**ABSTRACTS**

**Friday, 27 January 2017**

**Oral Abstract presentations**

**09:20 - 11:00 Innate Immune Responses**

**O01 Carbohydrate A10 (CA10) From Ehrlich Tumor Cells Promote Tolerance By Acting On Human Dendritic Cells And Generating Functional Regulatory T Cells**

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**Keywords**: Carbohydrate A10, Ehrlich Tumor, Dendritic Cells, Regulatory T Cells

**Introduction**

During tumor development, alterations in glycosylation pattern of tumor cell surface carbohydrates may condition the immune response against the tumor. Therefore, the aim of this work is to study the capacity of the carbohydrate A10 (CA10) shed from the plasma membrane of the murine Ehrlich tumor (ET) cells and certain human adenocarcinomas to modulate the phenotype and function of human dendritic cells (DCs) and their capacity to induce T cell responses.

**Methods**

The activation of NF-κB/AP-1 with CA10 was studied in THP1 cells. Flow cytometry, cocultures and ELISA assays were performed to assess the effect of CA10 in human monocyte-derived DCs (hmoDCs). Allogeneic co-cultures of hmoDCs and naïve CD4⁺ T cells, CFSE-dilution assays, real-time quantitative PCR and ELISA were also performed. The *in vitro* generation of FOXP3⁺ regulatory T (Treg) cells was monitored by flow cytometry and their functional properties assayed by conventional suppression assays. The ET was generated by intramuscular inoculation of ET cells in the left groin of mice and the FOXP3⁺ Treg cells generated *in vivo* were analyzed in inguinal lymph nodes by flow cytometry.

**Results**

Although CA10 does not activate NF-κB/AP-1 in THP1 cells, it partially impairs the activation of these transcription factors induced by the TLR2 ligand Pam3CSK in the reported cell line. CA10 induces the expression of the tolerogenic marker PDL1 in hmoDCs as well as the production of IL-6 and IL-10. CA10-activated hmoDCs generate functional IL-10-producing CD4⁺CD25⁺CD127⁻FOXP3⁺ Treg cells that were able to block the proliferation of CD4⁺ T cells from PBMCs in a dose-dependent manner. Supporting the role of CA10 in the induction of Treg cells, our *in vivo* data showed that the percentage of FOXP3⁺ Treg cells is increased in inguinal lymph nodes from mice bearing Ehrlich tumors compared to controls.

**Conclusion**

Our results showed that the carbohydrate CA10 promotes the generation of FOXP3⁺ Treg cells both *in vitro* and *in vivo* by acting on DCs, which might well contribute to favor tolerance induction as a mechanism of tumor escape.
Human Rhinovirus Triggers Activation Of Inflammasome In Airway Epithelium In Asthma

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Keywords: Asthma, Rhinovirus, Pattern Recognition Receptors, Inflammasome

Introduction

There is increasing recognition of the importance of early environmental exposures in asthma development and exacerbations. Evidence supporting this relationship is particularly strong for house dust mite (HDM) and human rhinovirus. Bronchial epithelium provides protection against inhaled environmental factors, such as infectious agents, allergens and pollutants through the formation of a highly regulated epithelial barrier. Many of these processes are controlled by pattern recognition receptors (PRRs). Some of PRRs have been reported to assemble into large multiprotein complexes called inflammasomes, whose activation leads to secretion of proinflammatory cytokine IL-1ß. There is limited information about the role of inflammasome activation in human asthmatic bronchial epithelium in asthma development and exacerbations. Thus, we aimed to understand the role of several PRRs in the pathogenesis of allergic asthma and upon viral infections.

Methods

We performed next generation sequencing transcriptome analysis of bronchial epithelial cells from healthy individuals and allergic asthma patients donors. Air-liquid-interface cultures of differentiated bronchial epithelial cells from asthmatic patients and healthy control donors were investigated by quantitative RT-PCR, ELISA, Western-blot and confocal microscopy after HDM extract stimulation and human rhinovirus (HRV-16) infection.

Results

In asthmatic patients several canonical pathways and bio-functions connected to innate immune responses and inflammasome-related mechanisms were dysregulated. We found that AIM2, RIG-I, MDA5 and ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD) were expressed in human airway epithelial cells and their expression was regulated by HDM and HRV-16. These findings were accompanied by the presence of ASC specks and increased expression of IL-1ß mRNA, protein (pro-IL-1ß) and released mature IL-1ß levels in asthmatics patients upon HRV-16 infection alone and combined HDM and HRV-16 stimulation. Moreover, IL-1ß expression was decreased by caspase-1 inhibitor.
Conclusion
Inflammasome activation in response to HDM and HRV highlights the role of bronchial epithelium as essential inflammatory cells upon human rhinovirus infection combined with house dust mite exposure. Induction of rapid IL-1ß secretion might have an impact on asthma susceptibility, symptoms, exacerbations and represents a potential target for novel therapies.

O03 Modulation Of Respiratory Syncytial Virus-Induced IL-33-Dependent Type 2 Immune Response By Parasite Products

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Keywords: RSV, IL-33 Pathway, ILC2, Type 2 Immune Response, Allergic Asthma

Introduction
 Genome-wide association studies strongly implicate the IL-33 pathway in asthma development. IL-33 is a cytokine released in the airways upon Respiratory Syncytial virus (RSV) infection, upon which it activates type 2 Innate Lymphoid cells (ILC2s) to initiate a type 2 immune response, leading to the development of an asthmatic phenotype. Recent publications indicate that IL-33 released during primary RSV infection in a neonatal mouse model has a pivotal role for the development of the asthmatic hallmarks upon re-infection later in life. Recently, our group showed that the secretory/excretory products of the murine parasite Heligmosomoides polygyrus (HES) suppresses both IL-33 release and IL-33 receptor (IL-33R) expression. We subsequently identified the protein in HES responsible for suppressing IL-33 release, naming it H. polygyrus Alarmin Release Inhibitor (HpARI). With this project, we are seeking to identify another molecule contained in HES involved in the downregulation of the IL-33R.

Methods
Parasite products were fractionated by size or charge and tested for IL-33R suppression. Candidates were identified by mass spectrometry and expressed in HEK293 cells. These, and HpARI were tested in neonatal mouse RSV infection, and human in vitro models of RSV responses.

Results
Administration of HpARI reduced ILC2 activation 24 h after RSV infection, and reduced type 2 response after re-infection. We now plan to assess the effects of IL-33R-suppressing molecule of HES in this model, and further investigate its mechanism of action.

Conclusion
The IL-33 pathway is a strongly inducer of a type 2 immune response. Modulating this pathway using parasite-derived products would improve our knowledge in the mechanism involved in the development RSV-associated asthma, and in developing new therapies targeting the IL-33 pathway.
**004 The Staphylococcal Protease SplD Induces Allergic Asthma Via The IL-33/ST2 Axis In Mice**

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**Keywords**: S. Aureus, Th2 Inflammation, Airways, Sensitisation, Allergy

**Introduction**
The colonization with *Staphylococcus aureus* in the nasal mucosa is associated with nasal polyposis and asthma. Serine protease like protein D (SplD) and other closely related proteases secreted by *S. aureus* have recently been identified to induce allergic asthma in humans and mice but their mode of action is largely unknown. We aimed to describe this response mechanism to SplD in mice *in vivo*.

**Methods**
Wild type or Rag2 deficient C57BL/6 mice received either repeated intratracheal applications of SplD, SplD and soluble ST2 (sST2), ovalbumin (OVA) or PBS. The inflammatory profile of lungs, bronchoalveolar lavage and lymph nodes was tested by flow cytometry, Luminex and ELISA. Periodic Acid-Schiff, toluidine blue and immunohistochemistry stainings were performed on lung tissue sections. Airway hyperreactivity was measured and SplD- or OVA-specific serum IgE levels were determined by ELISA.

**Results**
SplD treated mice had significantly higher amounts of IL-33 and eosinixin in their lungs compared to the PBS or OVA treated control mice. This was accompanied by a strong eosinophilic lung inflammation, airway hyperreactivity, goblet cell hyperplasia, increased numbers of mast cells, IL-13 producing ILC2s and T cells and the formation of SplD-specific IgE. Higher numbers of dendritic cells and IL-5 and IL-13 releasing T cells were found in the local draining lymph nodes of the SplD-treated mice compared to the control mice. The inhibition of IL-33 due to sST2 treatment dampened the eosinophilic response and the cytokine production of the T-cells in the lymph nodes significantly, while SplD-specific IgE production was unchanged. Rag2 deficient mice treated with SplD showed no infiltration of eosinophils or goblet cells in the lungs or airways.

**Conclusion**
The eosinophilic response induced by SplD in mice was mainly dependent on IL-33 as it could be dampened by sST2 treatment. Furthermore, our data on Rag2 deficient mice shows that the response to SplD is highly dependent on the adaptive immune system and that ILCs, without mature T and B cells, cannot mediate the inflammatory response to SplD. These results strengthen the hypothetical involvement of *S. aureus* in asthma development and provide new potential treatment options.
**Friday, 27 January 2017**  
Oral Abstract presentations  
17:50 - 19:30 Immune Regulation in Tissues

**O05 Monocytes Accumulate In The Airways Of Children With Fatal Asthma**

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**Keywords**: Monocytes, Pediatric Asthma, Fatal Asthma, Matrix Metalloproteinase

**Introduction**
Activated Th2 cells are believed to play a pivotal role in allergic airway inflammation, but which cells attract and activate Th2 cells locally has not been fully determined. Recently, it was shown in an experimental human model of allergic rhinitis (AR) that activated monocytes rapidly accumulated in the nasal mucosa after local allergen challenge, where they promoted recruitment of Th2 cells and eosinophils. The objective of this study was to investigate whether monocytes are recruited to the lungs in paediatric asthma.

**Methods**
Tissue samples obtained from children and adolescents with fatal asthma attack (n=12), age-matched non-atopic controls (n=9), and allergen-challenged AR patients (n=8) were subjected to in situ immunostaining.

**Results**
Monocytes, identified as CD68+S100A8/A9+ cells, were significantly increased in the lower airway mucosa and in the alveoli of fatal asthma patients compared with control individuals. Interestingly, aggregates of CD68+S100A8/A9+ monocytes obstructing the lumen of bronchioles were found in asthmatics (8 out of 12) but not in controls. Analysing tissue specimens from challenged AR patients we confirmed that co-staining with CD68 and S100A8/A9 was a valid method to identify recently recruited monocytes. We also showed that the vast majority of accumulating monocytes both in the lungs and nasal mucosa expressed matrix metalloproteinase 10, suggesting that this protein may be involved in their migration within the tissue.

**Conclusion**
Monocytes accumulated in the lungs of children and adolescents with fatal asthma attack. This finding strongly suggests that monocytes are directly involved in the immunopathology of asthma and that these proinflammatory cells are potential targets for therapy.
Interaction Between The Microbiome And The Transcriptome In Lesional And Non-Lesional Skin In Atopic Dermatitis Patients


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Introduction
Host-microbe interaction plays a critical role in the pathogenesis of atopic dermatitis (AD). It is unclear if changes in the microbiota affect the host immune status and/or skin barrier function, or vice versa, and how they together influence AD. We studied differences in the microbiome of lesional and neighboring non-lesional skin in AD patients and correlated them to changes in epidermal barrier and immune response-related gene expression in RNA sequencing transcriptome.

Methods
The microbiome in skin swab samples from AD patients (N=14, lesional site and adjacent non-lesional site) and 7 healthy controls was sequenced using amplicon based 16S analyses of hyper variable regions V1 to V3. The transcriptome was assessed by RNA sequencing from punch biopsies taken at the same lesional and non-lesional sites as the microbiome swabs.

Results
The microbiota diversity in lesional skin was significantly low compared to non-lesional skin of AD patients. This was due to significantly higher frequency of the most abundant species in lesional AD samples, which is in 90% of the cases of the Staphylococcus genus. The frequency of several taxonomic units of Staphylococcus aureus is significantly higher in AD lesional samples, whereas in the AD non-lesional and healthy skin Staphylococcus epidermidis is the dominating species. In contrast, transcriptome analysis showed a global difference between AD lesional and non-lesional skin samples. The majority of tight junction genes show a significant downregulation in AD lesional skin, whereas the cytokines IL36G, IL38 and IL37 show upregulation. Correlation studies between microbiome and transcriptome demonstrated significant positive association between the abundance of S. aureus with pro-inflammatory gene expression as well as with down regulation of tight junction expression. We further found a mixed pattern of negative and positive correlations between the abundance of S. epidermidis and the expression of interleukins and tight junction genes.

Conclusion
Our results show how gene expression differences in microbiome are correlated with inflammation status and skin barrier function. Furthermore, different Staphylococci species have opposite relationship to immune response and tight junction gene expression. Bioinformatics analysis allowed us to identify several groups of tight junction and immune related genes that differently correlated to the microbiome, potentially indicating distinct positive and negative influences on lesion development in AD.
Extensive Skin Barrier Damage And Inflammatory Changes Upon Exposure To P-Phenylenediamine In Hair Dye Allergic Individuals

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Keywords: Allergic Contact Dermatitis, PPD, Tight Junctions, Skin Barrier, Filaggrin

Introduction
p-Phenylenediamine (PPD) is a strong contact allergen used in hair dye products and often the cause of allergic contact dermatitis among consumers and hairdressers. Previous studies have established PPD as a hapten capable of sensitization through penetration of skin, however, the full extent of changes PPD causes in skin of exposed individuals remains unknown.

Methods
Skin biopsies were collected from 9 PPD allergic individuals and 7 hairdressers with no allergy to PPD, on day 4 after patch test with 1% PPD in vaseline or vaseline alone for 48 hours. RNA-sequencing and transcriptomics analysis were performed on skin biopsies and confirmed with quantitative RT-PCR. Protein expression were analysed with immunofluorescence staining. Reconstructed human epidermis cultures were used to test the effects of PPD in vitro where both RNA analysis and immunofluorescent staining were performed.

Results
PPD challenged allergic skin showed a highly different transcriptional profile compared to non-allergic skin and the vaseline controls. Analysis of tight junctions (TJ) related genes among PPD challenged and vaseline challenged skin in allergic individuals showed the majority of TJ genes to be downregulated together with Filaggrin-1 and Filaggrin-2. Furthermore, it showed downregulation of Filaggrin-2 in the PPD challenged skin from non-allergic hairdressers. Immunofluorescence staining and MFI calculation confirmed protein downregulation of Claudin-1 and Filaggrin-1 protein expression in PPD challenged allergic skin and a change in protein organisation of Claudin-1 in PPD challenged skin from non-allergic hairdressers. In Vitro PPD exposure caused dysregulation of TJ proteins, but not downregulation of Filaggrin-1.

Conclusion
PPD causes widespread epidermal barrier dysregulation, especially in tight junctions. Understanding the mechanisms behind strong allergens like PPD and what makes them so potent sensitizers may help with prevention and treatment of contact allergy, especially among risk occupations like hairdressers.
**Introduction**

The incidence of chronic inflammatory airway diseases has dramatically increased in recent years in developing countries, exceeding 5% of the Western European and North American populations. This contrast with much lower asthma incidence in tropical countries that have significant levels of parasite infections, suggesting a possible role for worms in suppressing allergies. In contrast to the well-defined roles of lipid mediators in allergy, much less is known about their role during parasitic infection. This study provides insights into how parasites differentially modulate the eicosanoid profile of myeloid cells in vivo and in vitro.

**Methods**

Wildtype mice or eosinophil deficient (ΔdblGATA) mice were infected with Heligmosomoides polygyrus bakeri larvae by oral gavage. Worms were cleared by treatment with the antihelminthic Cobantril at day 28 and mice were re-infected with 200 larvae at day 44. 14 days after re-infection, sections of small intestines were obtained for immunohistochemistry. Concentrations of lipid mediators in intestinal secretions were measured by LC-MS/MS. Human myeloid cells (eosinophils, neutrophils and monocyte-derived macrophages) were treated with parasite products from different parasitic nematodes. The lipid mediator profile was analyzed by LC-MS/MS.

**Results**

Using a mouse model of intestinal helminth infection, we observed that large numbers of 12/15-lipoxygenase (12/15-LO) expressing cells infiltrated parasite-induced lesions in the small intestine. This was associated with a pronounced increase in the production of 12/15-LO metabolites (12-HETE, 15-HETE) in small intestinal tissue, whilst 5-LO products remained close to the detection limit. Making use of eosinophil deficient mice, we further showed that eosinophils were the major source of 12/15-LO metabolites in the intestine. Despite intact protective immunity in the absence of eosinophils, we observed a striking defect in the capacity of eosinophil deficient mice to degrade the debris of dead parasites within the intestinal mucosa. In human myeloid cells (eosinophils, neutrophils and macrophages) products from different parasitic nematodes differentially modulated the production of eicosanoids involved in granulocyte recruitment, tissue repair and mucus production

**Conclusion**

Our data suggest that parasites may evade host immunity and initiate repair by modulating the eicosanoid profile of innate host cells.
**P02 Urban Fine And Coarse Mode Particulate Matter Differentially Alter The Phenotype Of Monocyte-Derived Dendritic Cells Independent Of Their Oxidative Characteristics.**

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**Keywords**: Monocyte Derived Dendritic Cells, Air Pollution, Oxidative Potential, London

**Introduction**
Ambient particulate matter (PM) is associated with poor respiratory health in asthmatics and patients with chronic obstructive pulmonary disease. A PM-induced pro-inflammatory response via dendritic cell (DC) maturation and activation has been proposed as a central mechanism for this adverse response. Here, we investigated the capacity of PM collected from heavily trafficked and urban background locations in London (2013) to drive the maturation of human monocyte derived DCs (MDDC), to evaluate their potential impact of the adaptive immune response. In addition, we examined whether the oxidative properties of the particles themselves played a role in mediating their effect on DCs.

**Methods**
Multiple PM$_{2.5}$ and PM$_{10}$ filters collected from Marylebone Road (roadside) and North Kensington (background) were collected, extracted and pooled to provide a set of annual samples (2013). The capacity of these PM samples to drive damaging oxidation reactions was assessed through their capacity to deplete glutathione (GSH) and ascorbate (AA) from a synthetic respiratory tract lining fluid. Peripheral blood human MDDCs were then exposed *in-vitro* to the ambient PM$_{2.5}$ and PM$_{10}$ samples at various concentration (1.25-20 mg/mL) for 24h, with the expression of CD83, MHC Class I and II analysed by flow cytometry.

**Results**
Roadside PM$_{10}$ had enhanced ascorbate dependent oxidative potential (OP$_{AA}$) (p<0.01) compared to background PM$_{10}$, with a similar increment (p<0.053) apparent for the glutathione dependent metric (OP$_{GSH}$). No differences in OP$_{AA}$ and OP$_{GSH}$ were noted between the two fractions at each location. MDDCs exposed to PM$_{10}$ increased expression of MHC Class II and CD83 (p<0.01, based on the response across the full concentration range). In contrast all PM samples decreased the expression of MHC Class I to a similar extent.

**Conclusion**
Two PM-fraction dependent responses were observed with ambient PM$_{10}$ and PM$_{2.5}$. MDDC maturation was largely restricted to the coarse mode PM, with little evidence of a PM$_{2.5}$ effect. In contrast, both PM$_{10}$ and PM$_{2.5}$ suppressed MHC Class I expression. Thus the pattern of response could not be simply explained by the oxidative properties of the particles, suggesting that other components were driving the responses observed. Overall however these results suggest that MDDCs exposed to PM may be less able to stimulate CD8$^+$ T cells, increasing susceptibility to recurring respiratory tract infections.
**P03 Microbial-Derived Biogenic Amines Can Influence Host Immune Responses**

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**Keywords**: Microbiome, Biogenic Amines, Immune Regulation

**Introduction**

Body surfaces are colonized by diverse microbial communities that interact with the host. Not only the microbiota composition but also its metabolic activity has profound effects on immune tolerance and control of inflammatory responses. We are interested in biogenic amines (BAs), that can be generated following microbial decarboxylation of amino acids. The aims of this project are (1) to identify bacteria in the gut that secrete BAs and (2) to determine the influence of BAs on mucosal immune responses.

**Methods**

Bacterial cell culture from fecal samples, THP-1-Blue cells, cytokine multiplex assay, flow cytometry

**Results**

Following in vitro culture of bacterial isolates from human fecal samples, spermine, spermidine, cadaverine, tyramine, agmatine, putrescine and histamine-secreting microbes were identified. However, only spermidine (SPD) and spermine (SPM) suppressed LPS-induced activation of NF-kB and AP-1 in THP-1 cells. In addition, SPD and SPM also suppressed PBMC secretion of pro-inflammatory cytokines (MCP-1, MIP-1β, G-SCF, GM-SCF, IL-6, TNF-α, IFN-γ) and dendritic cell surface expression of CD80 and CD86 following LPS stimulation

**Conclusion**

In conclusion, we demonstrate that the human gut microbiome harbors many different BA-producing microbes and some of these metabolites (i.e. SPD and SPM) display immunoregulatory effects. Future studies will determine if these microbes and metabolite levels are altered in allergic individuals and will further examine the SPD and SPM mechanisms.

**P04 ILCs Display Resistance To Dexamethasone Through The Expression Of The Multi-Drug Efflux Pump MDR1**

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**Keywords**: Steroids, ILCs, Asthma, Resistance

**Introduction**

Innate lymphocyte cells (ILCs) play an important immune regulatory role in a variety of inflammatory disorders such as asthma. Corticosteroids have long been the standard form of treatment for these diseases. However, a significant proportion of patients respond poorly even at maximal doses. The potential for human ILCs to resist the immunomodulatory effect of corticosteroids, and the
mechanisms by which this may be achieved, are currently unknown. Multi drug resistance 1 (MDR1) is a promiscuous drug efflux pump which can mediate efflux of various drugs, including corticosteroids.

**Methods**

Using a combination of flow cytometry, quantitative PCR and functional assays we determined the expression and function of MDR1 in circulating and lymphoid tissue ILCs, as a potential mechanism of steroid resistance. Then, by *in vitro* culture, we assessed the potential of ILCs to resist exposure to the corticosteroid dexamethasone, as mediated through MDR1 expression.

**Results**

ILC3s both in blood and lymphoid tissue expressed high levels of functional MDR1 which enabled them to resist apoptosis induced by dexamethasone *in vitro*. ILC2s, on the other hand, did not express MDR1 at steady-state and remained susceptible. Preliminary data, however, suggests that levels of MDR1 may be upregulated in ILC2s from patients with severe asthma, who are resistant to inhaled corticosteroids.

**Conclusion**

This data demonstrates MDR1 to be variably expressed in ILC subsets, with expression mediating steroid resistance. Expression may be modulated during disease, with the factors regulating expression currently under investigation. Identifying the mechanisms of resistance in key immune mediators such as ILCs represents a critical point of investigation in developing globally effective treatments.

**P05 Electrical Impedance Measurements For The Assessment Of Epithelial Barrier Defects**

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**Keywords**: Epithelial Barrier, Electrical Impedance, Tight Junctions, Skin

**Introduction**

Defects in the epithelial barriers, mainly due to disruptions of tight junctions (TJs), allow allergens, pollutants or microbes, to enter the tissue and thus activate the immune response. The aim of this study is to establish a method to assess the epithelial barrier function *in vivo* by electrical impedance (EI) spectroscopy, a relatively new technique under investigation as an early diagnostic tool for skin and mucosal barrier defects.

**Methods**

The method was tested on C57BL/6 nude mice skin after damaging the epithelial barrier by tape stripping, an efficient approach through which the cell layers of the stratum corneum are successively removed using adhesive films. EI measurements were performed before tape stripping and after 5, 10, 15 and 20 tape strips. The same protocol was performed in human subjects. In addition, skin from a second group of C57BL/6 nude and wild type mice was treated *in vivo* or *ex vivo* with proteases (papain and trypsin), or microbial products (e.g. cholera toxin), which can specifically destroy the epithelial TJs. The results were confirmed by immunofluorescence staining for TJs and qPCR.

**Results**
In mice already after 5 tape strips a significant reduction of EI was detected, reflecting the decreased epithelial barrier function. In humans similar results were observed. Furthermore, mice skin was treated ex vivo with trypsin and cholera toxin and a significant decrease of EI was shown after 1 hour. In in vivo experiments, papain was locally delivered by means of a hydrophilic cream and after 2 days from the application an important reduction of EI could be detected. A compromised expression of TJs was shown by immunofluorescence staining for TJs and qPCR, confirming the reduced epithelial barrier function.

**Conclusion**
EI spectroscopy can be used as an in vivo diagnostic method to determine barrier defects.

**P06 Recurrent Bacterial Complications Of Upper Respiratory Infections In Children: Some Etiological And Immunological Features**

Olha Shvaratska  
DMA, Dnipro, Ukraine  

**Keywords**: Upper Respiratory Infections, Acute Otitis Media, Rhinosinusitis, Lysozyme, Children

**Introduction**
Upper respiratory infections (URIs) are one of the most common diseases of childhood. Some children tend to have recurrent bacterial complications of URIs which increase the duration of the disease and lead to repeated administration of antibiotics. It was assumed that frequent bacterial complications of URIs in children might be associated with impaired local resistance state of mucous membranes of the upper respiratory tract (URT). Spectrum of causative pathogens might also differ in such children which should be considered when appointing a rational antimicrobial therapy.

**Methods**
We enrolled 110 children aged 3 to 18 years with URIs complicated with acute purulent otitis media (APOM) or rhinosinusitis (RS). Frequency of bacterial complication of URI in 40 children (main group) met the criteria of recurrent course (4 or more documented episodes of APOM and/or RS during the previous year); 70 children with lower frequency of APOM and/or RS were the comparison group. The study investigated concentrations of human cathelicidin (hCAP/LL-37), secretory immunoglobulin A (sIgA), lactoferrin (La) and lysozyme in oropharyngeal discharge twice during the disease, and after recovery. Microbiological examination was used for determining the causative agent. Reference values were obtained in 30 healthy children.

**Results**
Upper airways mucosal local resistance status data are presented in Table 1. In children with recurrent (versus episodic) bacterial complications of URIs signs of malfunction of local protective mucosal anti-infection mechanisms of the URT were noted: reduction of hCap/LL-37 level during the whole disease, reduction of La in the early development of bacterial process, and reduced concentrations of lysozyme in all phases of the study. It’s noteworthy that concentrations of hCAP/LL-37 and La during the disease exceeded control values in both groups (hCAP/LL-37 1.27 (0.56; 5.60) ng/ml, La 5.79 (3.99; 8.50) ug/ml, p< 0.001); in opposite, lysozyme levels obtained from children of the main group were significantly lower compared to control patients (40.37 (33.98; 43.81) pg/ml,
p<0.001) both initially and after recovery. In main group St. aureus appeared as co-agent more often than in comparison group (45.0 % vs. 28.1 %, respectively, p <0.01), and S. pneumoniae was found more rarely (15.0 % vs 31.3 %, p<0.01).

**Conclusion**

Recurrent bacterial complications of URIs are associated with local mucous resistance insufficiency, and higher rate of St. aureus colonization.

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<th>Main group, n=40</th>
<th>Comparison group, n=70</th>
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<tr>
<td><strong>Early period of bacterial process</strong></td>
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<tr>
<td>hCAP/LL-37, ng/ml</td>
<td>34.02 (25.54; 64.70)</td>
<td>66.89 (36.12; 98.70)</td>
<td>p=0.009</td>
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<td>sIgA, ug/ml</td>
<td>119.15 (87.56; 165.76)</td>
<td>117.50 (72.16; 172.43)</td>
<td>p=0.628</td>
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<td>La, ug/ml</td>
<td>55.56 (39.12; 63.76)</td>
<td>64.77 (49.18; 76.44)</td>
<td>p=0.002</td>
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<td>Lysozyme, pg/ml</td>
<td>21.86 (12.89; 28.72)</td>
<td>39.82 (26.49; 45.29)</td>
<td>p&lt;001</td>
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<td><strong>Late disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hCAP/LL-37, ng/ml</td>
<td>8.63 (3.47; 11.92)</td>
<td>9.95 (6.34; 24.56)</td>
<td>p=0.022</td>
</tr>
<tr>
<td>sIgA, ug/ml</td>
<td>371.10 (297.38; 459.70)</td>
<td>402.23 (307.49; 506.18)</td>
<td>p=0.426</td>
</tr>
<tr>
<td>La, ug/ml</td>
<td>12.44 (10.02; 15.58)</td>
<td>13.44 (9.99; 22.49)</td>
<td>p=0.168</td>
</tr>
<tr>
<td>Lysozyme, pg/ml</td>
<td>47.27 (36.29; 56.20)</td>
<td>65.39 (55.93; 76.52)</td>
<td>p&lt;001</td>
</tr>
<tr>
<td><strong>After recovery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hCAP/LL-37, ng/ml</td>
<td>1.37 (0.61; 3.89)</td>
<td>0.97 (0.55; 2.47)</td>
<td>p=0.205</td>
</tr>
<tr>
<td>sIgA, ug/ml</td>
<td>197.84 (174.47; 254.73)</td>
<td>201.51 (176.15; 229.75)</td>
<td>p=0.682</td>
</tr>
<tr>
<td>La, ug/ml</td>
<td>4.51 (3.08; 7.25)</td>
<td>3.80 (3.02; 5.68)</td>
<td>p=0.326</td>
</tr>
<tr>
<td>Lysozyme, pg/ml</td>
<td>19.19 (16.80; 22.88)</td>
<td>26.58 (17.43; 34.98)</td>
<td>p=0.002</td>
</tr>
</tbody>
</table>

Note: p* – Mann-Whitney U-test

**P07 Coupling Of Carbohydrates And Allergen To Silica Nanoparticles Facilitates Antigen Uptake And Activation Of Dendritic Cells**

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**Keywords**: Silica Nanoparticles, Allergen-Neoglycoconjugates

**Introduction**

Nowadays allergy is one of the most common chronic diseases in developed countries. Today, no effective treatment exists to overcome these disorders. Currently, subcutaneous allergen-specific immunotherapy (SCIT) is considered to be the most successful approach to treat allergies. However, such therapy is associated with severe side effects and patient compliance is low due to the long
treatment schedules. Therefore, new methods with higher efficacy and shorter immunization schedules are urgently needed. One promising alternative to SCIT is to use allergen-neoglycoconjugates to target antigen presenting cells via C-type lectin receptors (CLRs). Studies on allergen-neoglycoconjugates have demonstrated that they are able to induce high IgG titers, while preventing IgE binding. The strong immunogenicity and hypoallergenic properties of neoglycoconjugates make them ideal candidates for allergen-specific immunotherapy. However, they are poorly defined and lack molecular homogeneity, which complicates their production in a reproducible manner. The use of silica nanoparticles (SNPs) as a core element and polysaccharides with modified reducing ends allows to overcome most of the aforementioned limitations of neoglycoconjugates.

Methods
In this work, we report the functionalization of the reducing end of polysaccharides Mannan, Dextran and Laminarin with an alkyne group and their coupling via click chemistry to the SNPs. As a model allergen, we used Bet v 1 that was coupled to the SiO₂ NPs via its N-terminus.

Results
Resulting Bet v 1-glyconanoparticles were homogeneous in size as determined by dynamic light scattering. Nanoparticles were efficiently internalized and showed dendritic cell activation in vitro. In addition, immunodominant epitope of Bet v 1 was efficiently presented to Bet v 1 specific T cells which indicates a strong immunogenicity for the Bet v 1-glyconanoparticles.

Conclusion
Our preliminary data suggest that these allergen-glyco-SNPs are suitable candidates for allergy vaccines. Our future plans include performing a comprehensive characterization of these NPs by using electron microscopy, Fourier-transformed infrared spectroscopy and NMR. We will also test the APC targeting capacity of these SNPs in human skin explants and their potential for allergy immunotherapy in relevant mouse models.

P08 Immune Responses In A Murine Device-Related Infection Model: Role Of IL-17A

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² AO Research Institute Davos, Davos, Switzerland

Keywords: Infection, S. Epidermidis, IL-17A, Mouse Model

Introduction
Infection associated with implanted devices is a major complication of surgically fixed fractures. In this study, a murine implant-associated infection model was established with a clinical isolate of Staphylococcus epidermidis as a contaminating agent. The development of infection over time and associated immune responses were assessed, with emphasis on IL-17A responses.

Methods
Titanium plates, with or without S. epidermidis (10⁴ CFU), were used to fix a femoral osteotomy in wild-type (WT) C57Bl/6 mice (female, 20-28 weeks old). Mice were sacrificed at 3, 7 and 14 days after surgery. Additionally, WT and IL-17A KO C57Bl/6 mice (female, 20-28 weeks old) were operated and sacrificed at
day 14. Live bacteria from the tissues were quantified. Bone single cell suspensions were re-stimulated ex-vivo for cytokine and chemokine measurements. Lymph node and bone cells were characterized by flow cytometry.

**Results**
From day 7 on, no bacteria were cultured from some animals (9/16 in total), indicating that the immune system was capable of clearing the infection. IL-17A was associated with the inoculation of bacteria and IL-17A+ T lymphocytes were increased in local lymph node, especially on those animals where bacteria were not cultured. When using IL-17A KO mouse strain, it was observed that 100% of the animals remained infected at day 14 while 25% of WT cleared bacteria. However, the differences were not significant. IL-17A sources in bone marrow were assessed, being predominantly CD3+CD4+ and γδ+ T lymphocytes with some ILC-like cells.

**Conclusion**
IL-17A was a landmark of the infection process and high levels of IL-17A+ cells in animals that cleared bacteria suggested a link between such responses and infection resolution. Further experiments with an IL-17A KO mouse strain indicate that, while IL-17A responses partially contribute to infection clearance, other factors are also involved.

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**Friday, 27 January 2017**
21:00 - 22:00 Poster session I
Topic 2 - Immune Mechanisms: Poster P10 - P18

**P10 Frequency And Regulation Of Allergen-Specific T And B Cells Linked To Mechanism Of Immune Tolerance In Allergen-Specific Immunotherapy**

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**Keywords:** Allergen-Specific T Cells, Allergen-Specific B Cells, Allergen-Specific Immunotherapy

**Introduction**
Allergen-specific immunotherapy (AIT) is the only available treatment option for allergic disease that can induce immune tolerance to allergens. The mechanisms involved in this process include changes in allergen-specific T and B cell response. The aim of this study was to identify the role of allergen-specific T and B cells in allergen tolerance induction.

**Methods**
Twenty-three house dust mite (HDM) immunotherapy patients were recruited at the Phramongkutklao Hospital (Bangkok, Thailand). Peripheral blood was taken at baseline (W0) and after week 10 (w10) and week 30 (W30). We used Derp 1-pMHC-II-tetramers to identify Derp 1-specific T cells. B cells specific for the major HDM allergen Derp 1 were identified using Derp 1 labeled with two different fluorescent dyes.
Results
Twelve (52.2%) of our patients were matched with their MHC-II expression to peptide MHC-II tetramers. The number of Derp 1-tetramer+ CD45RO+CD4+ T cells were increased significantly higher after AIT. Within the T regulatory cell population, the percentage of Derp 1-tetramer+ CD25+CD127+CD4+ T cells and a subset showing activation, such as Derp 1-tetramer+ FOXP3+Helios+CD25+CD127+CD4+ T cells increased substantially from baseline on W10. After AIT, Derp 1-specific TH2 cells producing IL-4 and IL-17 significantly decreased. In contrast, Derp 1-specific TH2 cells producing IL-10, IL-22 and IFN-γ, significantly increased during the course of AIT. The frequency of Derp 1-specific class-switched memory B cells (CD19+IgM-CD27+) increased significantly and Derp 1 specific IgG4+ and IgA+ switched cells expanded significantly after AIT. We also observed an increase in frequency of Derp 1+ plasmablast (CD19+CD27hiCD38hi) and IL-10 secreting Bregulatory type1 cells (CD19+CD73 CD71+CD25+) after AIT.

Conclusion
AIT-induced allergen immune tolerance by increased frequency of Derp 1-specific T and B cell subsets. Particularly increased Derp 1-specific T and B regulatory cells have an important role in immune tolerance.

P11 Is The Skin Essential For Peanut Sensitization? Circulating Peanut-Responsive Th2 Cells Lack Expression Of Cutaneous Lymphocyte Antigen

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Keywords: Peanut-Responsive T-Cells, CD154, Th2, CLA

Introduction
It has been suggested that sensitization to peanut and perhaps other foods occurs by exposure through non-oral routes as the skin. Peanut allergic subjects have a dominant Th2 response characterized by production of the cytokines IL-4, IL-5 and IL-13. However, the tissue homing phenotype of peanut–specific Th2 cells is poorly understood, but could give an indication of the exposure route. The aim of this investigation was to characterize the tissue homing phenotype for human peanut-specific CD4+ T cells.

Methods
45 × 10^6 PBMCs (5-7.5 × 10^6 cells/mL) from peanut allergic subjects (n = 9) and healthy controls (n = 13) were stimulated with an in the absence of 100 mg/mL peanut extract and brefeldin A for 14-16h. Peanut-responsive CD4+ T cells were identified by CD154 expression. The acquisition was performed with a BD LSR II Fortessa.

Results
PBMCs from peanut allergic subjects had higher frequencies of circulating peanut-specific T cells per million CD4+ T cells than healthy controls (113 vs 35). The CD154+ peanut-specific CD4+ T cells of peanut allergic subjects and healthy controls showed a primary Th2 (IL-4 and IL-5) and Th1 (IFN-γ) profile, respectively. Compared to controls, peanut-responsive T cells of peanut allergic subjects had increased expression of the Th2 skin homing chemokine receptor CCR4 (8% vs 24%), but not the signature skin homing antigen CLA (14% vs 16%) or the other skin homing chemokine receptor CCR10 (7% vs 13%). Moreover, no difference in expression of the gut-homing marker β7 was found in the peanut-
responsive cells of healthy and peanut allergic subjects (3.1% vs 2.5%). Interestingly, cytokine responses of the peanut-specific CLA-positive T cells of peanut allergic subjects showed a low percentage T cells with an early (IL-4+) or highly differentiated (IL-4+IL-5+) Th2 phenotype (14% and 5%, respectively).

**Conclusion**
Circulating peanut-specific CD4+ T cells of peanut allergic subjects have a predominance Th2 profile but lack co-expression of the signature skin homing antigen CLA. These results are not supportive of the hypothesis that peanut sensitization occurs through the skin.

**P12 Linking KIT Signalling To Actin Cytoskeleton Reorganization In Mast Cells.**

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Universitat de Barcelona, Barcelona, Spain

**Keywords**: Mast Cells, Cytoskeleton, KIT

**Introduction**
Microtubules and microfilaments influence a variety of processes during mast cells activation. For example, during the degranulation process leading to proinflammatory mediators release, granules translocate along microtubules from the interior of the cell towards the plasma membrane and the cortical actin needs to be depolymerized. The dynamic reorganization of the actin cytoskeleton is also essential for many cellular processes, including endocytosis, migration, polarization, and adhesion.

Several adaptors proteins like 3BP2, an adaptor protein that modulates Kit and FceRI signaling cascade in mast cells, can play a role in these processes. With three-hybrid assays, we found that 3BP2 binds to Myo1f, an unconventional type I myosin, via 3BP2-SH3 domain. Myo1f is expressed predominantly and differentially in the immune system, and it has been described as an important player in neutrophil migration.

**Methods**
FceRI stimulation
KIT stimulation
ShRNA silencing and overexpression by lentiviral infection
Immunofluorescence
Cell migration
Flow cytometry
Adhesion assays
RhoA / Rac1 / Cdc42 Activation Assays

**Results**
We found that Myo1f is a protein expressed in mast cells, and it colocalizes with the cortical actin. We used immunocytochemistry assays to describe the molecular pattern of Myo1f in the KIT-3BP2 pathway. As KIT receptor has been described as an important chemotactic receptor we also assayed two of the most important functions of mast cells regulated by actin reorganization: migration as well as their capacity to perform degranulation in Myo1f and 3BP2-silenced mast cells. Myo1f knockout mouse shows an abnormally increased adhesion and reduced motility in neutrophils, decreasing mice survival after infection. This results from an augmented exocytosis of β2 integrin-containing granules. For this reason, we
investigated the integrin patterns in Myo1f and 3BP2-silenced mast cells under different conditions, as well as their adhesion capacity to fibronectin. Furthermore, as 3BP2 also links to Vav1, a Rho family protein that regulates mast cell signalling, we investigated the activation of several GTPase proteins in our mast cell models, such as RhoA, Rac1 and Cdc42, which are implicated in mast cell migration and degranulation.

**Conclusion**

3BP2-Myo1f interaction could play an important role in cytoskeleton regulation of mast cells, especially in cortical actin regulation, by these means affecting several mast cell function.

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**P14 Immunological Ways Of Action Of MV140, A Polyvalent Bacterial Preparation Used For The Treatment Of Recurrent Urinary Tract Infections (RUTIs)**

_Cristina Benito-Villalvilla_1, Cristina Cirauqui_1, Alba Angelina_1, Leticia Martín-Cruz_1, Carmen M. Diez-Rivero_2, Miguel Casanovas_3, José Luis Subiza_4, Oscar Palomares_1

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**Keywords:** Polyvalent Bacterial Preparation, Sublingual Vaccine, Human Dendritic Cells, Human T Cells, Whole Heat-Inactivated Cells

**Introduction**

MV140 is a polyvalent bacterial preparation composed of entire heat-inactivated bacteria (*K. pneumoniae*, *E. coli*, *P. vulgaris* and *E. faecalis*, 25% each) used as a novel sublingual vaccine. MV140 has been shown to prevent recurrent urinary tract infections (RUTIs) in two clinical retrospective observational studies, however, the immunological ways of action remain unknown. Therefore, the aim of this work is to study the capacity of MV140 and its individual bacterial components to immunomodulate the phenotype and function of human DCs at the molecular level.

**Methods**

MV140, negative control (containing all the excipients without bacteria) and each individual bacterium were from Inmunotek S.L. The cytokine signature was determined by ELISA. Allogeneic co-cultures of MV140-activated hmoDCs and naïve CD4<sup>+</sup> T cells, CFSE-dilution assays, flow cytometry, real-time quantitative PCR, blocking and pharmacological inhibition experiments were performed. BALB/c mice were sublingually immunized four times with MV140 or control. Proliferation of CFSE-labeled CD4<sup>+</sup> T cells and cytokine production of splenocytes, lymph nodes cells and bladder cells were measured.

**Results**

MV140-activated hmoDCs produce significant higher levels of pro-inflammatory cytokines (IL-1β, IL-6, IL-23 or TNF-α) as well as IL-10 than control-treated cells. Significant differences were also demonstrated between the Gram negative and positive bacteria. HmoDCs activated with MV140 or its individual bacterial components promote the generation of T<sub>H</sub>1 and T<sub>H</sub>17 as well as IL-10-producing T cells. MV140-activated hmoDCs induced significantly higher levels of CD4<sup>+</sup> T cells simultaneously producing IL-17A and IFN-γ than control-treated hmoDCs. In
addition, a significant increase in FOXP3⁺IL-10⁺ T cells but not GATA3⁺IL-10⁺ T cells by hmoDCs treated with MV140 was observed. Blocking experiments with specific pharmacological inhibitors revealed that ERK, p38, JNK and NFκB significantly contribute to the observed effects, acting downstream of Toll-like receptors via MyD88 and C-type lectin receptors via Syk. In vivo sublingual immunization of mice with MV140 promotes the generation of potent systemic Th1, Th17 and IL-10 immune responses. Initial experiments in humans showed that trained immunity mechanisms might be involved in the immunological action of MV140 in human DCs.

**Conclusion**
This study uncovers the potential way of action by which MV140 might exert its clinical efficacy in patients suffering from RUTIs.

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**P15 Assessment Of CD1d Presentation Of Food Lipid Allergens**

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² Swiss Institute for Allergy and Asthma Research, Davos, Switzerland
³ Technical University of Madrid, Madrid, Spain

**Introduction**
Peach allergy is the most prevalent plant food allergy in the Mediterranean area, and its major allergen, the non specific lipid transfer protein is Pru p 3. However, increasing evidence suggests that Pru p 3 is not enough to explain the allergenic activity but perhaps the lipid transported by Pru p 3. Many major allergens have been described as lipid carriers, such as Pru p 3, Bet v 1 and Ara h 2 and this feature could be linked to their allergenic activity. Thus, the allergic sensitization could be based on the recognition of lipid ligands by receptors on cells of immune system. Preliminary data generated by our lab in Madrid suggests that the lipid antigens contained within Pru p 3 may be transported and presented to the immune system by CD1d.

**Methods**
Two methods were used:
1) Generation of CD1d-based Artificial Antigen Presenting cell (aAPC) using a magnetic beads model and their presentation and activation of iNKT cells (which usually respond to lipid antigens). Then, the production of IL-2 was measured by ELISA.
2) BMDC’s from CD1d-KO and WT mice were used to observe the difference in the intracellular levels of cytokines (IL-4, IFN-γ, IL-10, IL-17) in iNKT cells and dendritic cell CD80/CD86 costimulatory molecule expression.

**Results**
- At the beads model, there was a clear increase in the production of IL-2
- In mice, a decrease of costimulatory expression CD80 / CD86 in KO mice was observed in the percentage of BMDC’s.
Besides, the IL-4 and INF-γ production levels were also reduced in the KO mice according to the percentage of iNKT cells. These results are not observed so clearly with the IL-10 and IL-17.

**Conclusion**
Pru p 3-ligand activates de iNKT cells and the CD1d receptor seems to be involved in this process.
**P16 Reciprocal Regulation Of GRK2 And Bradykinin Receptor Stimulation Modulate Ca2+ Intracellular Level In Endothelial Cells**

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**Keywords:** Bradykinin, GRK2, Calcium, Endothelial Cells

**Introduction**

BK (Bradykinin) has a pivotal role in mediating Angioedema in patients with C1-INH deficiency, through activation of its endothelial receptors. Indeed, several endothelial mediators are under its control, through the Gq protein coupled receptors B1 and B2, using Ca²⁺ as second messenger. The G protein coupled receptor kinases GRK2, can modulate B1 and B2 receptors through desensitization. The purpose of the study is to verify the effects of GRK2 inhibition in regulating bradykinin signaling.

**Methods**

In BAEC (bovine aortic endothelial cells) we evaluated GRK2 expression as well as intracellular Ca²⁺ accumulation (Fluo 4AM) in response to BK. To inhibit GRK2, we either reduced its cellular levels by silencing with specific siRNA, or by inhibition with KRX-C9 administration, a specific HJ-loop derived peptide inhibitor of GRK2.

**Results**

5 minutes after BAEC stimulation, BK induces an increase of cell GRK2 levels, which reverberates in several cellular compartments (Cytosol, Membrane and Mitochondria); at 15 minutes GRK2 returns to baseline levels. BK induced GRK2 accumulation is proteasome dependent, since GRK2 ubiquitination is significantly reduced post BK stimulation and the interaction between GRK2 and mdm2, its specific E3 ligase, decreases. Consistently, GRK2 accumulation can be prevented by proteasome inhibition (MG132). BK causes Ca²⁺ cytosolic accumulation, which is sensitive to GRK2 expression activity and levels, as it is enhanced by specific inhibition with KRXC9 (CTRL: 50,4% vs KRXC9: 72% of fluorescence intensity over basal) and delayed cytosolic removal with siRNA (Scramble-siRNA: 23% vs siRNA-GRK2:46% of fluorescence intensity over basal) at 15 min post BK stimulation.

**Conclusion**

BK induces GRK2 intracellular accumulation, which in turn can desensitize BK receptors and reduce BK induced calcium intracellular signaling. Proteasome plays a key role by acutely regulating GRK2 cellular levels. These data demonstrate that GRK2 participates in the modulation of the BK signaling in the endothelium, thus targeting GRK2 activity may represent an innovative strategy to regulate biological effects of BK in specific physio-pathological conditions such as Angioedema with C1-INH deficiency.

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**P17 Composition And Functionality Of The Intra-Hepatic Innate Lymphoid Cell Compartment**

Marianne Forkel¹, Lena Berglin¹, Eliisa Kekäläinen¹, Adrian Carlsson¹, Emma Svedin¹, Jakob Michaelsson¹, Malin Flodström-Tullberg¹, Annika Bergquist², Hans-Gustaf Ljunggren³, Magnus Westgren⁴, Ulrik Lindforss⁵, Danielle Friberg⁶, Carl Jorns⁷, Ewa Ellis⁷, Niklas Björkström⁸, Jenny Mjösberg³
Introduction
Although the human liver is known to host unique subsets of tissue resident NK cells, the more recently described CD127⁺ innate lymphocytes (ILCs) have not been explored in the human liver.

Methods
Flow cytometry, immunohistochemistry, cell culture, ELISA, qPCR

Results
Here, we performed a detailed analysis of the phenotype, tissue residency and functionality of group 1, 2 and 3 innate lymphoid cells (ILC1-3) in the human liver at steady state and in disease. At steady state, the human liver shows a unique ILC composition with a dominance of tissue resident ILC3, followed by ILC1 and ILC2. Although ILC2 were rare at steady state, using two separate cohorts of liver tissue samples, we show a direct correlation between the frequency of intrahepatic ILC2 and the severity of liver fibrosis. Intrahepatic ILC2 secreted the pro-fibrotic cytokine IL-13 when exposed to IL-33 and thymic stromal lymphopoetin (TSLP). These cytokines were expressed by TLR3-stimulated hepatocytes, hepatic stellate cells (HSC), and Kupffer cells.

Conclusion
Our data provide a first detailed characterization of ILCs in the human liver in health and disease and indicate a role for ILC2 in human liver fibrosis. These findings may have important clinical implications, as targeting ILC2 might be a novel therapeutic strategy for the treatment of liver fibrosis patients.

P18 Sensitisation Including Staphylococcus Aureus In Atopic Dermatitis, STAT3-, And DOCK8-Hyper-IgE Syndrome

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³ Dermatology Hospital; Ludwig Maximilian University, Munich, Germany

Keywords: Hyper-IgE-Syndrome, Atopic Dermatitis, IgE, Staphylococcus Aureus

Introduction
Severe atopic dermatitis (AD) overlaps with hyper-IgE syndromes (HIES) regarding eczema, eosinophilia, elevated serum-IgE levels, and recurrent Staphylococcus aureus (S. aureus) infections. HIES are primary
immunodeficiencies due to monogenetic defects such as in the genes DOCK8 and STAT3. So far the contribution of S. aureus to allergy is not completely understood.

Methods
We assessed the specificity of serum IgE of AD and HIES patients in the context of clinical and immunological findings.

Results
Total serum-IgE levels were similarly elevated in STAT3-HIES, DOCK8-HIES, and AD patients whereas sensitization pattern differed between the disease groups. AD patients were mainly sensitized against aeroallergens, whereas DOCK8-HIES patients showed clinical allergy and specific IgE against food allergens. STAT3-HIES patients had food- and aeroallergen-specific IgE comparable to non-allergic controls and overall no clinical allergy or Th2-shift. All patient groups presented with significantly higher serum concentrations of IgE specific to S. aureus enterotoxin A, B, C, and TSST compared to healthy controls. The ratio of IgE specific to S. aureus toxins to total IgE was highest in STAT3-HIES patients.

Conclusion
Though total serum IgE is elevated in HIES and AD patients, all disease groups show a different and specific IgE-based sensitization pattern correlating with specific clinical disease and T cell subset phenotypes. We expect these findings to be important to elucidate the role of STAT3 signaling as well as of S. aureus in regards to allergic disease.

Friday, 27 January 2017
21:00 - 22:00 Poster session I
Topic 3 - Allergic Immune Response: Poster P19 - P27

P19 A ROLE OF IMMUNOTHERAPY IN ALLERGIC CONJUNCTIVITIS

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Introduction
Allergic conjunctivitis is many times an ophthalmologist’s nightmare. Anti allergic eye drops, which often contain corticosteroids, may temporarily bring relief of symptoms but may bring an early cataract, among other side effects. Allergy testing and immunotherapy based on the test results can cure a significant number of these patients and provide relief to others.

Methods
24 patients of allergic conjunctivitis were subjected to modified skin allergy testing. Out of these 10 patients also had associated allergic rhinitis and asthma. Common allergens found in these patients were: Dust, Dust mites, Pollen, Fungi & Food. Based on test results, vaccines were prepared for individual patients and immunotherapy given. The patients who did not significantly respond to conventional medicines and vaccine, we reviewed the history to discover any missed out allergen. A specially designed indigenous “personal volumetric air sampler” with safranine stained glycerin coated slides inside, was kept in patients home office and surrounding atmosphere.

Results
Out of 24, 18 patients (75%) started improving within 3 months of starting immunotherapy. After 6 months their eye drops and other medicines were stopped. Restricted food items started one by one after 6 months. They are
symptoms free for last 1 1/2 to 2 years. For 6 patients who did not respond to above therapy, specially designed “air sampler” brought great surprises. The fungi and pollens, which were not tested, previously were found. Also three new pollens were discovered. Appropriate immunotherapy made 5 out of 6 patients symptoms free.

**Conclusion**
Allergy testing and immunotherapy can cure allergic conjunctivitis. Personal volumetric air sampler is a very useful tool to find out additional allergens in case of resistant cases.

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**P20 Developing A 3D Co-Culture Model That Allows Assessment Of The Potential To Use The Microbiome To Prevent Asthma, By Assessing The Interaction Between The Airway Microbiome And Mucosal Immune-System**

**Tamar Smulders**
Academic Medical Centre, Amsterdam, Netherlands, The

**Keywords**: Airway Microbiome, Mucosal Immunity, Asthma Development, Air Liquid Interface

**Introduction**
The collective microbes on our body (microbiome) support the development of an immune system protective against the development of asthma. Previous research suggests that lifestyle changes in westernized countries may increase asthma prevalence through a reduction of early life microbial exposures. Similarly, early life exposure to beneficial microbiota was able to prevent asthma development in mice. These results are however not based on understanding of the function of microbiota in interaction with the immune system in humans. Herefor an ex vivo model is needed.

The aim of this study is to develop a 3D co-culture system that models the communication between the immune system and the nasal epithelial cells (airway mucosal immune system) of individual children in interaction with different microbiomes. This will help us to study potential therapeutic effects of microbiome modulations on asthma immunity.

**Methods**
To achieve this, we are developing a 3D co-culture model of Perihperal Blood Mononuclear Cells (PBMCs) and primary human nasal epithelial cells (HNECs) that can be stimulated with microbes and full microbiomes. In this model HNECs and PBMCs will be cultured together and need to be in the same medium. We have tested if PBMCs thrive well in Air Liquid Interface (ALI) medium, in which HNECs differentiate to pseudostratified ciliated epithelium. Furthermore, we followed survival of different subpopulations in IMDM (standard PBMC medium) and ALI medium. The next step will be to test if HNECs thrive well in IMDM and what their survival is.

**Results**
When PBMCs are cultured in ALI medium, compared to IMDM, they have a largely different cytokine expression profile. Survival for the different subpopulations is similar in both media. Since the cytokine profile differs largely in ALI medium, the co-culture model will preferably be performed in IMDM. The next step will be testing the behaviour and survival of HNECs in IMDM.
**Conclusion**

This research lays the foundation for the 3D co-culture model I will study during my PhD. The ex vivo co-culture model will give us the opportunity to study the interaction between the airway microbiome and airway mucosal immune system. Ultimately, this co-culture model will greatly improve our understanding of the relevance the airway microbiome has in asthma development and its potential use as a novel therapeutic approach.

**I: Single Microbe  II: Complete Microbiome**

*Figure 1: Graphical depiction of the co-culture model consisting of epithelial cells and PBMCs allowing stimulation experiments with known microbes or whole communities.*

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**P21 Characterisation Of Antigen-Specific T Cells To Milk And Egg In Atopic Eczema.**

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**Keywords:** T-Cells, Milk Allergy, Food Allergy, Atopic Eczema, Atopic Dermatitis

**Introduction**

Atopic eczema (AE) is a prevalent inflammatory skin disease which can have a severe negative effect on quality of life. Food allergy is strongly implicated in AE. Allergy to foods causing delayed flares in atopic eczema (AE) is mediated by T-cells (Tc).

Our study aimed to utilise established methods to enumerate food-specific Tc responses in AE by answering:

i. Can food-specific Tc responses can be detected in AE?
ii. Is the frequency of food-specific Tc different in AE and controls?

**Methods**

Peripheral blood mononuclear cells from non-atopic controls (n= 9) and AE (n= 12) were stimulated with egg and milk proteins (Ovalbumin, Ovomucoid, Beta-lactoglobulin (BLG), Casein (CAS), and formula milk) and IFN-γ, IL4, and IL10 assayed with Enzyme-Linked ImmunoSpot (ELISpot). BLG and CAS short-term T-cell lines (TCLs) were tested for expansion of antigen-specific responses.

**Results**

Both AE and controls demonstrated positive IFN-γ responses to BLG but responses were significantly lower in AE (p<0.05). Positive IFN-γ responses to CAS were only detected in controls (p<0.05). Only AE TCLs cultured with BLG and CAS demonstrated expansion of antigen-specific Tc. Evidence of cross-reactive responses by BLG-specific T cells for CAS was detected but not vice versa.

**Conclusion**

Food-specific (BLG/CAS) Th1 but not Th2 cells are detectable in both AE and controls, but Th1 responses to milk proteins are significantly impaired in AE. However, on culture with milk proteins, only AE patients showed antigen-specific Th1 proliferation which may suggest that there is a skew towards food-specific memory Tc in AE versus effector Tc in controls. Taken together, these data imply that food-specific effector Th1 responses may be protective against AE which may be more important than any increased risk conferred by food-specific Th2 cells.
**P22 Changes In The Allergenicity Of Cashew And Pistachio Nuts After Thermal Treatments.**

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**Keywords**: Cashew, Pistachio, Food Allergy, Thermal Processing, Mediator Release Assay

**Introduction**
Despite the rich nutritional profile and beneficial effects, the consumption of tree nuts can lead to severe allergic reactions in sensitized patients. Food processing has been proposed as a methodology to obtain food products with altered allergenicity, but the consequent modification of proteins and the changes on the allergenic capacity of tree nuts have been not thoroughly studied. The aim of this work was to elucidate the influence of moist thermal treatments (boiling and autoclave) on the IgE reactivity of pistachio and cashew proteins, by means of *in vitro* techniques. Rat basophilic leukaemia cell line (RBL-48), which expresses the human receptor FcεRI (α subunit) in its surface, was also used for mediator release assays (MRA) to analyse cashew and pistachio IgE cross-linking capability.

**Methods**
The allergenicity of untreated and treated cashew and pistachio nuts was evaluated by IgE-ELISA, IgE-immunoblot and ELISA inhibition assays using serum from Spanish patients with clinical cashew and pistachio allergy. RBL-48 sensitized with a pool of sera was used for MRA. Sensitized cells were stimulated with untreated and treated protein extracts, in order to investigate the ability of untreated and treated cashew and pistachio proteins to cross-link IgE on effector cells. Expression of the human receptor FcεRI in RBL-48 was firstly corroborated by means of flow-cytometry.

**Results**
Autoclaving at 2.56 atm for 30 minutes strongly decrease the IgE reactivity of cashew, compared to boiling and softer autoclave treatments (1.18 atm). The results obtained from ELISA inhibition and IgE-ELISA assays showed that boiling without pressure had a very low effect on the reduction of cashew allergenicity, and this outcome was similar in almost all patients, according to IgE-ELISA tests. In the case of pistachio, autoclave treatments also reduced its IgE-binding ability, although the effect was weaker than in cashew. Boiled pistachio showed significant reduction on the IgE reactivity in 4 out to 6 patients measured by ELISA tests. The β-hexosaminidase release after cell line stimulation with treated cashew and pistachio was halved compared to the result with untreated protein extracts.
Conclusion
The results of this study indicate that thermal/pressure treatment (autoclaving) was able to decrease not only IgE-binding properties of cashew and pistachio protein extracts but also their IgE cross-linking properties.

P23 The Acute Stress Hormone Epinephrine Promotes A Shift Of Human Monocyte-Derived Macrophages (MDMs) Towards The Regulatory M2b Phenotype

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Keywords: Epinephrine, Monocyte-Derived Macrophages, Allergic Inflammation

Introduction
M2 or alternatively activated macrophages are immune cells with high phenotypic heterogeneity. Among them, the M2a subpopulation is known to promote the Th2 environment in allergic inflammation. Catecholamines, on the other hand, are well-known immune modulators and data from mouse studies suggest that these stress hormones may induce regulatory macrophages. However, no data exist on the potential immunoregulatory role of catecholamines on human macrophages in the context of allergy.

Aims: Thus, the aim of our study was to understand the effect of epinephrine on M2 polarized monocyte-derived macrophages (MDMs).

Methods
For that purpose, isolated monocytes from healthy PBMCs were differentiated into M2 macrophages by M-CSF (M0) or further stimulated into the M2a phenotype in the presence of IL-4 and IL-13 cytokines. After overnight incubation with epinephrine, supernatants were collected and analyzed by ELISA for IL-10, TNF-α, IL-6, CCL-1, IL-12 and IFN-γ, whereas cell surface markers including CD206, CD163, CD86 and CD11b were evaluated using flow cytometry.

Results
Both M0 and M2a macrophages after overnight epinephrine treatment showed an increase in IL-10, TNF-α and IL-6 production, but no IFN-γ and IL-12 expression; only the M2a macrophages showed additional CCL-1 expression. Epinephrine treatment induced a downregulation of surface markers CD206, CD163 and CD11b and upregulated CD86. Collectively, our data suggest that epinephrine may promote a phenotypic shift of M0 and M2a polarized primary human macrophages, redirecting M2a macrophages toward the M2b regulatory phenotype.

Conclusion
We conclude that epinephrine in acute stress or when applied therapeutically in anaphylaxis, may direct macrophages towards a tolerogenic phenotype.

P24 Understanding The Relationship Between Interleukin-10 And Airway Macrophages In Regulating Allergic Airways Disease

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Introduction
AMs (airway macrophages) contribute to maintenance of immune tolerance in the healthy lung and their dysregulation may facilitate allergic airway inflammation such as that in asthma. Although the factors underlying AM tolerance are incompletely understood, they are thought to include production of immunoregulatory cytokines. Here we sought to investigate the role of the prototypic regulatory cytokine IL-10 in AAD (allergic airways disease) and assess the potential of AMs as a source of IL-10.

Methods
AAD was induced in adult mice by intranasal administration of HDM (house dust mite) extract 5 times weekly for 1 or 3 weeks. In some experiments, mice received intranasal anti-IL10RB blocking antibody or isotype control twice weekly throughout the regimen. AMs, PMs (peritoneal macrophages) and BMDMs (bone marrow-derived macrophages) were obtained from naïve mice and stimulated in vitro with LPS (lipopolysaccharide).

Results
Using IL-10 reporter mice we found less than 2% of AMs to be IL-10-producers in vivo at homeostasis and during AAD. Instead, inflammatory macrophages/monocytes and lymphocytes were the most abundant IL-10-producing cells in the allergic lung. Accordingly, stimulation of purified AMs with HDM extract or LPS, an immunologically active component of HDM, induced little to no IL-10 mRNA or protein expression. This was in marked contrast to other macrophage populations, PMs and BMDMs, which contained readily detectable levels of IL-10 mRNA at baseline and secreted IL-10 following stimulation with LPS.

Although pulmonary IL-10 was largely not AM-derived, it was a key regulator of the immune response to inhaled allergen. Local blockade of IL-10 signalling throughout continuous HDM exposure induced a distinct, severe AAD phenotype marked by enhanced airway hyperresponsiveness; concomitant with decreased eosinophilia, exacerbated Th1 and Th17 responses and a lung-specific deficiency in Tregs. This T cell imbalance was also accompanied by elevated pulmonary IFNγ levels and increased MHC-II expression on AMs.

Conclusion
Local pulmonary IL-10 signalling controls the balance of T cell populations in the allergic lung and mitigates the severity of AAD. However, IL-10 production is unlikely to be a major immunoregulatory mechanism employed by AMs during AAD. We are currently exploring further regulatory mechanisms utilised by AMs and the role of IL-10 from other cellular sources in regulating airway inflammation.
Interaction Of Natural And Mutant Fish Parvalbumins And Fish-Derived Food Matrix With Bronchial Epithelial Cells

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Keywords: Epithelial Cells, Fish Allergy, Fish Parvalbumins, Food Matrix

Introduction
Inhalation of aerosolized fish allergens and fish matrix components is often associated with severe IgE-mediated reactions in sensitized individuals. The calcium-binding proteins parvalbumins are major fish allergens. The role of epithelial cells in allergic reactions to fish is not well understood. We explored interactions of the human bronchial epithelial cell line 16HBE14o- with natural fish parvalbumins in presence or absence of fish-derived food matrix. Furthermore, in order to explore the role of calcium binding to parvalbumins in their interaction with the cells, we included in our study a mutant carp parvalbumin in which two functional calcium-binding sites were mutated.

Methods
We used the natural parvalbumins Gad m 1 and Cyp c 1 purified from cod and carp, respectively. As a model for a mutant fish parvalbin, a non-calcium-binding recombinant Cyp c 1 expressed in E. coli was used. A <3kDa fraction of fish extract was used as a fish matrix. Polarized 16HBE14o- cells were treated apically with parvalbumins with or without the respective fish-derived food matrix. Fluorescently labelled parvalbumins were detected by confocal microscopy. Concentrations of IL-6, IL-8, CCL2 and GM-CSF in the basolateral cell culture medium were measured by the Luminex-based multiplex assay.

Results
Apical exposure of the cells to parvalbumins resulted in their internalization. Parvalbumins localized mostly to the basal part of the cells below the nuclei. Mutant Cyp c 1 decreased IL-6, IL-8 and GM-CSF release to basolateral medium, whereas natural parvalbumins had no effect. CCL2 release was decreased after treatment with natural and mutant Cyp c 1, but not with natural Gad m 1. Carp matrix strongly increased basolateral release of IL-6 and IL-8. This effect was reversed by adding natural or mutant carp parvalbumin. In contrast, cod matrix had no effect on IL-6 and IL-8 release.

Conclusion
We observed internalization of fish parvalbumins by bronchial epithelial cells. Mutant carp parvalbumin induced a different pattern of cytokine release compared to the natural allergens which indicates a possible role of calcium binding to parvalbumins in their interaction with epithelial cells.

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Human Neutrophils Serve As Antigen-Presenting Cells For Allergen-Specific Effector T-Cells

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Keywords: Neutrophils, Antigen-Presenting Cells, Late-Phase, T-Cell

Introduction
Late-phase reactions (LPR) of IgE-mediated allergy are infiltrations of eosinophils, neutrophils and allergen-specific T-cells. As human neutrophils express MHC class II molecules upon stimulation with certain cytokines they might act as antigen-presenting cells (APC) in LPR. However, their ability to process antigen is still unclear. Therefore, we assessed whether human neutrophils internalize, process and present the major birch pollen allergen Bet v 1 to allergen specific T-cells.

Methods
Neutrophils isolated from the peripheral blood of allergic donors were cultured in the presence of GM-CSF, IL-3 and IFN-gamma and analyzed for their life span and the expression of HLA-DR, CD80 and CD86, by flow cytometry. Bet v 1 labelled with pHrodo was employed to determine its internalization by neutrophils and monocytes. Microsomal proteases were isolated from either cell type and the degradation of Bet v 1 was monitored over time by SDS-PAGE and quantified by ELISA. The resulting proteolytic fragments were sequenced by mass spectrometry. Bet v 1-specific T-cell clones generated from birch-pollen allergic donors and specific for relevant epitopes of the major birch pollen allergen were incubated with highly pure (>99%) neutrophils or monocytes pulsed with Bet v 1. After 48 hours, T-cell proliferation and cytokine response were assessed.

Results
A cocktail of cytokines significantly prolonged the lifespan of neutrophils and significantly upregulated expression of HLA-DR. The expression of CD80 was only marginally enhanced and CD86 was not detected. Neutrophils also effectively internalized and rapidly processed Bet v 1. In contrast to endolysosomal proteases from monocytes, neutrophilic proteases started to degrade Bet v 1 within seconds and digested it completely within 3-6 hours. The Bet v 1-derived peptides resulting from endolysosomal proteolysis were similar between neutrophils and monocytes and contained the most relevant T-cell epitopes of Bet v 1. Neutrophils pulsed with Bet v 1 induced proliferation of all T-cell specific for various epitopes distributed over the entire amino acid sequence of Bet v 1. The clones produced a similar cytokine profile as observed with monocytes. Nevertheless, monocytes were the more potent antigen-presenting cells.

Conclusion
Our results provide evidence for a potential role of neutrophils as APC in allergic LPR. Since they lack relevant costimulatory molecules we are currently investigating whether they induce anergy in allergic-specific T-cells.
**P27 AdipoR1+ Regulatory T-Cells Are An Important Source Of IL-10: Implications During Allergic Inflammation?**

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**Keywords**: Adiponectin, AdipoR1, Regulatory-T Cells, Allergic Inflammation, IL-10

**Introduction**
Adiponectin has an important potential anti-inflammatory role in allergic asthma. It attenuates allergen induced airway inflammation in mice, and induces the secretion of interleukin (IL)-10 via its adiponectin receptor 1 (AdipoR1). We showed recently that AdipoR1 is expressed on murine regulatory T-cells (Tregs), nevertheless, AdipoR1 expression on human Tregs, and its function is still unknown. The aim of this study was to evaluate the AdipoR1 expression on human Tregs and the effect of allergic inflammation on their IL-10 production.

**Methods**
Heparinized blood samples were obtained from healthy volunteers. Foxp3⁺ Tregs were defined as Helios⁺ and Helios⁻ cells, and AdipoR1 expression was assessed by flow cytometry. CD4⁺ T cells were isolated from buffy coats, and cultured under Th2-like cytokine milieu in presence or not of adiponectin. IL-10-producing Helios⁺ and Helios⁻ AdipoR1⁺ Tregs were identified using intracellular flow cytometry and levels of IL-10 in culture supernatants were analyzed by ELISA.

**Results**
AdipoR1 was expressed on human circulating Foxp3⁺ Tregs (6.4% of CD4⁺ CD25⁺ T cells), mainly on Helios⁻ cells (5% Helios⁻ vs 1% Helios⁺, P<0.01). Helios⁺ AdipoR1⁺ Tregs contained high frequency of IL-10-intracellular cells compared to Helios⁻ AdipoR1⁺ Tregs (35% vs 15%, respectively, P<0.01). Adiponectin treatment or Th2-like conditions did not change intracellular expression of IL-10 in both Helios⁺ and Helios⁻ AdipoR1⁺ Tregs. However, cells cultured under Th2-like conditions in presence of adiponectin displayed increased frequency of IL-10-intracellular in Helios⁺ but not in Helios⁻ AdipoR1⁺ Tregs. Interestingly, AdipoR1⁺ non-Tregs showed increased frequency of IL10-intracellular in response to adiponectin but no under Th2-like or combined conditions. Adiponectin augmented IL-10 concentration in supernatants of CD4⁺ T cells cultured under Th2-like conditions, whereas individual treatments of adiponectin or Th2-like conditions did not alter IL-10 levels.

**Conclusion**
These findings suggest that adiponectin is a key regulator of mediated-IL-10 anti-inflammatory function of Tregs in response to asthmatic/allergic inflammation.
O09 T Follicular Helper Cells; Janus Cells Of Chronic Allergic Airway Disease

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Keywords: T Follicular Helper Cells, Adaptive Immunity, Allergic Disease, Chronic Disease

Introduction
T\(_{\text{FH}}\) (T follicular helper cells) are a specialised subset of CD4\(^+\) T cells, which are important for the generation of both protective and pathogenic antibody responses. In the context of allergic airway disease, T\(_{\text{FH}}\) are indispensable for the development of allergen specific IgE. However, their contribution to the overall allergic disease phenotype is controversial, with both protective and pathogenic roles being described. To date current models of studying T\(_{\text{FH}}\) and allergy have focused on using prime and boost models of allergic disease, rather than chronic exposure models with clinically relevant common aeroallergens.

Methods
To assess the T\(_{\text{FH}}\) response induced during allergic airway disease BALB/C mice were chronically exposed to inhaled HDM (house dust mite extract) for up to 5 weeks. To determine the impact of impaired T\(_{\text{FH}}\) responses, CD4\(^{\text{cre-}}\)Bcl\(-6^{\text{fl/fl}}\)T\(_{\text{FH}}\) KO (T\(_{\text{FH}}\) knockout) or WT (littermate controls) mice were exposed to HDM for 5 weeks.

Results
T\(_{\text{FH}}\) appeared in the mediastinal lymph nodes of HDM treated animals within 1 week of allergen exposure and in the lungs after 3 weeks. T\(_{\text{FH}}\) accumulated at both sites with continued allergen challenge consistent with the emergence of GC (germinal centre) B cells and allergen specific IgE and IgG1 from 3 weeks onwards. T\(_{\text{FH}}\) loss during allergic airway disease development resulted in complete ablation of GC B cell responses and absence of allergen specific IgE and IgG1. Concomitant with the lack of allergen specific immunoglobulin, T\(_{\text{FH}}\) KO mice displayed increased lung and airway eosinophilia, Th2 cells and airway hyper-responsiveness compared to WT controls.

Conclusion
Chronic HDM exposure resulted in local and systemic T\(_{\text{FH}}\) responses. Loss of T\(_{\text{FH}}\) responses was associated with impaired antibody responses but enhanced Th2 based inflammation. Together this implicates T\(_{\text{FH}}\) as both critical drivers and regulators of distinct aspects of chronic allergic airway disease.

O10 Peripheral Blood Innate Lymphoid Cells Of Patients Suffering From Severe Pulmonary Inflammation Display An Activated Phenotype

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**Keywords:** Innate Lymphoid Cells, Asthma, COPD

**Introduction**
In recent years the importance of innate lymphoid cells (ILCs) in the regulation of immune responses has become evident. These cells are enriched at barrier surfaces and can rapidly respond to pathogens by producing cytokines and interacting with other cell types. Murine studies have defined ILCs as tissue resident cells that exert their function locally. However, these cells are also present in the circulation and elevated numbers of ILC2s have been found in the blood of asthma patients, whereas in COPD an increase in peripheral blood ILC1s was reported.

**Methods**
We isolated ILCs from the blood of severe asthma patients and patients suffering from COPD and compared the expression of CD45RA, RO, CD62L, CD69 and KLRG1 on all ILC subsets to those from healthy controls. The expression of these markers was also analysed on tissue ILCs. The effects of cytokine stimulation of the regulation of these markers were studied.

**Results**
Here we report on the presence of activated ILCs in the blood of severe asthma patients and patients suffering from COPD. The ILC2s and ILC3s in the blood of severe asthma patients express CD45RO instead of CD45RA and lost CD62L and KLRG1 (for ILC2s) expression, indicative of an activated state. The same was observed for ILC1s in the blood of COPD patients. In this manner these cells resembled their tissue counterparts. Interestingly, in mild to moderate asthma patients we did not observe this phenotype change. In vitro we were able to reproduce this activated phenotype upon culture of naïve ILCs with subtype specific stimulating cytokines.

**Conclusion**
Taken together this is indicative that in severe forms of pulmonary inflammation there is also a systemic component which affects the ILCs. We propose that the activation state of peripheral blood ILCs can be used as a marker for disease severity.

**O11 Human Rhinoviruses Infect And Activate B Cells**

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**Keywords:** B Lymphocytes, Human Rhinovirus

**Introduction**
Infections with human rhinoviruses (HRV) are highly associated with asthma exacerbations and an increased risk of developing asthma later in life. Many cell types play a role during HRV infection, including epithelial cells lining the airways.
and lymphocytes, such as CD4+ T cells. However, the role the role of B cells during HRV infection has not been clear and is focused in the present study.

**Methods**

B cells were purified from peripheral blood mononuclear cells (PBMC) using magnetic-activated and fluorescent-activated cell sorting. Cell proliferation was assessed using proliferation dyes for flow cytometry. Viral RNA was measured in these cells using quantitative PCR. Antibodies and cytokines produced by B cells were measured using ELISA and multiplex assays.

**Results**

When PBMC from non-allergic individuals were stimulated with HRV16, a virus dose-dependent proliferation of B lymphocytes was observed. Blocking the surface receptor for RV16 (ICAM-1) reduced the B cell proliferation. UV-irradiated virus did not induce any proliferation in B cells. Blocking the endosomal activity of Toll-like receptors by chloroquine during HRV-stimulation decreased proliferation and viability of the B cells. A change in antibody production towards elevated IgM was observed, when cells were stimulated with HRV, but not UV-HRV. B cells produced only low levels of inflammatory cytokines in parallel cultures. Positive (+) and negative (-) strands of viral RNA of RV16 were detected by quantitative PCR. Virus load stayed constant in the B lymphocyte compartment over a period of seven days, while it decreased in PBMC.

**Conclusion**

This study shows that HRV can infect B cells. For infection, ICAM-1 is used while signaling via endosomal receptors leads to proliferation of B cells. The demonstration of increased positive and negative HRV strands suggests that RV is entering and also actively replicating inside B cells, suggesting that B cells could act as a natural reservoir of RV for chronicity.

**O12 Role Of T-Lymphocytes In Rhinovirus-Induced Asthma Exacerbations**

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**Keywords**: CD4, CD8, RHINOVIRUS, ASTHMA, TRANSCRIPTOME

**Introduction**

Asthma is a chronic respiratory disease and acute attacks or exacerbations can be triggered by rhinovirus (RV) infection. A malfunction in the adaptive immune response is believed contribute to this process. T-helper cells are essential components of the adaptive immune response, however their role in asthma exacerbations remains uncertain.

**Methods**

In this study we have used multi-color flow cytometry and microarray techniques to characterise the phenotype and transcriptional profile of CD4+ T-cells in Broncho-alveolar Lavage (BAL) from Asthmatic (n=11) and Healthy (n=12) individuals prior to (Baseline) and Day 3 (D3) and Day 8 (D8) after experimental RV infection.
Results
Infection increased the total number of BAL cells in Healthy but not in asthmatic subjects and CD4+ and CD8+ populations (D3 and D8) in both groups. D3 after infection revealed down-regulation of activation signalling pathways (e.g. NF-Kb) and pro-apoptotic pathways (e.g. Rac). On D8, asthmatics down-regulated multiple genes encoding histone proteins (e.g. HIST1h) whilst up-regulated Nitric Oxide/Reactive Oxygen Species signalling pathways and Th2-related genes (STAT6, IL-4R). In the CD8+ population, EIF2 and mTOR signalling pathways were down-regulated and several KIR genes overexpressed on D3 and D8. Moreover, we identified novel genes (e.g. BATF, GZMB) whose expression profiles correlated positively with the severity of RV-induced symptoms.

Conclusion
The characterisation of T-lymphocytes in BAL during RV-induced exacerbation in this study showed a deep defect on: 1) activation/proliferation pathways in the CD4+ subset and 2) protein synthesis-associated pathways in CD8+. Furthermore we revealed the expression of genes not previously implicated in asthma that could help us to clarify the pathology of RV induced exacerbations.

Saturday, 28 January 2017
Oral Abstract presentations
17:50 - 19:30 Allergens and Allergy Mechanisms

O13 Basophil Activation As A Measure Of Allergy Sensitivity To Peanut During Treatment By Omalizumab

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Keywords: Basophil, Basophil Activation, Allergy, Allergy Sensitivity, Allergy Treatment

Introduction
Treatment with omalizumab has shown a positive effect on food allergies, but no dosages are established. Basophil allergen threshold sensitivity (CD-sens) can be used to objectively measure omalizumab treatment efficacy and correlates with the outcome of Double Blind Placebo Controlled Food Challenge to peanut.

Methods
Severely peanut allergic adolescents (n=23) were treated with omalizumab for 8 weeks and CD-sens was analysed before and after. Based on whether CD-sens was suppressed after 8 weeks, the patients either were subject to a peanut challenge or received 8 more weeks with increased dose of omalizumab, followed by peanut challenge or another 8-week cycle of omalizumab. IgE and IgE-antibodies to peanut and its components were analysed before treatment.

Results
After individualized omalizumab-treatment (8-24 weeks) all patients continued with an open peanut challenge with no (n=18) or mild (n=5) objective allergic symptoms. Patients (n=15) needing an elevated omalizumab-dose (ED) to supress CD-sens, had significantly higher CD-sens values at baseline 1.49 (0.44-20.5)
compared to those (n=8) who managed with normal dose (ND) 0.32 (0.24-5.5) (p<0.01). Median ratios for Ara h 2 IgE-ab/IgE were significantly higher in the ED group (17 %) compared to the ND group (11 %).

Conclusion
Individually dosed omalizumab, monitored by CD-sens, is an effective and safe treatment for severe peanut allergy. The ratio of IgE-ab to storage protein Ara h 2/IgE as well as CD-sens to peanut may predict the need of a higher omalizumab dose.

O14 Identification And Immunological Characterization Of Novel Allergens In Polistes Venom

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Keywords: Polistes, Hymenoptera, Venom, Basophil-Activation, Mass-Spectrometry

Introduction
Allergies due to venoms of hymenoptera can cause severe anaphylaxis. In the last years, progress of component-resolution advanced the differential diagnosis of honeybee and wasp venom allergic patients. The discrimination between clinically relevant sensitization to venom of different wasp species, especially between *Vespula* and *Polistes* species, is still challenging. Both species live side to side in Mediterranean regions and the US, but with *Polistes dominula* being an invasive species, *Polistes* venom allergy is likely to evolve in northern regions of Europe. Amongst others, the diagnostic challenge is due to extensive cross-reactivity between the venoms. Additionally, for *Polistes dominula* only a small fraction of venom components is known. In this study, *Polistes* venom was analyzed for additional allergens, which were subsequently characterized in detail regarding their potential to trigger allergic reactions.

Methods
*Polistes* venom was extensively analyzed by mass spectrometry and de novo peptide sequencing following 1D or 2D gel electrophoresis. Identified components were cloned from venom gland mRNA and expressed in insect cells. The resulting purified proteins, together with their homologues of different hymenoptera species, were characterized by immunoblotting and assessed for IgE cross-reactivity. Moreover, their capacity to activate basophils of either honeybee or wasp venom allergic patients was evaluated.

Results
Newly identified *Polistes* venom components and homologues from other hymenoptera species were successfully produced in Sf9 insect cells and thus were
devoid of cross-reactive carbohydrate determinants. The analysis of sera from honeybee, Vespula and Polistes venom allergic patients revealed extensive IgE cross-reactivity between homologous proteins, independent of glycosylation. Additionally, basophil activation tests could reveal comparable cross-reactivity. This indicates the presence of shared IgE epitopes, probably in conserved regions of venom proteins.

**Conclusion**
The detailed mass spectrometry analysis of Polistes venom led to yet unknown venom components and serologic as well as cellular tests demonstrated their importance in Polistes venom allergy. However, as the newly identified proteins show high homology on amino acid level with homologous allergens of other hymenoptera species, component-resolved analyses are affected by extensive immunological cross-reactivity.

**O15 INFLUENCE OF CONFORMATIONAL AND LINEAR IgE EPITOPES ON ARA H 2 SPECIFIC IgE-BINDING**

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**Keywords**: Recombinant Ara H 2, Conformational IgE Epitopes, Linear IgE Epitopes, Ara H 2 Specific IgE-Binding

**Introduction**
Ara h 2 is the most important peanut allergen. It is stable against proteolysis and thermal denaturation. Little is known about the role of conformational versus linear IgE epitopes of this molecule.

**Methods**
An Ara h 2 mutant, mtAra h 2 (lacking the surface exposed loops that contain most linear IgE epitopes), and the wild-type protein (wt) were expressed in the baculovirus insect cell system. The proteins were purified from the cell culture supernatants and purity of the proteins was verified by SDS-PAGE. Aliquots of wt, mt and natural (n) Ara h 2 were reduced with dithiothreitol and alkylated with iodoacetamide. Physicochemical characteristics were determined by mass spectrometry, N-terminal sequencing and CD spectroscopy. IgE-binding was tested by direct and inhibition ELISA using ten sera of peanut allergic patients.

**Results**
Mass spectrometry and N-terminal sequencing of mt, wtAra h 2 and nAra h 2 yielded masses corresponding to the predicted sizes and the correct N-termini. CD spectroscopy revealed the characteristic alpha-helical structure of the proteins. The complete reduction of all three reduced and alkylated proteins was also confirmed by CD spectroscopy. In direct as well as inhibition ELISA, allergic patients’ sera revealed a 20-50% reduced IgE-binding to the mutant compared with wt and nAra h 2. Upon reduction, wtAra h 2 revealed patient-specific
decreases in IgE-binding. Relative amounts of IgE-binding to reduced wtAra h 2 (containing mostly conformational IgE-binding epitopes) and the native mtAra h2 (containing mostly linear IgE-binding epitopes) showed a high extent of patient dependent variability. The reduced and alkylated mutant showed almost no IgE-binding.

**Conclusion**
These results indicate that both conformational and linear IgE-binding epitopes are important for Ara h 2 specific IgE-binding. Relative contributions of linear and conformational epitopes to Ara h 2 allergenicity are variable among patients with peanut allergy.

Supported by the Austrian Science Fund doctoral program W1248-B13 (Doctoral Program Molecular, Cellular and Clinical Allergology, MCCA).

**O16 Molecular Signaling For The Inhibition Of Allergic Sensitization And Induction Of Spectral T Helper Phenotypes By Alum/CpG-ODN Formulation**

**Ricardo Wesley Alberca-Custódio**, Eliane Gomes, Fernanda Nunes, Denise Fonseca, Luciana Mirotti, Momtchilo Russo
University of Sao Paulo, Sao Paulo, Brazil

**Keywords**: Allergy, TLR, Lung Inflammation

**Introduction**
Alum adjuvants are considered a T helper (Th)2 adjuvants while agonists of toll-like receptors (TLR) are viewed as Th1/Th17 adjuvants. Combining alum with TLR adjuvants might dampen the pro-Th2 activity and improve the effectiveness of vaccines formulations. Using the OVA model of lung inflammation, we found that sensitization with the synthetic TLR9 agonist(CpG) adsorbed to alum inhibited the development of lung Th2 allergic responses without shifting towards a Th1 pattern, through MyD88 signaling in myeloid cells. Notably, sensitization of IL-10-KO mice with OVA/alum/CpG resulted in a Th1 lung inflammation. Conversely, in IL-10/IL-12-KO mice resulted in a Th17 lung inflammation. We conclude that OVA/alum/CpG sensitization signalling via IL-10 molecules results in non-polarized immunity, and in absence of IL-10 or IL-10/IL-12, results respectively in Th1 and Th17 immunity.

**Methods**
8-week-old C57BL/6 and deficient(KO) mice were sensitized on days 0 and 7 subcutaneously with 4ug of ovalbumin(OVA) adsorbed to 1.6 mg of Al(OH)3 (alum) or 10ug CPG(oligonucleotide ODN1925) adsorbed onto OVA/alum. On days 14 and 21, mice were intra-nasally challenged with OVA(10ug). Samples were collected on day 22.

**Results**
Administration of Ova/Alum/CpG during sensitization on WT mice attenuate lung inflammation by the reduction of total cells(p<0.01) and eosinophils(p<0.01), without the increase of neutrophils(p>0.05). This results were supported by the reduction in lung T cells IL-5+ cells without the increase in IFNy+/IL-17+, levels of Ova-specific IgE(P<0.05) were reduced with an increase in IgG2c(P<0.05), histological analysis demonstrated an inhibition of lung infiltrate(p<0.05) compared with OVA/Alum. This inhibition was abrogated in
MyD88-KO(p>0.05), but not in RAG mice reconstituted with T and B cells from MyD88-KO(P<0.05).
This inhibition was sustained in IL-12p40/IFNγ-KO, type 1 or 2 interferon receptors(P<0.05).
In IL-10 KO mice, CpG reduced lung eosinophils(p<0.05) but increased neutrophils(p<0.05), with an increase in lung T cells IFNγ+ but not IL-17+.
In IL-10/IL-12 KO mice, CpG reduced lung eosinophils(p<0.05) but increased the neutrophils(p<0.05), with an increase in lung T cells IL-17+ but not IFNγ+.

**Conclusion**
In conclusion, Alum/CpG formulation decrease IgE and increase IgG2c and could induce spectral adjuvant activities ranging from non-polarized to Th1/Th17 immunity. Therefore, Alum/CpG might be of potential use in anti-allergic treatments.

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**Saturday, 28 January 2017**
21:00 - 22:00 Poster session II
**Topic 4 - Allergens and Allergic Inflammation: Poster P28 - P38**

**P28 Characterization Of Major Parietaria Pollen Allergen Par J 2 Obtained By Insect Cell Expression And Its Advantages For Diagnosis Of Allergy.**

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**Keywords**: Allergy, Allergens, Parietaria Pollen, Par J 2

**Introduction**
The weed *Parietaria judaica* is one of the most important pollen allergen sources in the Mediterranean area and certain areas of the UK, Australia, California and Eastern Europe.
Par j 2 the major *Parietaria* allergen has a molecular weight of 11.3 kDa, belongs to the family of lipid-transfer proteins and is recognized by more than 80% of *Parietaria*-allergic patients.
Aim of this study was to express and purify a correctly folded recombinant Par j 2 molecule which mimics the structural and immunological features of the natural allergen.

**Methods**
Recombinant Par j 2 was expressed in baculovirus-infected insect cells as well as in *Escherichia coli* and purified by affinity chromatography. Both proteins were characterized by SDS-PAGE, mass spectrometry (MALDI MS) size exclusion chromatography, circular dichroism (CD) and ELISA.

**Results**
Recombinant soluble Par j 2 expressed in baculovirus-infected insect cells was characterized by SDS-PAGE under reducing and non-reducing conditions. It
migrated as single band of approximately 14 kDa and the molecular mass determined by MALDI MS matched the mass calculated according to the amino acid sequences of the protein. Size exclusion chromatography showed that both, the insect cells and *E. coli* expressed recombinant proteins, occurred mainly as monomeric forms and also contained an oligomeric form of approximately 60 kDa. The analysis by CD showed that insect cell-expressed rPar j 2 assumed mainly α-helical structure whereas bacterially-expressed rPar j 2 contained mainly unordered species. When IgE reactivity of the recombinant Par j 2 proteins were compared with natural Par j 2 by ELISA using sera from Parietaria allergic patients from Mediterranean region and Austria, we found that insect cell-expressed Par j 2 showed higher IgE reactivity than *E. coli*-expressed Par j 2 and equally well as natural Par j 2.

**Conclusion**

Our results thus show that the eukaryotic expression of Par j 2 in insect cells yielded a folded recombinant protein with superior IgE reactivity over *E. coli* expressed Par j 2. The insect cell-expressed Par j 2 can now be used to study the three-dimensional structure of the allergen and for IgE-based diagnostic testing for identifying Parietaria allergic patients.

_The work is supported by the PhD program DKW1248-B13 MCCA, funded by the grant F4605 (FWF)._
in *Pichia pastoris* yeast was synthesized and used to express the recombinant protein. Recombinant and natural Ole e 7 were immunologically compared by WB and ELISA.

**Results**

rOle e 7 synthetic cDNA has been produced in *P. pastoris* with a final yield of 1mg/L of cell culture. After rOle e 7 purification and extensive immunological analysis, we confirmed that the recombinant allergen resembled most of the allergenic and antigen properties of the natural allergen isolated from pollen.

**Conclusion**

rOle e 7 spanned the immunological properties of the natural allergen. The recombinant allergen will permit the analysis and characterization in detail of this protein and might be used for a more effective clinical diagnosis of sensitized patients.

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**P30 INFLUENCE OF CONFORMATIONAL AND LINEAR IgE EPITOPES ON ARA H 2 SPECIFIC IgE-BINDING**

Angelika Tscheppe1, Dieter Palmberger2, Christian Radauer1, Merima Bublin1, Christine Hafner2, Chiara Palladino1, Barbara Gepp1, Nina Lengger1, Vanessa Mayr1, Reingard Grabherr2, Heimo Breiteneder1

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**Keywords:** Recombinant Ara H 2, Conformational IgE Epitopes, Linear IgE Epitopes, Ara H 2 Specific IgE-Binding

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**Results**

Mass spectrometry and N-terminal sequencing of mt, wtAra h 2 and nAra h 2 yielded masses corresponding to the predicted sizes and the correct N-termini. CD spectroscopy revealed the characteristic alpha-helical structure of the proteins. The complete reduction of all three reduced and alkylated proteins was also confirmed by CD spectroscopy. In direct as well as inhibition ELISA, allergic patients’ sera revealed a 20-50% reduced IgE-binding to the mutant compared with wt and nAra h 2. Upon reduction, wtAra h 2 revealed patient-specific
decreases in IgE-binding. Relative amounts of IgE-binding to reduced wtAra h 2 (containing mostly conformational IgE-binding epitopes) and the native mtAra h2 (containing mostly linear IgE-binding epitopes) showed a high extend of patient dependent variability. The reduced and alkylated mutant showed almost no IgE-binding.

**Conclusion**

These results indicate that both conformational and linear IgE-binding epitopes are important for Ara h 2 specific IgE-binding. Relative contributions of linear and conformational epitopes to Ara h 2 allergenicity are variable among patients with peanut allergy.

Supported by the Austrian Science Fund doctoral program W1248-B13 (Doctoral Program Molecular, Cellular and Clinical Allergology, MCCA).

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**P31 Increased Type-2 Inflammation In Asthma With Fixed Airflow Obstruction**

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**Keywords:** Asthma, S-ECP, S-Periostin, FeNO, U-EDN

**Introduction**

Long-term complications in asthma are airway remodeling and fixed airflow obstruction. Airway inflammation is believed to play a crucial role in these changes. There is increased interest in markers for the broader measurement of type-2 inflammation in order to better understand the inflammatory process. We analyzed four proposed markers of type-2 inflammation in relation to fixed airflow obstruction (fixed-AO) in asthma subjects.

**Methods**

A total of 483 individuals from the Swedish GA2LEN study, aged 17-76 years, were included and divided into asthmatics without (n=405) and with (n=78) fixed-AO, defined as a postbronchodilator FEV1/FVC < lower limit of normal. Markers of inflammation measured were serum periostin (S-periostin) (elevated defined as ≥75 ng/mL), urinary eosinophil-derived neurotoxin (U-EDN) (elevated >60.3 mg/mol creatinine), fraction of exhaled NO (FeNO) (elevated ≥25 ppb), and serum eosinophil cationic protein (S-ECP) (elevated ≥20 µg/mL). Asthma symptoms during the past year were self-reported. Chi-squared and t-tests were used to compare the levels of inflammatory markers and asthma symptoms between the groups. Thereafter, multiple logistic regressions were done with elevated markers and symptoms as outcomes, adjusted for sex, age, atopy, smoking, study center, use of inhaled corticosteroids and antileukotrienes, BMI, age of asthma onset and COPD.

**Results**
Levels of U-EDN and S-ECP were higher in asthma with fixed-AO than in asthma without fixed-AO in univariate analyses, whereas there was no difference in the levels of S-periostin and FeNO. In the multiple logistic regression models, fixed-AO was related to elevated S-periostin (OR: 2.2 (95% CI: 1.1-4.4 ), U-EDN (OR: 3.1 (1.6-6.2)) and S-ECP (OR: 2.9 (1.4-6.0)), but not to elevated FeNO (OR: 0.75 (0.40-1.4)). The fixed-AO group reported breathlessness at night more often than the group without fixed-AO. This difference disappeared in the multiple logistic regression models.

**Conclusion**

U-EDN and S-ECP are increased in asthmatics with fixed-AO compared to asthmatics without fixed-AO. Elevated S-periostin related to fixed-AO in the adjusted model, whereas FeNO was not related to FAO and the groups had only minor differences in symptoms. This could indicate that despite a similar clinical picture in terms of symptoms, increased type-2 inflammation relates to fixed-AO. Further studies are warranted to examine whether controlling type-2 inflammation might prevent fixed airflow obstruction.

**P33 A Critical Role Of Mast Cells In The Inflammatory Mechanism Of Experimental Allergic Enteritis**

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**Keywords**: Allergic Enteritis, Mast Cells, CCR8, CCL1

**Introduction**

The complexity of food allergies is expressed in multiple clinical phenotypes including allergic asthma, atopic dermatitis, anaphylaxis, and allergic enteritis. To develop curative immunotherapy of food allergies, it is crucial to understand the pathogenic mechanisms for each clinical phenotype. However, the pathological mechanism of allergic enteritis (AE) is still not well known, partly due to difficulty in access to inflammatory tissues.

**Methods**

In order to elucidate cellular and molecular mechanisms of AE, we established a murine model of AE by intraperitoneal sensitization with ovalbumin (OVA, egg white allergen) plus ALUM and feeding with egg white (EW) diet.

**Results**

In this model, BALB/c mice develop inflammatory features, e.g. accumulation of eosinophils and goblet hyperplasia in small intestines, which are similar to those in AE patients. Microarray analysis of inflammatory tissues of AE mice showed upregulation of gene expression of CC chemokine receptor (CCR) 8 and its chemokine ligand 1 (CCL1) as well as the Th2 cytokine IL-4 in the jejunum. Leukocyte trafficking is controlled by tissue-specific expression of chemokines and chemokine receptor expression on the leukocyte surface. Since it has been suggested that the main source of CCL1 are mast cells, we set up a hypothesis that mast cells play a crucial role in induction of AE. To verify it, we used KitW-sh/KitW-sh mice (mast cell deficient mice) and CCR8 knockout
(CCR8KO) mice on BALB/c background. In KitW-sh/KitW-sh mice, the concentration of CCL1 and the accumulation of eosinophils in small intestines were lower compared to wild type mice, although goblet hyperplasia was still observed. In CCR8KO mice, inflammatory response was shifted to an increased number of neutrophils than eosinophils.

**Conclusion**
The results suggest that mast cells play a crucial role to recruit eosinophils into small intestines via axis of CCR8 and CCL1 and hence induce AE. Our study will provide the basis to establish a novel anti-inflammatory strategy for treatment of AE.

**P34 Induction Of Functional Foxp3+ Regulatory T Cells And Galectin-9 By Oral Immunotherapy In Combination With A Fructo-Oligosaccharide Supplemented Diet In A Murine Model**

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**Keywords:** Oral Immunotherapy, Fructo-Oligosaccharides, Mast Cells, Short Chain Fatty Acids, Galectin-9

**Introduction**
In addition to a prebiotic effect, non-digestible oligosaccharides exert a direct effect on the intestinal epithelium and immune cells. Recent studies showed that fructo-oligosaccharides (FOS) increase the efficacy of oral immunotherapy (OIT) in a mouse model for cow’s milk allergy, however, the mechanism is unknown.

**Objective:** Investigating the effect of OIT+FOS on the effector response and the process of tolerance induction.

**Methods**
Female C3H/HeOuJ mice (5-6 weeks old, n=8/group) were sensitized to the cow’s milk protein whey (20 mg in PBS, intragastrically (i.g.) with cholera toxin (15 μg) once a week for 5 weeks (d0-d28). The mice received a diet with 1% FOS or a control diet from d35-d70. OIT (10 mg in PBS or PBS alone) was provided 5 days a week for 3 weeks (d41-d59). Intradermal (i.d.) and i.g. challenges were performed to measure the acute allergic response. Serum, bone marrow, caecum content, small intestines and mesenteric lymph nodes (MLN) were collected at d50, d63 and d70. Spleen-derived T cell fractions (whole spleen, CD4+CD25- and CD4+CD25+) were transferred to naïve recipient mice at d70. The recipients were sensitized and challenged as described for the donor mice.

**Results**
Early induction of CD4+CD25+foxp3+ Tregs was observed in the MLN of the OIT+FOS group compared to FOS alone (d50). After OIT (d63), Treg levels in the MLN decreased in the OIT+FOS mice. It was hypothesized that Tregs moved to the lamina propria, but no difference was observed. Transfer of Tregs from OIT+FOS mice protected recipients against anaphylaxis upon challenge, suggesting functional protection. OIT+FOS increased butyric acid levels in the caecum after 3 weeks (d63), while at d70 galectin-9 was increased in the serum. Intragastric challenge resulted in reduced mast cell degranulation in OIT+FOS mice. Interestingly, preliminary research showed that mast cells cultured from
bone marrow of FOS and OIT+FOS mice have reduced expression of c-Kit, FcεRI and showed reduced activation.

**Conclusion**
This study shows that OIT+FOS results in an early induction of functional Tregs and a reduction of mast cell degranulation upon challenge. The latter may be caused by inhibition of mast cell activation by galectin-9 and/or butyric acid. Moreover, the effect of FOS on bone marrow suggests possible epigenetic changes reducing development of mast cells. Further research is needed to investigate if this approach may be of potential value to treat food allergies.

**P36 Impact Of Free Fatty Acids Binding On The 3D Structure And Allergenic Activity Of The Major Peach Allergen Pru P 3**

**Pawel Dubiela**\(^1\), Roberta Aina\(^1\), Piotr Humeniuk\(^1\), Sabine Geiselhart\(^1\), Merima Bublin\(^1\), Dominika Polak\(^1\), Rebecca Del Conte\(^2\), Francesca Cantini\(^2\), Stefano Alessandri\(^3\), Barbara Bohle\(^1\), Tomasz Borowski\(^4\), Karin Hoffmann Sommergruber\(^1\)

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**Keywords**: NsLTP, Pru P 3, Lipids, Interaction, 3D Structure

**Introduction**
Non-specific lipid transfer proteins (nsLTPs) are relevant plant food allergens e.g. from peach (Pru p 3). They share a conserved fold with an internal cavity. Different lipid–protein complexes showed that the tunnel adapts its volume while binding a broad range of hydrophobic molecules. The aim of the study was to investigate the specificity of Pru p 3 - ligand interaction. Furthermore, we investigated the ability of lipid binding to affect the tertiary structure of the allergen and its IgE binding activity.

**Methods**
Pru p 3 was purified from peach peel and subsequently expressed in *Pichia pastoris*. Binding of lipids to Pru p 3 was monitored by fluorescent based ANS assay and W-LOGSY (Water-Ligand Observed via Gradient Spectroscopy). Furthermore, molecular dynamic analysis (MD) was applied to explore the nature of interaction between nsLTP and tested ligands. The impact of lipid binding on the allergenicity of the protein was investigated by ELISA and BAT.

**Results**
Due to pre-incubation of Pru p 3 with lipids a concentration dependent reduction of ANS binding was observed. Pru p 3 incubated (1:1; 1:10) with stearic acid showed 27% and 39% of ANS fluorescence reduction respectively, compared with Pru p 3 without lipids. For oleic acid (1:1; 1:10) reduction was 57% and 77%, respectively. Pru p 3 preferably bound unsaturated free fatty acids that was confirmed with W-LOGSY. MD suggests changes in protein structure due to binding to certain ligands. Interaction between oleic acid and Pru p 3, moved the C-terminal loop out towards the surface of the molecule, while the same region of Pru p 3 with other ligands is closer to the core of the molecule. Pre-incubation of oleic acid with Pru p 3 significantly increased IgE binding in ELISA and BAT assay as compared to stearic acid and allergen alone.
Conclusion
In this study, we observed differences in the binding capacity of Pru p 3 to the free fatty acids. MD simulation showed that interaction between Pru p 3 and tested ligands can lead to conformational changes that increased allergenic activity of nsLTP in vitro. The capacity of lipids to modify the conformation of nsLTPs may help us to understand how such interactions affect the allergenic potential of individual protein.

**P37 Mast cell-derived Extracellular Vesicles Induce Innate Immunity**

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Krefting Research Centre, Gothenburg, Sweden

**Keywords:** Exosomes, Vesicles, Innate Immunity

**Introduction**
Cell releases various secretory molecules including various cytokines and membrane bound extracellular vesicles (EVs) with various RNA, DNA and proteins in fluid and tissue as well. EVs are shown to have transfer of biomarker potential and ability to transfer biological function to recipient cells. In this part of our study we aim to evaluate the immunological signaling activated by nucleic acid present in mast cell derived EVs.

**Methods**
EVs from mast cells (HMC1.2) were isolated by differential ultra-centrifugation protocol. Isolated EVs were subjected to various nuclease, floated on optiprep and downstream analysis of zeta potential and particle number were made using nano-particle tacking technology. Functional analysis of uptake and signaling activation were performed on human mesenchymal stem cells (hMSC), macrophage cells.

**Results**
Our study demonstrates that nuclease sensitive nucleic acids were present on the surface of EV isolates from mast cell lines. Association of EV nucleic acid was revealed by increase of zeta potential and particle number upon nuclease treatment indicating its aggregation. Additionally, cytoplasmic nucleic acid traces were found in recipient cells. Activation of innate immune signaling was observed by activation of nucleic acid sensor and secreted cytokines

**Conclusion**
We define that surface association of nucleic acid EVs and that could assist its uptake by recipient cells and activate innate immune signaling.

**P38 Eosinophil Cationic Protein And Eosinophilia In The Clinical Setting Of Urticaria**

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**Keywords:** Eosinophil Cationic Protein, Urticaria-Like Lesions, Infections

**Introduction**
Urticaria is a heterogenous entity with increased prevalence: up to 20% of the world's population presented with an urticaria-like eruption once in their life. The lesions usually disappear in less than 24h and even faster with antihistamines. The
vascular reaction could be modulated by eosinophils and their products, chief among these being the eosinophil cationic protein (ECP), recognized as a marker of eosinophilic inflammation in asthma and other diseases.

**Methods**
A retrospective study was performed using the Allergology Clinic patients' records, selecting cases with urticaria-like cutaneous lesions during 2013-2016. The recommended set for laboratory tests included complete blood count, ECP level, diaminoxidase (DAO) level, stool exam, nasopharyngeal culture and investigation of other causes for skin eruptions (focused on ENT, dental, gastrointestinal and urinary infections). Patients were also recommended follow-up consults.

**Results**
A set of 121 patients met the selection criteria (64 of male sex, 57 of female sex). Patients were analyzed by 3 age groups with rather different diagnostic patterns (0 to 4 years, dominant atopic dermatitis; 5 to 17 years, over 17 years with an increased prevalence of parasitosis, angioedema, and dermographism as well as decreased prevalence of food sensitization). A score was built, based mainly on the severity of cutaneous changes, and analysed versus relative ECP and relative eosinophil levels. A moderate level of correlation was detected between ECP relative level (as multiple of upper normal limit) and the score on one side, and the relative eosinophilia (in % of the WBC count) on the other side (Spearman rho=0.475 p<0.0001 and 0.401 p=0.0006 respectively). There was no significant correlation between the score and relative eosinophilia (rho=0.061 p>0.05).

**Conclusion**
1. There is age-related variability of skin changes induced by immunoinflammatory mechanisms, the most striking case being atopic dermatitis, specific for young age, as well as food allergies with decreasing trend towards adulthood.

2. The eosinophil's role in inflammation is revealed rather from its activation status (indirectly determined from ECP level) than from peripheral eosinophilia. The latter does not have a significant use in estimating the clinical severity of the investigated pathological entities.

3. Although food sensitization and infections are frequent comorbidities, their pathogenic mechanisms do not traditionally involve ECP.

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**Saturday, 28 January 2017**
21:00 - 22:00 Poster session II
**Topic 5 - Adaptive Immunity: P39 - P48**

**P39 Immune Cell Subsets, Cytokine And Cortisol Levels During The First Week Of Life In Neonates Born To Preeclamptic Mothers**

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**Keywords:** Cortisol, HLA-DR, Memory T Cell, Preeclampsia, Preterm Neonate
Introduction
Preeclampsia (PE) is characterized by a systemic maternal inflammatory response and reduced immune tolerance towards the developing fetus. Yet, little is known about the impact of maternal PE on the fetal and neonatal immune system. We investigated the prevalence of distinct immune cell subsets along with plasma cortisol and cytokine levels in preterm newborns of PE mothers during the first week of life and compared them to preterm neonates born from pregnancies not complicated by PE.

Methods
Cord blood and peripheral blood samples on the 1st, 3rd, and 7th postnatal (PN) days of life were collected from 14 preterm infants affected by PE and 14 non-PE pregnancies. We measured plasma cortisol and cytokine levels with immunoassays and also assessed the prevalence of T, natural killer (NK) and dendritic cell (DC) subsets and activation markers using flow cytometry.

Result
The prevalence of CD4+ T lymphocytes and CD4+HLA-DR+ T cells was significantly lower in preterm infants of PE mothers on PN day 3 compared to controls. The prevalence of memory T cells was significantly higher in PE on PN day 7. The prevalence of CD8+CXCR3+ cells was significantly lower in PE on PN days 1 and 7. CD8+CD69+ T lymphocytes had a lower prevalence on PN days 0 and 1 in preterm neonates born to PE mothers. Myeloid DCs had a lower prevalence on PN days 1 and 3 in PE neonates.

MCP-1 and IL-4 had significantly higher levels on all 3 postnatal days in neonates of PE mothers, while cytokine levels were generally lower at birth compared to controls. Cortisol levels were lower in PE neonates on day 1 and 7, respectively.

Conclusion
Our observations show that PE pregnancies are associated with altered newborn immune status during the first week of life as represented by altered immune phenotype and plasma cytokine and cortisol levels.

P40 Th17 Lymphocytes In Children With Chronic Obstructive Lung Diseases

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Keywords: Childhood Asthma, Severe Asthma, Cystic Fibrosis, Th17 Lymphocytes, IL-17A

Introduction
The pathogenesis of bronchial asthma (BA) and other chronic obstructive pulmonary diseases such as cystic fibrosis (CF) is widely believed to involve Th17 lymphocytes. However, there is still a small number of investigations regarding children. We aimed to assess the Th17 in children with BA and CF.

Methods
We enrolled 32 children at mean age 10 ± 3 years (4 – 17 years) distributed in the following groups: with BA (n = 20; 10 with severe and 10 with moderate BA),
Analyses of Th17 cells in peripheral blood were performed by a 4-color FacsCalibur flow cytometry (Lise-Wash Protocol with the following fluorescence-labeled antibodies: anti-CD3-FITC, anti-CD4-PerCP, anti-CD161-AlexaFluor 647, anti-CCR6-PE) and the concentration of IL-17A (pg/ml) was measured by ELISA.

**Results**

Patients with BA had a significantly higher percentage of Th17 lymphocytes (12.40 ± 1.16) compared to those with CF (7.64 ± 0.87) (p = 0.0035) and compared to the healthy children (7.25 ± 0.45) (p < 0.008). Stratifying the BA group according to the severity, we found higher levels of Th17 in patients with severe BA (p=0.03). Patients with moderate asthma had Th17 values close to those in CF children. We found that patients with better control of asthma had values of Th17 closer to those with CF (p = 0.98) than children with poor control (p = 0.000) (post hoc, Bonferroni correction). We did not find a significant difference in the serum concentrations of IL-17 in different groups of children (p> 0.05), nor significant correlation between the levels of Th17 in peripheral blood and the corresponding concentration of IL-17 in serum (p> 0.05).

**Conclusion**

The number of Th17 cells is significantly increased in the peripheral blood of children with severe BA compared to the children with moderate BA. Severe BA patients could have possible benefit from the future target therapies.

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**P41 AAT As An Immunoregulatory Mediator In Chronic Airway Disease And Asthma**

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**Keywords**: Th17, Immune Regulation, Asthma

**Introduction**

Alpha 1 antitrypsin (AAT) is a serine protease inhibitor which protects the lungs from proteases such as neutrophil elastase (NE). Recent data indicates that AAT may exert immune regulatory functions, of particular interest is its ability to increase IL-10 production. Preliminary data has shown that the AAT gene is one of the most highly upregulated genes in CD4+ T cells treated with vitamin D. Furthermore, there is a significant positive correlation between circulating levels of vitamin D and levels of AAT in the airways (unpublished data). Our laboratory has shown that steroid resistant asthma has a pro-inflammatory phenotype with high IL-17A and low IL-10 and this axis can be modified to a more anti-inflammatory phenotype with calcitriol (active vitamin D) supplementation. We therefore hypothesise that the NE/AAT axis controls the balance of anti-inflammatory and pro-inflammatory cytokines and that vitamin D can induce AAT as an intermediate in the modification of this balance.

**Methods**

To study the effect of AAT deficiency (AATD) on the immune system we isolated peripheral blood mononuclear cells (PBMCs) from AATD patients, COPD patients and healthy controls. PBMCs were either stimulated with PMA and ionomycin directly ex-vivo or following 7 days of culture with αCD3 and IL-2 in the presence or absence of 1,25(OH)_{2}D_{3}. Cells were stained for surface markers and intracellular cytokines for flow cytometric analysis. PBMCs were co-cultured with
polymorphonuclear cells (PMNs) for 24 hours. Following stimulation cells were again stained for cytokine expression.

**Results**

PBMC cultures from PiZZ patients showed significantly increased frequency of IL-17+ IL-22+ \((p=0.034)\) and IL-17+ IL-17F+ \((p=0.0127)\) cells compared to healthy controls, suggesting a lack of immune modulation. This could be significantly reduced with vitamin D treatment in culture. An increase in the pro-inflammatory cytokine IL-17A was also seen in PiZZ PBMCs directly ex-vivo. To investigate what is driving this increase in inflammatory cytokines PBMC and PMN co-cultures were performed. At 24h of culture increasing PMNs showed a trend towards an increase in IL-17+ cells.

**Conclusion**

Overall, these data indicate a lack of immune modulation in AATD which may be overcome with vitamin D. This lends weight to the hypothesis that the NE/AAT axis controls the balance of pro- and anti-inflammatory cytokines and that this may be controlled by vitamin D.

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**P42 Role Of Lingo4 In Th17 Differentiation**

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**Keywords:** Lingo4, IL-17, Th17, RORC, Lentiviral Transduction

**Introduction**

T helper (Th) cells encompass a complex network of different subsets. As one of them Th17 cells, named by their signature cytokine IL-17, were first described over a decade ago. They play important roles in host defense against different pathogens as well as in the pathogenesis of several inflammatory conditions. Moreover, accumulating evidence suggests that the human Th17 cell subset is a heterogeneous population with different functions, but the regulatory factors driving this heterogeneity remain elusive.

Whole genome expression arrays of human T cell clones revealed an increased expression of Lingo4 in IL-17 producing cells, a gene not associated with Th17 cells so far or T cells at all.

**Methods**

Whole genome expression arrays revealed Lingo4 as a new gene in association with human Th17 cells. Findings were validated by real-time PCR on mRNA level. Further, lentiviral mediated overexpression was used to investigate the impact of Lingo4 on Th17 differentiation.

**Results**

Different immune and non-immune cells were tested for Lingo4 mRNA expression. PBMCs and in vitro differentiated Th17 cells showed increased levels of Lingo4 mRNA. Further, kinetic of in vitro Th17 differentiation showed a time-dependent increase of Lingo4 expression with oscillatory dynamics. Of note, expression of RORC, the key transcription factor of Th17 cells, was tightly correlated with Lingo4 expression, whereas secretion of IL-17 was anticyclical. Lentiviral transfection of naive T cells with Lingo4 achieved a strong overexpression of Lingo4 on mRNA level, but not on protein level, whereas in other cell types overexpression increased Lingo4 also on protein level.
**Conclusion**

The time-dependent increase of Lingo4/RORC and the anticyclical course of IL-17 secretion may indicate strict regulation of Lingo4/RORC expression dependent on IL-17 level. Further, the fact that Lingo4 mRNA but no protein was detected after overexpression suggests a strong T cell specific regulation of Lingo4 that prevents its translation into a protein.

Our data show for the first time specific Lingo4 expression in Th17 cells and clearly indicate an association of Lingo4 and Th17 differentiation. The regulatory role of Lingo4 during the differentiation process and contribution to Th17 heterogeneity has to be addressed in future studies.

**P43 DNA Methylation Changes During T Helper Cell Differentiation.**

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**Keywords**: Epigenetics, Asthma, TCF7, IL6R

**Introduction**

Asthma is a heterogeneous disease partly due to the variety of T helper cells contributing to its pathology. During an immune response, naïve T helper cells differentiate into distinct lineages including Th1, Th2 and Th17 cells. Each lineage contributes to different asthma phenotypes due to their distinct transcriptomes, which are regulated by the cells' epigenetic architecture. One such epigenetic mark is DNA methylation, which can be altered by environmental conditions and changes during the differentiation of Th1 and Th2 cells at key cytokine loci, such as IFNγ and IL-13 respectively. In this study, an unbiased approach was used to discover other genes with altered DNA methylation status after differentiation.

**Methods**

Primary human naïve T cells were differentiated into Th1 and Th2 cells. DNA methylation of these cells was studied using the Infinium HumanMethylation450K and MethylationEPIC BeadChip arrays (Illumina, San Diego, USA). Gene expression was studied using the HumanHT-12 v4 Expression BeadChip array (Illumina).

**Results**

Consistent with previous studies, there was profound demethylation of CpG (cytosine-phosphate-guanine) dinucleotides at IFNγ and IL-13 promoters after Th1 and Th2 differentiation respectively. After Th1 differentiation 71 genes were significantly upregulated or downregulated by two-fold or greater (p<0.05 with FDR) and 4400 CpG dinucleotides had a 20% or greater change in absolute methylation. TCF7 and IL6R, amongst other genes, displayed a significant reduction in RNA levels and showed a marked increase in DNA methylation at numerous CpG dinucleotides clustered around their respective transcriptional start sites. ChIPseq experiments reveal that the IL6R locus is also marked by H3K27me3 in differentiated Th1 cells suggesting epigenetic silencing of the locus. Since IL6R signalling is involved in the establishment of the Th17 lineage our data begin to explain how phenotypic restriction is established in these differentiating T cells.

**Conclusion**

This study shows that changes in DNA methylation are not restricted to the key T helper cell cytokines with DNA methyltransferases being recruited to the TCF7 and IL6R loci during Th1 differentiation. Understanding the epigenome of T helper cells will contribute to the endotyping and studying of lineage plasticity in asthma.
**P44 Eradication Of Unwanted CD8+ T-Cell Responses To Abacavir Via The Modification Of The 6-Amino Cyclopropyl Moiety.**

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**Introduction**

**Background:** Treatment with the reverse transcriptase inhibitor abacavir is associated with hypersensitivity reactions. This reaction is mediated by CD8+ T-cells and is seen exclusively in individuals carrying the HLA-B*57:01 risk allele. Abacavir alters the conformation of the antigen binding cleft which causes an alteration of self-peptides displayed on the cell surface. This altered peptide repertoire is hypothesized to initiate a T-cell response in hypersensitive patients.

**Methods**

**Aims:** To examine the potential for synthesis of novel compounds that maintain antiviral activity but do not evoke a CD8+ T-cell response

**Methods:** Forty three abacavir analogues have been synthesized with modifications to the cyclopropyl group, which were categorised into two groups. Abacavir responsive CD8+ T-cell clones were generated from healthy donors positive for the HLA-B*57:01 risk allele. The cytokine secretion profile from these clones was measured by culturing the clones in the presence of autologous antigen presenting cells and abacavir or abacavir analogues using an IFN-γ Eli-Spot assay. Antiviral activity of the analogues was assessed using a range of established assays. In silico docking studies were carried out to find potential binding orientations of the abacavir analogues within the F-pocket of HLA-B*57:01.

**Results**

Major histocompatibility complex class I restricted CD8+ T-cell clones proliferated and secreted IFN-γ in response to abacavir. Several analogues in the first group displayed promising antiviral activity without triggering CD8+ T-cell responses. Molecular docking studies of these analogues to HLA-B*57:01 demonstrated a relationship between the protein binding and the T-cell response. These findings led to the synthesis of another 18 compounds with further modifications to the cyclopropyl group, namely azetidine derivatives. This series was subjected to additional antiviral activity and T-cell response assays and several molecules were shown to be devoid of T-cell activity, whilst maintaining a favourable antiviral profile.

**Conclusion**

These studies demonstrate that modification of the cyclopropyl group of abacavir has the potential to generate compounds with favourable antiviral activity, which are devoid of the unwanted T-cell responses. This represents an exciting paradigm to the design of safe antiviral drugs removing the need for personalised medicine therapy regimes.

**P45 Amoxicillin-Protein Adducts Activate T-Cells In Patients And Volunteers.**

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**Keywords:** Amoxicillin, Hypersensitivity, Adduct, HSA, HLA

**Introduction**
Exposure to the antibiotic amoxicillin (AX) is associated with the development of a number of adverse drug reactions including liver injury and skin injury. AX specific T-cells have been detected in patients presenting with both liver and skin reactions, suggestive of an immune disease aetiology. These reactions do not occur in every drug administered patient therefore their origin may be attributed to the individual biology of a patient. This theory has gained credence with the discovery of a number genetic associations including the HLA DRB1*15:01-DQB1*06:02 haplotype. β-lactam antibiotics form drug-protein adducts that are postulated to activate T-cells from hypersensitive patients, following protein processing and the liberation of peptide epitopes. A number of AX-modified proteins have been found including AX conjugated with human serum albumin (HSA). However, as yet, a role for such adducts in the activation of T-cells has not been described. This study aimed to investigate the role of AX-protein adducts in the activation of a drug-specific T-cell response.

**Methods**
AX was incubated with HSA at 100:1 molar ratio for 24 hours and free AX was removed using ultrafiltration columns. LC/MS was conducted using an API 4000 or TripleTOF 5600 mass spectrometer (AB Sciex). AX-HSA was incubated with PBMCs from hypersensitive patients and healthy volunteers for two weeks to generate antigen-responsive T-cell lines. Subsequently, T-cells were cloned by serial dilution and repetitive mitogen stimulation. Well growing clones were tested with 1.5 mg/ml AX-HSA and a stimulation index above 1.7 was used to indicate drug specificity. This was replicated with free AX and also AX modified peptides. Clones were expanded and characterized in terms of cellular phenotype and cytokine release.

**Results**
AX-HSA was successfully generated *in vitro* and characterised using LC/MS to find a number of lysine residues modified including K190 and K195. Remaining free AX was quantified to be less than 0.5% in the purified adduct. A number of CD4+ and CD8+ AX and AX-HSA specific clones were generated from both patients and volunteers. Clones proliferated in a dose-dependent manner and secreted a mixture of Th1 and Th2 cytokines.

**Conclusion**
Free AX and AX-HSA adducts can activate T-cells in both patients and healthy volunteers. Thus, we are now conducting additional studies to identify the precise drug-modified peptide which is recognised by the T-cell and its relationship with the known HLA associations.

**P46 The Role Of Allergen-Specific IgG Antibodies In Allergen-Specific T Cell Responses**

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Keywords: Allergen-Specific Immunotherapy, Allergen-Specific IgG Antibodies

Introduction
Allergen-specific immunotherapy (AIT) is to date the only causative treatment for allergic disorders. A beneficial response in AIT-treated patients has been associated with the production of high levels of allergen-specific of IgG4 and IgG1 antibodies, which may compete with IgE for allergen binding. However, allergen-IgG complexes bind and cross-link Fcγ-receptors expressed on the surface of antigen-presenting cells. The aim of this study is to investigate whether allergen-IgG complexes modulate allergen uptake and presentation as well as the resulting T cell response.

Methods
The major grass pollen allergen Phl p 5 was expressed in E. coli, purified and used to generate allergen-specific T cell lines from patients with grass pollen allergy. Human Phl p 5-specific IgG1 and IgG4 monoclonal antibodies (mAb) presenting the same paratope were produced in CHO-K1 cells and purified. In a first approach, we employed neutrophils as antigen presenting cells as they constitutively express FcgRII and FcgRII as well as FcgRI upon stimulation with IFNg. In addition, their phagocytic activity has been shown to be modulated by IgG-opsonization of antigens.

Results
Phl p 5-specific T cell lines were incubated with autologous neutrophils pulsed with Phl p 5 plus/minus specific IgG1 and IgG4 mAb. We observed enhanced T cell proliferation in the presence of Phl p 5-specific mAb.

Conclusion
These results provide preliminary evidence that allergen-IgG1 and IgG4 complexes alter T cell activation by neutrophils. Currently, we are testing the effect of mAb on the uptake and processing of Phl p 5.

P47 Role Of T-Lymphocytes In Rhinovirus-Induced Asthma Exacerbations.

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Keywords: CD4, CD8, RHINOVIRUS, ASTHMA, TRANSCRIPTOME

Introduction
Asthma is a chronic respiratory disease and acute attacks or exacerbations can be triggered by rhinovirus (RV) infection. A malfunction in the adaptive immune response is believed contribute to this process. T-helper cells are essential components of the adaptive immune response, however their role in asthma exacerbations remains uncertain.

Methods
In this study we have used multi-color flow cytometry and microarray techniques to characterise the phenotype and transcriptional profile of CD4+ T-cells in Broncho-alveolar Lavage (BAL) from Asthmatic (n=11) and Healthy (n=12)
individuals prior to (Baseline) and Day 3 (D3) and Day 8 (D8) after experimental RV infection.

**Results**
Infection increased the total number of BAL cells in Healthy but not in asthmatic subjects and CD4⁺ and CD8⁺ populations (D3 and D8) in both groups. D3 after infection revealed down-regulation of activation signalling pathways (e.g. NF-Kb) and pro-apoptotic pathways (e.g. Rac). On D8, asthmatics down-regulated multiple genes encoding histone proteins (e.g. HIST1h) whilst up-regulated Nitric Oxide/Reactive Oxygen Species signalling pathways and Th2-related genes (STAT6, IL-4R). In the CD8⁺ population, EIF2 and mTOR signalling pathways were down-regulated and several KIR genes overexpressed on D3 and D8. Moreover, we identified novel genes (e.g. BATF, GZMB) whose expression profiles correlated positively with the severity of RV-induced symptoms.

**Conclusion**
The characterisation of T-lymphocytes in BAL during RV-induced exacerbation in this study showed a deep defect on: 1) activation/proliferation pathways in the CD4⁺ subset and 2) protein synthesis-associated pathways in CD8⁺. Furthermore we revealed the expression of genes not previously implicated in asthma that could help us to clarify the pathology of RV induced exacerbations.

**P48 Lymphocyte Proliferation Test: 3H-Thymidine Uptake Versus CFSE-Based Assay**
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**Keywords:** Lymphocyte Proliferation Test; 3H-Thymidine, CFSE

**Introduction**
Lymphocyte transformation test (LTT) is the most widely used test to detect T-cell sensitization to drugs *in vitro*, by measuring ³H-thymidine uptake of dividing cells. Although it has been in use for more than four decades, its sensitivity is limited. Carboxyfluorescein succinimidyl ester (CFSE) is a membrane permeating dye that binds the amino groups of cytoplasmic proteins with its succinimidyl-reactive group. When cells divide, CFSE-labeled proteins are similarly distributed between the daughter cells, thus halving cell fluorescence with each division.

The aim of this study was to compare the CFSE-based assay with the classical ³H-thymidine incorporation method and characterize the phenotype of the dividing cell populations.

**Methods**
In this study were included 33 subjects without immunodeficiency, 18 patients who presented with a clinical history of drug hypersensitivity reactions and 15 healthy individuals for control group. Peripheral blood mononuclear cells were used and cell cultures were performed for assessment of mitogenic and antigenic T cell proliferation, using phytohaemagglutinin and *Candida albicans* as stimuli, respectively. Stimulation indices by CFSE-based assays were calculated focusing on particular lymphocytes subsets defined by expressed cell membrane antigens, as well as complexity and fluorescence intensity of the staining.
Results
The CFSE-based assay employing flow cytometry allowed an evaluation of T cell responsiveness to both mitogen and antigen stimuli in every subject. However, the CFSE-derived values did not correlate well with the $^3$H-thymidine uptake, being the stimulation indices of CFSE method lowest than the classical one. Thus, our results revealed the potential of the CFSE analysis, by being able to determine the phenotype, generations of dividing cells and number of cells in each one.

Conclusion
The CFSE-based assay might provide an analysis on particular lymphocyte subsets, measuring expression of activation markers directly. It is also a non-radioactive, less labour intensive, cheaper and might be an alternative for the in vitro detection of drug sensitization. However, this work showed that sensitivity of the CFSE-based assay must be improved, with studies involving a larger number of subjects with well-defined phenotypes, in order to assess cut-off values of stimulation indices to stimuli with less antigenic potential as drugs.

Sunday, 29 January 2017
Oral Abstract presentations
09:20 - 11:00 Regulation of Allergic Inflammation

O17 Raw Cow’s Milk Prevents The Development Of Airway Inflammation In A Murine House Dust Mite-Induced Asthma Model

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Introduction
Numerous epidemiological studies show an inverse relation between raw cow’s milk consumption and the development of asthma. This protective effect seems to be abolished by milk processing. Evidence for a causal relationship is however still lacking and also direct comparisons between raw and heat treated milk are hardly studied. In the present study we therefore investigated the preventive capacity of raw milk and heated raw milk on the development of house dust mite (HDM)-induced allergic asthma in mice.

Methods
Six- to seven-week-old, male BALB/c mice were intranasally (i.n.) sensitized with 1 µg HDM or PBS on day 0, followed by an i.n. challenge with 10 µm HDM or PBS on days 7 to 11. In addition, mice were orally treated with 0,5 mL raw cow’s milk, heated raw cow’s milk (10 minutes, 80°C) or PBS three times a week throughout the study. At the end of the study (day 14), airway hyperresponsiveness (AHR) in response to increasing doses of methacholine was measured in order to assess lung function and bronchoalveolar lavage fluid (BALF) was examined to study the extent of airway inflammation. T helper (Th) cell subpopulations were quantified in lung cell suspensions using flow cytometry and chemokine and cytokine
concentrations were determined in lung homogenates and supernatants of ex vivo HDM re-stimulated lung cells.

**Results**

Sensitization and challenge with HDM resulted in AHR and pulmonary eosinophilic inflammation. Raw milk prevented both typical features of allergic asthma, whereas heated raw milk did not. Epithelial- and DC-derived mediators, IL-33, CCL20, CCL17 and CCL22, were significantly increased in the lungs of HDM-mice. Both milk types reduced the concentration of CCL17. Pulmonary concentrations of Th2 cytokines, IL-5 and IL-13 were also increased in HDM-mice, but only raw milk prevented this increase. Upon re-stimulation of lung cells with HDM, both raw and heated raw milk were able to significantly reduce the production of IL-4 and IL-13. The percentage of Th2 cells in lung cells suspensions was also significantly reduced by both milk types.

**Conclusion**

Raw cow's milk prevents the development of asthma in a murine HDM-induced allergic asthma model. Heat treated raw milk did not show this protective effect. Besides an abundant amount of epidemiological evidence, this study now also suggests a causal relationship between raw cow's milk consumption and the prevention of allergic asthma.

**O18 Antigens Of The Helminth Schistosoma Mansoni Suppress Allergic Airway Inflammation**

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**Keywords:** Allergic Airway Inflammation, Helminths, Schistosoma Mansoni, Lung Dendritic Cells,

**Introduction**

The helminth *Schistosoma (S.) mansoni* establishes chronic infections in the host and is a strong driver of T-helper type 2 (Th2) as well as regulatory immunity. Our group has previously demonstrated that chronic *S. mansoni* infection protects against experimental allergic airway inflammation (AAI) via bystander suppression. We hypothesize that during infection helminths actively secrete molecules which drive the development of regulatory immunity. We previously found that intraperitoneal administration of schistosome eggs, the parasites life cycle stage most recognized by the immune system, induced T- and B-cell interleukin (IL)-10 production in the spleen. Here, we aim to study the protective effect of eggs and one of its major glycoproteins, omega-1, in the context of AAI.

**Methods**

Mice were treated twice with 5000 *S. mansoni* liver eggs or 50μg recombinant, plant-expressed omega-1 by i.p injection prior to allergic sensitisation. AAI was induced by injection of 10μg ovalbumin (OVA) emulsified in alum, and subsequent challenge by repeated exposure to aerosolized OVA. All analyses were carried out 24h after the last challenge.

**Results**

Egg administration significantly inhibited inflammatory cell influx into the airways
in response to allergic challenge, dampened local Th2 cytokine (IL-5, IL-13) production and reduced the levels of circulating, OVA-specific IgE- and IgG1. The number of regulatory (CD4+ Foxp3+ CD25+) T (Treg) cells in the airways was reduced in egg-treated compared to untreated allergic mice, suggesting that local Treg cells do not play a major role in protection. Egg administration resulted in increased levels of schistosome-specific Th2 cytokines indicating a fully developed Th2 response to the eggs, but concurrently reduced levels of OVA-specific Th2 cytokines produced by splenic CD4+ T cells. Strikingly, egg treatment ablated the accumulation of inflammatory, monocyte-derived dendritic cells (DCs) in the lung, whereas conventional DC populations remained unaffected. Preliminary data indicate that also omega-1 is capable of suppressing AAI.

**Conclusion**
We believe that early events in response to egg injection are critical factors for the protective outcome, and we are currently investigating these in more detail, especially with regards to DC migration and recruitment to the airways. Taken together, we show that the administration of *S. mansoni* eggs, and potentially its major glycoprotein omega-1, can protect mice from experimental AAI.

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**O19 Cannabinoid Receptors CB1/CB2 Promote Anti-Inflammatory Responses In Dendritic Cells: Implications For Allergic Diseases**

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**Keywords:** Cannabinoid Receptors, Dendritic Cells, Allergic Diseases

**Introduction**
The endocannabinoid system has been shown to play different functions in the context of allergic diseases. In humans, the mRNA expression levels of the cannabinoid receptor 1 (CB1) were upregulated in allergic patients, but the functional significance of such observation remains elusive. Human dendritic cells (DCs) express CB1 and cannabinoid receptor 2 (CB2), but their actual role in allergy and the underlying immunological mechanisms are not fully understood. Therefore, the aim of this work is to study the role played by cannabinoids receptors in the regulation of human DCs as potential therapeutic targets for immunotherapy of allergic diseases.

**Methods**
THP1-XBlue cell line was used to monitor NFkB/AP-1 activation. Surface markers and cytokine production in human monocyte-derived DCs (hmoDCs) in the absence or presence of WIN55, 212-2 were analyzed by flow cytometry and ELISA, respectively. Allogeneic co-cultures of hmoDCs and naïve CD4+T cells, CFSE-dilution assays and real-time quantitative PCR were also performed. CB1 expression on DCs subsets was determined with a fluorescence chemical probe in peripheral blood mononuclear cells and tonsils from healthy donors by flow cytometry and confocal microscopy.

**Results**
We showed that THP1 cells and hmoDCs express CB1 and CB2. The CB1/CB2 agonist WIN55, 212-2 inhibits the TLR2-L-induced NFκB activation and the production of IL-8 in THP1 cells. Pharmacological inhibition experiments with specific antagonist for CB1 or CB2 demonstrated that both CB1 and CB2 contributed to this inhibition. WIN55,212-2 down-regulates the expression of HLADR, CD86 and CD83 as well as the production of IL-6, IL-8, and TNFα in hmoDCs stimulated with LPS without altering cell viability. Similarly, WIN55, 212-2 inhibits cAMP levels and NFκB activation induced by LPS in hmoDCs. Human DCs activated in the presence of WIN55, 212-2 promoted the generation of IL-10-producing T cells. Interestingly, our data with a specific chemical probe revealed that both human myeloid and plasmacytoid DCs from peripheral blood and tonsils express significant levels of CB1 on the cell membrane.

Conclusion
The cannabinoid receptor agonist WIN55,212-2 promotes the generation of tolerogenic human DCs with potential anti-inflammatory properties. These findings have important implications for future therapeutic strategies in the treatment of immune regulation-related diseases such as autoimmunity, cancer, organ transplantation, chronic infections, allergy and asthma.

O20 IL-5 Dependent Mechanisms In IL-33 Induced Bone Marrow Eosinophilia

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Keywords: IL-33, ILC2, IL-5, Bone Marrow, Eosinophilia

Introduction
Interleukin (IL)-33 plays important roles in the immunopathogenesis of allergy and asthma. It acts by promoting IL-5 and IL-13 secretion from IL-33 responsive type 2 innate lymphoid cells (ILC2s) and T helper type 2 (Th2) cells or directly activates eosinophils and mast cells. IL-5 is a crucial component in eosinophilic inflammation by controlling differentiation and survival of eosinophils in bone marrow as well as release and recruitment of eosinophils to the airways. ILC2s and Th2 cells are major producers of IL-5 in airways, however, the cellular source in bone marrow is less explored. The aim of this study was therefore to identify the major producers of IL-5 in bone marrow in IL-33 mediated eosinophilia.

Methods
We investigated IL-5 producing bone marrow cells using intracellular flow cytometry in a murine model of IL-33 induced airway eosinophilia.

Results
Intranasal IL-33 administration resulted in eosinophil infiltration in airways and increased eosinophils in bone marrow. Airway and bone marrow eosinophils were dramatically reduced in mice pre-treated with anti-IL-5 antibodies followed by intranasal IL-33, demonstrating that IL-33 elicits IL-5 dependent eosinophilia in vivo. Bone marrow progenitors (CD34+) and ILC2s but not Th2 cells produced IL-5 in response to intranasal IL-33 administration. This was accompanied by increased IL-33 receptor expression on ILC2s but not on CD34+ cells which displayed low expression of the IL-33 receptor.

Conclusion
Our initial observations suggest that IL-33 promotes minimal eosinophil hematopoiesis via direct interactions with bone marrow progenitors, and opens the possibility that it takes place via indirect activation of bone marrow ILC2s.