove a reliable and dramatic reduction of the total IgE level in serum during IA associated with reduction of disease activity and improvement of the quality of life. Despite disappointing results of anti-IgE treatment in AD, IgE-IA might be highly beneficial for patients with severe AD.

V32

Proteomic patterns in chronic hand eczema

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Background: Chronic hand eczema (CHE) is a frequent skin disease and has a high socio-economic impact. Pathogenesis of CHE is multifactorial and depends on exogenous factors such as chronic irritant damage or contact allergy and on endogenous factors such as an atopic predisposition. In many
patients, however, factors triggering the eczema cannot be clearly identified, or potential triggers cannot explain the clinical presentation.

**Objectives:** Pathogenetic processes are often reflected by variations in protein expression. Little is known about protein expression patterns in the skin of patients with CHE. We therefore set out to systematically and comprehensively analyze the differential protein expression in CHE using modern mass spectrometry.

**Methods:** We analyzed palmar skin from hand eczema patients and healthy volunteers by performing LC–MS/MS analyses and label-free quantification. Subsequently, results were confirmed by immunohistochemistry of palmar skin from a separate cohort.

**Results:** In total, we were able to identify 185 candidate proteins that were significantly differentially expressed in the CHE samples. A detailed analysis of the mass spectrometric results revealed a characteristic proteomic pattern of epidermal barrier components and keratinocyte differentiation in CHE. Immunohistochemistry confirmed these findings.

**Conclusions:** Our results provide important clues to molecular factors and signaling pathways involved in the pathogenesis of chronic hand eczema.

**V33**

**How to address the functional role of innate lymphoid cells in TNCB contact hypersensitivity**

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**Background:** Innate lymphoid cells (ILCs) can be classified into three groups based on their dependency on transcription factors and expression of cytokines. The role of ILCs in the effector phase of allergic contact dermatitis has not been addressed so far. We have identified all groups of ILCs in ear skin, skin-draining lymph nodes (SDLN), blood and spleen of wild type animals. Mice injected with HOCl exhibited the most prominent skin thickness vs. the bleomycin-treated animals which was accompanied by a thick layer of subdermal fat in the HOCl-group. Histological analysis revealed an increase in cellular infiltrates in both Scl models which were characterized by using 7-color flow cytometry for DC and macrophage markers.

**Aim:** We want to evaluate the functional relevance of ILCs in both phases of CHS using targeted antibody depletion of ILCs and an adoptive transfer model.

**Methods:** For depletion of ILCs T-cell deficient Rag1-/- mice (CD90.2 genotype) were sensitized i.p. 4x with a CD90.2 specific monoclonal antibody. For adoptive transfer naïve donor mice (CD90.1 genotype) were sensitized with TNCB. After 5 days T-cells were FACS-sorted from SDLN and adoptively transferred i.v. in Rag1-/- mice (CD90.2 genotype). After transfer mice were challenged with TNCB on the ear skin and effective transfer of the CHS reaction was measured by increase in ear thickness.

**Results:** ILCs were efficiently depleted in the blood, spleen and SDLN using the above mentioned depletion protocol. Transfer of total lymph node cells or sorted T-cells into RAG1-/- mice was successful and significant hapten-specific ear swelling could be detected time dependent after the hapten challenge regardless of whether T cells were transferred one hour or three weeks prior to the challenge. Transferred lymph node cells showed hapten-specific proliferation in vitro.

**Outlook:** Given the efficient antibody mediated depletion of ILCs and the successful adoptive transfer of the hapten specific CHS in RAG1-/- mice, this approach should now allow us to address the functional role of ILCs during the elicitation phase of CHS.

**V34**

**Mouse models of chemically induced scleroderma show differences in early skin infiltration**

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The cellular and molecular events resulting in fibrosis development in scleroderma patients are not fully understood. While a multitude of data have been reported in terms of T cell and mast cell activation in the late phase of tissue fibrosis, the specific contribution of antigen presenting cells like dendritic cells and macrophages in the early phase of fibrosis induction remains unaddressed. For induction of fibrosis bleomycin and HOCL were administered s.c. in the neck area every day and skin punches were analyzed for quantification of skin thickness, collagen deposition, myofibroblast activation (α-SMA), inflammatory infiltrate (HE, flow cytometry) and for expression of inflammation and fibrosis related mediators (qRT-PCR).

At day 28 both models resulted in a significant increase in dermal thickness, total collagen levels and a prominent appearance of collagen fibers compared to PBS-treated control animals. Mice injected with HOCl exhibited the most prominent skin thickness vs. the bleomycin-treated animals which was accompanied by a thick layer of subdermal fat in the HOCl-group. Histological analysis revealed an increase in cellular infiltrates in both Scl models which were characterized by using 7-color flow cytometry for DC and macrophage markers.

Infiltrates peaked at day 7 in bleomycin-/ HOCl-treated skin, decreased and were absent at day 28 of continuous bleomycin or HOCl application. There was a significant increase in the number and percentage of CD11b+MHCII+ cells in the HOCl-model which points to an activated antigen presenting myeloid cell population. In addition, the percentage of CD11c+MHCII+ representing mostly DC and of Ly6C+MHCII+ and F4/80+MHCII+ monocytes/macrophages was significantly elevated in the skin of HOCl-injected animals. In both models, we found upregulated profibrotic parameters with a prominent induction of procollagen α1(I) in HOCl- and preferential development of α-SMA in bleomycin-treated mice.
V35
Proinflammatory human slanDCs recruitment by immune complexes
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There are a number of pathologic conditions where immune complex (IC) deposition causes Fc receptor-dependent inflammatory lesions, such as lupus nephritis or cutaneous vasculitis. 6-sulfo LacNAc dendritic cells (slanDCs) are equipped with a unique capacity to bind IC via the two IC receptors CD16 and CD32. slanDCs circulate in blood at high numbers, have an outstanding capacity to produce IL-12, IL-23, TNF-α and IL-1β, and can be found as inflammatory dermal DC in psoriasis and lupus erythematosus (LE).

In this study we provide strong evidence for the molecular and functional specialization of slanDCs as proinflammatory cells in LE-nephritis and cutaneous vasculitis. Histological studies revealed a strong accumulation of slanDCs in lesions with dense IC deposition, e.g. in the glomerulus from LE-nephritis patients and in the intra-/perivascular areas from cutaneous vasculitis patients.

To investigate the ICs-mediated slanDCs recruitment capacity, we applied a perfusion assay-based approach coupled with time-lapse video microscopy and measured the arrest functions of purified leukocyte subtypes on immobilized ICs. The flow conditions were adjusted to produce physiologically relevant surface shear stress of human venous capillaries. Under these conditions we observed a pronounced recruitment of FcyRIII (CD16) positive slanDCs which was completely dependent on CD16.

In a translational in vivo approach, immunodeficient Nonobese diabetic (NOD)-SCID interleukin-2 gamma chain receptor (NSG) mice were intravenously injected with preformed ICs and subsequently with fluorescent labeled slanDCs. Using Fc receptors blocking monoclonal antibodies, we show that glomerular deposition of ICs mediates recruitment of human slanDCs in a CD16-dependent manner.

Collectively, our findings demonstrate the IC capacity to recruit circulating slanDCs in vitro and in vivo. Modulation of ICs-mediated slanDCs recruitment may offer therapeutic benefits in patients with IC-mediated inflammatory and/or autoimmune diseases.

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V36
Anaphylactic reaction against amaranth in a cereal
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We report the case of a 43-year old female who developed two episodes of anaphylactic reactions after consumption of a breakfast including milk, oatmeal, fresh apples, strawberries and amaranth. She was eating breakfast in this composition for several months. In both cases she developed generalized urticaria, dyspnea and circulatory collapse within several minutes after meal. Emergency treatment with corticosteroids and antihistamins led to complete remission of symptoms within two hours. Her medical history was negative for any allergic reaction, she was not suffering from hayfever or asthma. There was no history of atopic eczema. Skin prick testing showed reactions to tree, grass and herbal pollen and amaranth. Prick testing with other ingredients of the cereal was completely negative. Serological investigation showed normal values for total IgE and basal serum tryptase. Slightly raised specific IgE antibodies could be detected against grass, tree and herbal pollen. No specific IgE was found for milk, rye, wheat, millet, rice, buckwheat, sesame, apple and strawberry. Amaranth is a cosmopolitan genus of annual or short-lived perennial plants. Some amaranth species are cultivated as leaf vegetables, cereals, and ornamental plants. To this day, amaranth grains are toasted much like popcorn and mixed with honey, molasses or chocolate or are added in cereal mixtures. In 2013 the first case of an anaphylactic reaction after consumption of amaranth grains has been published. This allergy therefore seems to be very rare. There seems to be no cross-reaction of amaranth seed extract and pollen, however knowledge concerning this allergy is limited. Furthermore, there is a multiplicity of amaranth species with a different composition of possible allergens. As the addition of amaranth to different cereals is increasing as a kind of fashion trend in nutrition, amaranth allergy should be kept in mind as a possible elicitor of anaphylaxis.

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V37
Isolation and characterization of different peanut oleosins: New tools for component-resolved diagnosis of peanut allergy.
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Background: Peanut allergy is one of the most severe class I food allergies with an increasing prevalence over the past de-
cades. The tight association between serious allergic symptoms and the presence of a class of lipophilic proteins, called oleosins, has been clearly shown for several oil-rich plants, but not for peanut. Since allergy diagnostic testing is based on aqueous extracts, potential allergenic proteins of lipophilic nature are usually underrepresented or even absent. To close the existing detection gap in the established peanut allergy diagnostics, our study focused on the isolation, molecular characterization and assessment of the allergenicity of peanut oleosins.

Methods: A comprehensive method for the isolation of oleosins was developed. This method comprised a step by step removal of seed storage proteins from peanut oleosins. Oleosin separation was carried out by preparative electrophoresis. Subsequent N-terminal sequencing, peptide mass fingerprinting and homology search were used for the identification of oleosins. Evaluation of the IgE-binding capacity was conducted by immunoblot analysis with sera of peanut-allergic individuals. Western blotting illustrated the allergenicity of these immunologically distinct oleosins.

Results: Highly purified oleosins were isolated from the complex lipophilic matrix of peanut. Further separation of the different oleosins was realized by a single run on an electrophoresis cell. All known peanut oleosins were identified by mass spectrometry and N-terminal sequencing, including the allergens Ara h 10, Ara h 11, the presumed allergen oleosin 3 and additional oleosins variants. Western blotting illustrated the allergenicity of these immunologically distinct oleosins.

Conclusion: Our method provides a novel strategy to isolate all known peanut oleosins simultaneously. Furthermore, there is some evidence that oleosins are relevant allergens linked to a severe peanut allergy. Hence, oleosins are introduced as important candidates for the component-resolved diagnostic.

V38 Development of a sensitive and specific sandwich-ELISA for the detection of the peanut marker allergen Ara h 2 in human breast milk

A. Scharf, F. Schocker, U. Jappe

A comprehensive method for the isolation of oleosins has been clearly shown for several oil-rich plants, but not for peanut. Since allergy diagnostic testing is based on aqueous extracts, potential allergenic proteins of lipophilic nature are usually underrepresented or even absent. To close the existing detection gap in the established peanut allergy diagnostics, our study focused on the isolation, molecular characterization and assessment of the allergenicity of peanut oleosins. A German cohort of 40 lactating women without peanut allergy was established. After a 24-hour legume-free diet, Ara h 2 levels were assessed in human breast milk before (as basal sample) and at different time points between 1 to 12 hours after ingestion of 100 g roasted peanuts. In order to develop a sandwich-ELISA directed against Ara h 2 for the detection in human breast milk, antibody-matching, cross-reactivity analysis and buffer evaluation were performed using anti-Ara h 2 antibodies of different origins and different Ara h 2 epitope-specificity.

Results: The optimized sandwich-ELISA for Ara h 2 detection in breast milk has a detection limit of 2.5 ng/mL. Additionally, no cross-reactivity against Ara h 6 and other legume extracts was found. The intra-assay coefficient of variation (CV) was 4.1%, and the inter-assay CV was 12.6%. Using this ELISA, Ara h 2 was detected in breast milk samples from 16/40 subjects (range: 2.6 to 194 ng/mL).

Conclusion: By means of a sandwich-ELISA, we established a sensitive and specific diagnostic tool for the determination of Ara h 2 in human breast milk. Thus, we are able to show the transfer of the peanut marker allergen into breast milk of 40% of the lactating women. These results are important with respect to an occult sensitization via breast milk and the implementation of prevention strategies for children at risk.
4-like conformation. 39% (18/46) of model proteins carrying individual IgE epitopes bound serum IgE of more than 50% of the study population. IgE binding to model proteins with individual and multiple substitutions could be inhibited with rGly m 4 and vice versa in a dose-dependent manner.

**Conclusion:** Levels of Gly m 4-specific IgE of sera of birch-sey allergic patients do not correlate with clinical reactivity to soy. 36 Gly m 4-specific amino acids that comprise a minimum number of 6 epitopes were identified and can be correlated to individual clinical symptoms to judge potential clinical relevance of IgE binding sites.

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**V40**

**Dietary Wheat Alpha-Amylase-Trypsin Inhibitors (ATIs)**

Worsen Intestinal Inflammation in Mice

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**Background and Aims:** The inflammatory bowel diseases (IBD) Crohn's disease and ulcerative colitis (UC) are characterized by a chronic self-destructive inflammation of the gastrointestinal tract. We have recently identified alpha-amylase-trypsin inhibitors (ATIs), non-gluten components of wheat, as potent activators of innate immunity by engaging the toll like receptor 4 (TLR4)-MD2-CD14 complex in monocytes, macrophages and dendritic cells (Junker Y et al, J Exp Med 2012). ATIs that are present even in „pure“ gluten preparations are implicated also as promoters in the pathogenesis of celiac disease and possibly other autoimmune / inflammatory diseases. Therefore, we studied the effect of dietary Gluten/ATIs on intestinal inflammation.

**Methods and Results:** C57BL/6 mice were set on a gluten-free diet (GFD) or (a usually gluten containing) standard-diet (STD) and subject to dextran sodium sulphate (DSS)- induced colitis (2.5% DSS in drinking water for 7-14 days). The GFD diet strongly reduced colonic inflammation compared to the STD. GFD fed mice maintained normal body weight, showed attenuated inflammation with reduced numbers of infiltrating inflammatory cells in the colonic mucosa and lacked epithelial erosions. Transcript levels of pro-inflammatory cytokines such as IL-1β and IL-6 in the intestine were significantly down-regulated in colitic C57BL/6 mice kept on a gluten- free diet compared to mice fed the STD. Furthermore the mouse model of T cell transfers DSS- induced colitis in RAG1-/- mice indicated a T cell mediated response in dietary gluten was an important trigger of T cell mediated inflammation. FACS analyses revealed that dietary ATI-containing) gluten altered the balance of pro-inflammatory (IL-17, IFN-g) and anti-inflammatory (IL-10) cytokines in T cells of lymphoid compartments (spleen, mesenteric lymph nodes) and in the mucosa towards a pro-inflammatory state. DSS- induced severe colitis in mice fed the STD was accompanied by a shift towards a proinflammatory microbiota.

**V41**

**Apical exposure to dietary non-digestible oligosaccharides and bacterial CpG DNA suppresses Th2 type chemokine release by activated intestinal epithelial cells**

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**Background:** Dietary short chain galacto- and long chain fructo-oligosaccharides (scGOS/lcFOS) and TLR9 ligand CpG DNA affect intestinal epithelial cell (IEC) function. Epithelial derived IL-1α is known to contribute to allergic sensitization in the lung.

**Aim:** To study the effect of IL-1α on Th2 polarizing chemokine release by IEC and the modulatory effect of scGOS/lcFOS and CpG DNA in presence or absence of monocyte derived dendritic cells (moDC).

**Methods:** HT-29 cells (IEC) cultured in transwells were pre-incubated basolaterally with IL-1α. IFNγ/TNFα and apically with scGOS/lcFOS ± CpG DNA for 6 hours, washed and basolaterally exposed to immature moDC or medium while apically exposed to scGOS/lcFOS ± CpG for 24-48 hours. Th2 driving IL-25, CCL2, CCL22 and regulatory galectin-9 and TGF were measured in basolateral supernatants. After 48h of co-culture, moDC were added to allogenic naïve T-cells for 6 days (MLR) and cytokines were measured.

**Results:** Combined IFNγ/TNFα activation induced the release of CCL2 and CCL22 by IEC, which was further enhanced by IL-1α. IFNγ/TNFα ± IL-1α activation also increased galectin-9 and TGF (24h). Exposure to scGOS/lcFOS ± CpG DNA reduced CCL2 and CCL22, while galectin-9 and TGF remained high. In the 48h supernatants of IEC/moDC co-cultures, scGOS/lcFOS enhanced galectin-9 in presence or absence of CpG DNA. scGOS/lcFOS plus CpG DNA reduced IL-25 in co-cultures pre-exposed to IFNγ/TNFβ/IL-1α while increasing IFNγ concentrations in the MLR.

**Conclusion:** IL-1α enhances Th2 polarizing chemokine release by IFNγ/TNFα activated IEC. Combined exposure to dietary scGOS/lcFOS plus CpG DNA suppresses this response skewing away from the allergic phenotype.

This study was performed within the framework of Dutch Top Institute Pharma (T1.501).

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**V42**

**Administration of recombinant GARP inhibits allergen-induced colitis in a humanized mouse model of allergy**

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Previously, we could demonstrate that the inhibition of allergen-induced IgE-dependent gut inflammation in PBMC-engrafted immunodeficient mice by regulatory T cells (Treg) was dependent on the Treg activation marker glycoprotein A repetitions predominant (GARP). Besides its role on the regulatory function of Treg, GARP has been shown to possess anti-inflammatory properties by enhancing Treg activity in vitro and in vivo. The aim of this study was to investigate whether GARP might be protective in our humanized mouse model of allergy, too. Therefore, NOD-scid-γc−/− mice were injected intraperitoneally with human PBMC from allergic donors together with the respective allergen in the presence or absence of recombinant GARP or, for comparison, activated Treg of the same donor. After an additional allergen boost one week later, mice were challenged with the allergen rectally on day 21 and gut inflammation was monitored by a high resolution video mini-endoscopic system evaluating translucency, granularity, fibrin production, vascularity, and stool. Administration of GARP reduced the number of migrated CD45+ human cells in mice. Furthermore, an increased amount of FoxP3+ cells could be observed. Consequently, total and allergen-specific human IgE production in mouse sera was almost as strongly inhibited in GARP-treated mice as shown for co-injection of activated Treg leading to a similar prevention of allergen-induced gut inflammation after rectal allergen challenge. These results demonstrate that activation of Treg by GARP may be an additional tool for therapeutic intervention of allergic diseases of the intestine.

Conclusion: The prevalence of pets in families of Luxembourgish school children is much higher than the European average. Allergen levels varied significantly between the 3 schools analysed. Levels of Can f 1 and Fel d 1 measured in some classes are comparable to values obtained in homes of pet owners and constitute a risk factor for allergic children.

P01 Evaluation of pet ownership and allergen exposure among school children in 3 Luxembourgish secondary schools
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Background: Exposure to cat and dog allergens is considered as important risk factor for development of asthma and rhinitis. The prevalence of small furry animals seems to be increasing in households of industrialized countries, but epidemiological data on small animals and exposure in schools are still lacking.

Methods: Questionnaires were distributed to 1761 pupils in three secondary schools in Luxembourg in order to record the prevalence of pets in families with children of school age. Electrostatic dust collectors were exposed for two weeks in representative classrooms with low to high pet prevalence. Additional dust collectors were exposed at 40 homes of children with and without pets. Dust samples were extracted and the marker allergens Can f 1, Fel d 1, Cav p 2 and Ory c 3 were quantified.

Results: Total pet prevalence was 66%, 36% kept a dog, 31% a cat, 14% a rabbit, 4% a guinea-pig and 3% a hamster. 11% had at least once a week contact with horses and 8% were living on a farm. Can f 1 and Fel d 1 levels were significantly different between the three schools. Levels between classes with presence of many pet owners and few pet owners were variable and differences were not significant. No guinea-pig allergens could be detected, but low levels of rabbit allergen were present in some classrooms. Can f 1 levels measured in schools (median 55.3 ng/m³, range 0-533) reached values similar to those measured in homes of dog owners (median 253.8 ng/m³, range 0-2201). Fel d 1 was measured at comparable levels as Can f 1 in schools (median 69.7 ng/m³, range 0-457) and in homes of cat owners (median 117.3 ng/m³, range 0-2448).

P02 Microbial colonization of pollen and their contribution to allergy
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All over the world the occurrence of allergic diseases increased during the last decades. One major causative factor are airborne pollen grains of amenable plants, e.g. diverse grasses and trees. The allergic potential of pollen depends on the amount of produced allergenic proteins (e.g. PALMs), which also seemed to be involved in the plant’s defense mechanisms. Soil or air pollution could affect the plant’s capability to defense itself against contamination with microbes. Up to now little is known about the composition of microbial communities colonizing allergenic plants (or their reproductive organs like pollen grains) and in what extent this contamination might influence the plant’s production of allergenic proteins. In this study we grew pure cultures of cultivable microorganisms on different media isolated from pollen of timothy grass and birch. For identification we used comparative 16S-rDNA and ITS1-2 sequence analysis, respectively. We also applied T-RFLP fingerprint techniques, a culture independent method based on the
amplification of the 16S-rRNA gen, to determine the composition of the microbial population on the pollen. Furthermore we did statistical correlation analysis of microbial contamination, several environmental parameters and the content of immune modulatory PALMs from pollen sampled on different locations as well as principal components analysis, analysis of similarity and cluster analysis.

So far affiliated microbial isolates associated with pollen belong to Bacillus sp. and Pseudomonas sp., but also to phytopathogenic genera like Pantoea sp. or the phytotoxic fungus Fusarium sp. After proving the reproducibility of the analytical microbiom-data, evidences of distinct colonizing patterns could be shown on different pollen species. The data were compared due to cultivable microbes from these pollens, the amount of produced immune-modulatory compounds (e.g. PGE2) as well as concerning the influence of environmental parameters.

### P03

**Airway surface dehydration impairs pulmonary allergen clearance**

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**Introduction:** Efficient clearance of inhaled allergens from the lungs of patients with allergic asthma is important to avoid prolonged allergen exposure and asthma exacerbation. We hypothesized that impaired mucociliary clearance constitutes a risk factor for inefficient intrapulmonary allergen clearance. Thus, we analyzed allergen clearance mechanisms in wild type (WT) mice and in mice with airway-specific overexpression of the sodium channel ENaC (βENaC-Tg), which suffer from airway surface dehydration, impaired mucociliary clearance and chronic airway inflammation [Mall et al., Nat Med, 2004].

**Methods:** Mucociliary clearance was determined by intratracheal application of 1 µm fluorescent beads and video-microscopic analysis of in vivo bead elimination. Following intrapulmonary application of fluorescently labeled *Aspergillus fumigatus* extract (A1), total allergen clearance was analyzed by measurement of residual fluorescence in lung homogenates after designated time points. Bronchoalveolar lavage (BAL) from WT and βENaC-Tg mice was analyzed by flow cytometry to identify immune cell populations with Af uptake.

**Results:** Mucociliary clearance and total lung allergen clearance were significantly reduced in βENaC-Tg mice compared to WT mice. Antigen-presenting cells including alveolar macrophages (AM) and conventional dendritic cells (cDCs) were identified as cell types with strongest Af uptake among immune airway cells. In addition to decreased total allergen clearance, βENaC-Tg mice showed reduced allergen uptake by AM when compared to WT mice. However, the level of allergen presenting airway cDCs was significantly elevated in βENaC-Tg mice.

**Conclusion:** We conclude that airway surface dehydration impairs pulmonary allergen clearance. Hence, we suggest that inefficient allergen clearance might contribute to increased allergen presentation by cDCs which could constitute a risk factor for allergic sensitization during chronic airway inflammation.

### P04

**Airway dehydration increases pulmonary IL-13 expression in vivo**

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**Rationale:** We have previously demonstrated that mice with airway specific overexpression of the epithelial Na+ channel (βENaC-Ctg) exhibit airway surface dehydration and eosinophilic airway inflammation in juvenile mice (Mall MA, 2008). We hypothesized that airway surface dehydration may increase the susceptibility for allergic airway inflammation and treatment with allergen could induce IL-13 expression in vivo. Here, we evaluated cellular sources of IL-13 expression in response to allergen treatment in wild-type and βENaC-Tg mice.

**Methods:** Juvenile wild-type and βENaC-Tg mice were challenged intratracheally with *Aspergillus fumigatus* (Af) extract. In vitro restimulated whole lung-derived leukocytes were analyzed for IL-13 expression by multicolor flow cytometry. Laser microdissection (LMD) of the airway epithelium and subsequent nested qPCR were performed for quantification of airway-specific mRNA expression of IL-13, IL-33, IL-25, TSLP and periostin. IL-13 expression was confirmed by immunohistochemistry with IL13-/- mice-derived lung tissue as negative control.

**Results:** Intrapulmonary exposure of Af significantly increased airway eosinophils and pulmonary IL-13 in βENaC-Tg mice. We observed elevated numbers of pulmonary IL-13+ cells among macrophages, Th2-, B- and ILC2-cells in βENaC-Tg mice by 11-color flow cytometry, which was further increased in response to Af treatment in Th2- and ILC2-cell populations. LMD revealed airway epithelial cell-specific expression of IL-13 and IL-33 but not TSLP and IL-25 in βENaC-Tg mice. Immunostaining confirmed epithelium-mediated IL-13 protein expression, which was enhanced after Af challenge in βENaC-Tg mice.

**Conclusion:** We demonstrate that airway surface dehydration is a risk factor for allergic airway inflammation including increased frequencies of IL-13+ cells in response to allergen. Expression of IL-13 is not limited to Th2 cells, but also ILC2 cells and airway epithelium provide substantial amounts of IL-13 in vivo.
P05
Acute Influenza Virus Infection protects of allergic airway inflammation in the OVA-mouse model
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Airway viral infections heavily affect allergic asthma and other chronic airway inflammatory diseases. Epidemiological and clinical data display contradictory effects in terms of asthma prevention and asthma exacerbation in already diseased patients. The molecular basis of these phenotype-modifying virus-host interactions, however, is still largely unknown. This leads us to the hypothesis that the impact of viral infections on the development of chronic inflammation depends on (i) virus origin and frequency of viral infection as well as (ii) on host immune status and localization of infection. Here, we describe a novel prevention model of influenza airway infection followed by succeeding OVA-sensitization and -challenge in Balb/c mice. A single infection using the pandemic Hamburg/09 H1N1 causes a quick influx and activation of CD4 and CD8 T-cell populations peaking at day 8-12 combined with a pronounced TNF-α, IFN-γ and IL-6 secretion pattern. Virus-specific tetramer-pos. cytotoxic T- and effector memory cells peak in the lung at day 12 followed by a migration into spleen. Even 60 days post infection, tetramer-pos cytotoxic T-cells were detected in both lung and spleen. A subsequent OVA-sensitization starting from 12 or 33 days post infection followed by challenge displayed a decreased severity of a wide range of allergic phenotypic parameters (e.g. eosinophilia p<0.05, goblet cell hyperplasia p<0.05, IL-5 p<0.05) compared to only OVA-treated group. CD8 memory cell subpopulations displayed a characteristic redistribution pattern, and further in-vitro analysis points towards a protective role based on cross-reactive sequence recognition leading to a virus induced dampening of a subsequent allergic phenotype. Delineation of the underlying molecular and cellular rearrangements will lead to a better understanding of the regulations of chronic inflammation and should identify novel targets for virus induced protection and exacerbation.

P06
Evaluation of individual sensitization patterns to shrimp allergens in patients with seafood allergy using ImmunoCAP ISAC microarray
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Background: Seafood is an important cause of anaphylaxis worldwide and considered to be closely associated with house dust mite allergy due to cross-reactivity between the tropomyosins of mites, shrimps and molluscs. We assessed the prevalence of sensitization to different shrimp allergens in seafood-allergic subjects and explored the relationship with house dust mite allergy.

Methods: Sera from 108 patients (43m/65f, mean age 37.7±15.7 yrs) with a history of shrimp allergy and a positive routine test result to seafood (skin prick test and/or sIgE) were retrospectively analyzed by ImmunoCAP ISAC+ for IgE antibodies against tropomyosin (nPen m 1, rDer p 10, nBla g 7), shrimp arginine kinase (nPen m 2), shrimp sarcoplasmatic calcium-binding protein (nPen m 4), as well as the major dust mite allergens nDer p 1 and rDer p 2.

Results: 67/108 sera (62%) reacted with at least one shrimp allergen in the microarray, whereas 38% were negative despite a positive CAP result to crude shrimp extracts. Sensitization to Pen m 1 was most prevalent (42.6%) followed by Pen m 4 (25.0%) and Pen m 2 (13.9%). 73% of the patients were monosensitized to only one molecule, mainly to Pen m 1 (65%) or Pen m 4 (63%) while sensitization to Pen m 2 was rarely monovalent (13%). Only 23/67 sera (34.3%) were positive to the major dust mite allergens Der p f 1 or Der p f 2. Correlating sensitization profiles with symptom severity after seafood ingestion revealed that tropomyosin sensitization is regularly associated with systemic reactions while symptoms are often milder in case of sensitization to Pen m 2 and 4. Reactions were most severe in those with negative ISAC.

Conclusions: Although tropomyosin can be confirmed as an important seafood allergen, it accounts for only 43% of cases. 38% of seafood-allergic subjects are sensitized to unknown allergens other that Pen m 1, 2 or 4. IgE to Der p f 1/2 is inconsistently found questioning a close causal relationship between house dust mite and seafood allergy.
cho-alveolar lavage, systemic and local proliferative and Th2 cytokine responses as well as allergen-specific IgE in serum were analyzed.

**Results:** Compared to the buffer-treated control group mice treated sublingually with allergen extract were refractory to the development of airway hyperresponsiveness, eosinophil attraction to broncho-alveolar lavage (BAL), allergen-specific IgE in serum and systemic proliferative responses following allergen challenge.

**Conclusions:** We could show that prophylactic sublingual treatment with allergen extract prevents the development of house dust mite induced allergic asthma in a mouse model. Clinical studies are required in order to determine whether this concept also holds true for humans.