## 226. T-cell subsets

**P1827**

**Pulmonary dendritic cells from chronic obstructive pulmonary disease patients suppress lung immune responses through induction of regulatory T cells**

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Defective Th immunity is considered to be implicated in the enhanced vulnerability of Chronic Obstructive Pulmonary Disease (COPD) patients to lower respiratory infections and lung cancer. Tolerogenic dendritic cells (DCs) and regulatory T cells (Tregs) are critical in the suppression of Th immunity. Their role in COPD is elusive. We hypothesized that pulmonary DCs in COPD exhibit tolerogenic properties and suppress lung Th responses through induction of Tregs. $CD1c^+$ DCs and $CD3^+$ T cells were isolated from the lungs of COPD patients (n=17), smokers (n=16) and never-smokers (n=4). DC maturation prior and upon LPS exposure were examined. The effects of pulmonary DCs on lung Th cell responses and on the induction of Tregs were investigated. Pulmonary DCs from COPD patients and smokers express decreased levels of co-stimulatory molecules (CD40/86) compared to never-smokers at baseline ($p<0.01$). Upon LPS exposure, only DCs from COPD patients fail to upregulate costimulatory molecules. Pulmonary DCs from COPD patients induce decreased proliferation of autologous lung CD4+ and CD8+ T cells compared to DCs from smokers ($p<0.001$). CD4+ T cells treated with DCs from COPD patients, but not from smokers, express increased levels of the immunosuppressive cytokine IL-10 ($p<0.01$) and suppress Th responses in *in vitro* suppression assays. Our results reveal that lung DCs from COPD patients suppress lung immune responses through induction of Tregs. This novel immunoregulatory circuit has important clinical implications for the enhanced vulnerability of COPD patients to respiratory infections and lung cancer.
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**P1828**

**Regulatory T cells in healthy and asthmatic subjects challenged with rhinovirus**

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**Background:** Allergic asthma is characterised by an imbalance in Th2/Th1 response and elevated secretion of Th2 cytokines. Regulatory T cells (Tregs), expressing FoxP3, are thought to play a prominent role in the suppression of Th1 lymphocytes in asthma patients.
**Objectives:** The aim was to address whether the number of Tregs differ between asthmatic patients and healthy controls and how these numbers are affected by a Th1 response, i.e. a provocation with rhinovirus type 16 (RV16).
**Methods:** Mild allergic asthma patients (n=9) and healthy non-allergic controls (n=14) were inoculated with RV16 (10 $TCID_{50}$). One day before and six days after the provocation, blood was drawn and bronchoalveolar lavage (BAL) fluid was collected. Lymphocytes and peripheral blood mononuclear cells (PBMCs) were analysed by multi-colour flow cytometry to quantify the $CD4^+CD25^+FoxP3^+$ Tregs.
**Results:** Mild asthma patients had more $CD4^+CD25^+FoxP3^+$ Tregs ($6.1\pm0.7\%$) in peripheral blood compared to the healthy controls ($3.7\pm1.1\%$, p=0.003). FoxP3 was expressed in a larger proportion of $CD4^+$ cells in the BAL than in peripheral blood of both asthmatic ($7.9\pm3.0$ in BAL and $5.1\pm1.4$ in blood) and control subjects ($7.5\pm2.6$ in BAL and $3.9\pm1.6$ in blood), but no difference between the prevalence in BAL lymphocytes from both groups was observed. RV16 provocation did not affect the $CD4^+CD25^+FoxP3^+$ Treg numbers in blood and BAL in either patients with mild asthma or healthy controls.
**Conclusion:** There is a higher number of $CD4^+CD25^+$ cells expressing $FoxP3^+$ in peripheral blood of mild allergic asthma patients. Rhinovirus challenge did not have an impact on PBMC and BAL Treg numbers in both healthy and asthmatic individuals.

**P1829**

**T-regulatory cells blood content in patients with asthma exacerbation**

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**Background:** Regulatory CD4+CD25+ T cells are important components of the immune system homeostasis and impairment of their activity can cause autoimmune diseases and allergy.
**Objective:** The aim of this study was to evaluate the blood content of naturally occurring T-regulatory cells (nTreg) in patients with different periods of asthma.
**Methods:** 48 steroid naïve asthmatic adults with asthma exacerbation and 48 with asthma remission were randomly selected and 30 matched control subjects were included. All were submitted to detailed clinical history and examination, pulmonary function testing. Investigation of CD4+, CD25+, CD4+CD25hi-lymphocytes in blood was carried out by flow cytometry. nTreg were defined as a fraction of CD4+CD25+-lymphocytes with a high level of CD25 expression (CD4+CD25hi-cells).
**Results:** Subjects with asthma exacerbation had significantly lower values of CD4+CD25hi-cells than subjects with asthma remission (1,56 (1,14-1,97)% vs 5,90 (4,96-6,71)%; p<0,001) and controls (1,56 (1,14-1,97)% vs 7,40 (6,41-8,52)%; p<0,001). Subjects with asthma remission had also significantly lower values of CD4+CD25hi-cells than controls (5,90 (4,96-6,71)% vs 7,40 (6,41-8,52)%; p<0,001).
**Conclusion:** CD4+CD25hi-cells blood content is decreased in asthma in comparison with healthy subjects. Blood content of CD4+CD25hi-cells in asthma exacerbation decreases 3 fold and more in comparison with remission and can be predictor for asthma exacerbation.

**P1830**

**Effector and regulatory lymphocytes in asthmatic pregnant women**

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Asthma is one of the most common diseases that may complicate pregnancy. Asthma and pregnancy show bidirectional interactions with unknown immunological mechanisms. The aim of this study was to evaluate CD4+ regulatory T (Treg), natural killer (NK), NKT, invariant natural killer (iNKT), memory and naive $CD4^+$T lymphocytes in mild to moderate partially or well controlled persistant asthmatic pregnant patients.
The prevalence of lymphocyte subsets was identified by cell surface markers and intracellular FoxP3 staining, in healthy non-pregnant (HNP; N=15), healthy pregnant (HP; N=33), asthmatic non-pregnant (ANP; N=62) and asthmatic pregnant (AP; N=61) women. Data are given in median and quartiles.
Treg cell prevalence was higher in HP than in HNP subjects (7.82 (5.27-10.24) vs. 4.88 (1.82-6.19), respectively; p=0.0052) and showed a positive correlation with fetal birth weight (p=0.02), which was blunted in AP group. AP patients had lower Treg (5.53 (2.45-7.83)) and higher iNKT (0.04 (0.01-0.18)) cell numbers than HP subjects (7.82 (5.27-10.24) and 0.01 (0.0-0.03), respectively; all p<0.05) that might suggest compromised pregnancy induced immunotolerance caused by allergic inflammation. However, lower effector/memory and higher naive T cell prevalence observed in AP (30.60 (24.42-44.18) and 57.24 (46.34-67.06), respectively) than in ANP group (43.11 (32.52-55.38) and 48.18 (38.07-58.62), respectively) suggested immunosuppression exerted by pregnancy on asthma.
Pregnancy induced Treg cell proliferation is absent in asthmatic pregnancy that may interfere with normal intrauterine growth. However, pregnancy induced inhibition of asthmatic immune responses can also be detected in uncomplicated asthmatic pregnancy.

**P1831**

**Comparative characteristics of regulatory T cells populations between patients with bronchial asthma (BA) and COPD**

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**Background:** T-regulatory cells (T-reg) are critical for controlling the degree of immune response to various types of antigens. Possibly due to late or insufficient activation of individual subpopulations of T-reg accumulation of activated immunocompetent cells and intensity of inflammation in COPD and BA is excessive.
**Aim and objectives:** To investigate differences in subpopulations of T-reg in COPD compared to that in BA and healthy donors (HD).
**Methods:** The circulating percentage of CD4+CD25high and CD4+FoxP3+ T-reg in peripheral blood was estimated by the flow cytometry analysis. We included 60 patients with COPD (57,8±1,09 years old), 20 patients with BA (54,1±2,2 years old) and 17 HD (54,1±2,2 years old).
**Results:** It was shown that COPD is associated with the increasing of percentage number of CD4+CD25high T-reg (2,82±0,24%) in the peripheral blood in comparison to BA (1,87±0,47%, p = 0,004) and HD (1,56±0,24%, p = 0,005). The patients with BA had the decreasing CD4+FoxP3+ T-reg (2,57±0,60, p<0,001) compared to COPD (8,37±0,38, p < 0,001) and HD (8,10±0,48, p < 0,001).
**Conclusions:** We consider that the observed increase of circulating in peripheral blood CD4+CD25high T-reg in COPD patients might be markers of disease severity. Our study suggests an association between differences in subpopulations of T-reg in patients with BA and COPD with varying degrees of inflammation.

**P1832**

**IL-17 and Th17-cells as markers of disease progression in pediatric allergic diseases. A therapeutic approach in an "*in vitro*" models**

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Th17 cells and IL-17 play a role in allergy development and progression. We tested IL-17 in plasma (P), nasal wash (NW) and induced sputum (IS) from 12 healthy (HC), 14 intermittent asthma (IA) and 28 mild-moderate asthma (MA) with concomitant intermittent-rhinitis (IR, n=12) or concomitant persistent-rhinitis (PR, n=16); 2) intracellular CD3+IL-17+, CD3+RORγt+ in peripheral blood (PB) T-cells from IA and MA children. In vitro we tested the effect of Budesonide ($10^{-8}$M) and Formoterol ($10^{-8}$M) alone or in combination on 1) intracellular CD3+IL-17+, CD3+RORγt+ expression in PBT-cells from children with MA and PR (n=10); 2) on the IL-8 release from nasal and bronchial epithelial cell lines pretreated with and without an anti-IL-17 receptor antibody and stimulated respectively with NW and IS from children with MA and PR (n=6). We observed significantly increased levels of 1) IL-17 in P, NW and IS from children with MA compared with IA and HC; 2) intracellular CD3+IL-17+ and CD3+RORγt+ in PBT-cells from subjects with MA compared with IA as well as in children with MA and PR when compared with MA and IR. In vitro experiments showed that Budesonide with Formoterol treatment significantly reduced 1) CD3+IL-17+ and CD3+RORγt+ in PBT-cells from children with MA and PR; 2) the effect of IL-17 present in NW and IS from children with MA and PR on IL-8 release in nasal and bronchial epithelial cell lines.
IL-17 and Th17-cells are markers of disease progression in children with allergic MA and concomitant PR. Budesonide with Formoterol might be useful for a therapeutic approach to control IL-17 mediated allergic disorders.
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**P1833**

**Lung and blood Th1 and Th17 responses against mycobacterial antigens in patients with pulmonary sarcoidosis**

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Sarcoidosis is an inflammatory disorder characterized by granulomas most commonly affecting the lungs. The presence of mycobacterial antigens, and recently a specific protein, *M. tuberculosis* catalase-peroxidase (mKatG), in sarcoidosis tissue has been reported. Th1 cell responses against mKatG have been observed in sarcoidosis lung and peripheral blood cells. In the present study, IL-17 and IFNγ production were evaluated by ELISPOT after stimulation of bronchoalveolar lavage (BAL) fluid and peripheral blood cells with mKatG and *M. tuberculosis* PPD proteins. Two groups of sarcoidosis patients were compared: HLA-DR3 positive (good prognosis) versus HLA-DR3 negative (bad prognosis). We also did immunohistochemistry on bronchial biopsies for further characterization of cytokine expression. Both mKatG and PPD stimulation of BAL cells resulted in higher frequencies of cells producing IFNγ compared to IL-17. We did not detect any significant difference between BAL and blood regarding IL-17 secretion after stimulation with both mKatG and PPD, while significantly more BAL cells produced IFNγ in comparison to blood after stimulation with mKatG and PPD (p<0.05). The existence of $IL\text{-}17^+$ cells in the granulomas also supports the role of IL-17 in sarcoidosis. The observed Th17 responses against mycobacterial antigens could contribute to the inflammation in sarcoidosis, although they generally occur at lower frequencies than corresponding Th1 responses.

**P1834**

**Characterization of lymphocyte subsets in the lungs of smokers and patients with COPD**

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**Background:** COPD is characterized by chronic inflammation in the lungs. CD8+ T cells but in more severe disease also CD4+ T cells, have been implicated in the pathogenesis. T cell maturation entails distinct stages which can be identified based on their expression of the surface markers CD45RA and CD27. By combining these markers it is possible to distinguish naïve and memory/effector subpopulations. We investigated whether these T cell subpopulations in bronchoalveolar lavage (BAL) differ in COPD patients compared to smokers with normal lung function and never-smokers.
**Methods:** Bronchoscopy and BAL was performed on 24 never-smokers, 20 smokers with normal lung function and 20 COPD patients (14 smokers and 6 ex-smokers). The frequencies of major lymphocyte subsets and the differentiation status of CD4+ and CD8+ T cells were analyzed by flow cytometry.
**Results:** There were higher percentages of CD8+ T cells in smokers and CD56+ T cells in smokers and COPD patients compared to never-smokers ($p<0.001$). A higher frequency of CD27+CD45RA- cells and a lower frequency of CD27-CD45RA- cells was found in the CD4+ T cell population of COPD current smokers compared to never-smokers and COPD ex-smokers ($p<0.05$). Smokers with normal lung function had a higher percentage of CD4+CD27-CD45RA+ cells compared to never-smokers ($p<0.05$).
**Conclusions:** The higher percentages of cytotoxic T cells in smokers and COPD patients may be related to smoking-induced tissue damage. The increased percentage of central memory cells in COPD smokers suggests an ongoing immune response. Smoking cessation resulted in a normalization of CD4+ central/effector memory cells in BAL, indicating a reversibility of smoke-induced changes.

**P1835**

**TCR rearrangement in patients with eosinophilic lung disease**

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**Background:** Expansion of monoclonal population of T-cells with T-cell receptor (TCR) rearrangements and aberrant cell surface immunophenotype produce IL-5 and contribute to the pathophysiology of the lymphocytic variant of the hypereosinophilic syndrome (HES).
**Objective:** To characterise T-cell expansion in eosinophilic lung disease.
**Methods:** T-cell expression profile was analysed by flow cytometry and TCR clonal gene rearrangements were studied by PCR in 45 consecutive patients with peripheral blood eosinophilia (greater than $1.0\times10^9$/L) and eosinophilic lung disease. Patients with myeloid or lymphocytic variants of HES or any identified cause of eosinophilia were excluded.
**Results:** Clonal TCR rearrangements were detected in 11/45 patients (24%): 4/18 with hypereosinophilic asthma, 3/17 with Churg-Strauss syndrome, 3/9 with idiopathic chronic eosinophilic pneumonia (ICEP), and 1/1 with allergic bronchopulmonary aspergillosis. Seven patients had 2 clonal rearrangements or more. TCR rearrangements involved the TCRγ chain in 10 patients and the TCRδ chain in one patient with ICEP. Aberrant T-cell immunophenotype usually found in HES ($CD3^-CD4^+$, $CD3^+CD4^-CD8^-$, or $CD3^+CD7^-$, and known to produce IL-5, was not detected. No significant clinical or biological difference was found between patients with or without clonal TCR gene rearrangements. IL-5 serum level was normal in all patients.
**Conclusion:** Expansion of TCR-Vγ or TCR-Vδ populations are present in a proportion of patients with eosinophilic lung disease without aberrant cell-surface immunophenotype. Further study is needed to evaluate whether T-cell clonality might contribute to the pathophysiology of these conditions or is only a transient reactive phenomenon.

**P1836**

**Reduced expression of PPAR-alpha in bronchoalveolar lavage CD4+ T cells of sarcoidosis patients**

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**Background:** Sarcoidosis is a systemic inflammatory disease characterized by noncaseating granulomas affecting many organs, especially the lungs and intrathoracic lymph nodes. Activated CD4+ T cells with a type 1 cytokine profile are considered to be of central importance for the pathogenesis. The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that play important regulatory roles in numerous cellular processes, including inflammation. Three PPARs have been described, namely PPAR-alpha, -beta/delta and –gamma. They are expressed in many cell types, including macrophages and T cells. The purpose of this study was to investigate on the mRNA and protein level the expression of PPARs in lung cells of sarcoidosis patients.
**Methods:** Seventeen sarcoidosis patients and nine healthy controls underwent bronchoscopy with broncoalveolar lavage (BAL) whereafter CD4+ T cells and alveolar macrophages (AMs) were sorted by flow cytometry and subjected to real-time PCR analysis for mRNA expression of PPARs. Immunofluorescence staining of BAL cytospin slides with anti-PPAR antibodies was also performed.
**Results:** PPAR-alpha relative gene expression was significantly downregulated in CD4+ T cells of sarcoidosis patients, but no differences were observed with regard to T cell expression of the other PPARs. PPAR expression in AMs did not differ between patients and controls. No differences were observed between patients with or without Löfgren's syndrome.
**Conclusion:** The observed downregulation of PPAR-alpha in T helper cells may contribute to ongoing inflammation in pulmonary sarcoidosis via failure to repress proinflammatory genes.

**P1837**

**Killer cells in asthma with incomplete airflow reversibility**

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Asthma with complete airflow reversibility (ACR) represents the majority of asthma cases, however recent attention has focussed increasingly on a small subset of asthmatics that display incomplete airflow reversibility despite optimal treatment (AIR), also a feature of COPD. A growing amount of literature has linked the three main types of killer cells, namely CD8 T cells, Natural Killer (NK) and NKT cells, to both ACR and COPD due to their cytotoxic and immunoregulatory functions. We aimed to study the role of these cells in AIR. Absolute cell counting, cytotoxic mediator and receptor profiling, activation time course assays as well as functional cytotoxic experiments were performed to compare the number and function of killer cell subsets in the peripheral blood of AIR and ACR patients and healthy controls. Our data suggest both the number and cytotoxic function of these killer cells are reduced in the peripheral blood of AIR patients compared to ACR patients, as quantified by perforin/granzyme B expression and functional cytotoxic assays. This indicates killer cells may be recruited to the AIR lung and have a subsequent role in the disease pathogenesis, as has been shown in COPD.

**P1838**

**Collagen receptor α2β1 integrin is overexpressed on CD4 and CD8 peripheral blood T lymphocytes in long-lasting clinically stable asthma**

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**Background:** Recruitment of the inflammatory cells from blood to the airways in asthma is mediated by adhesive molecules – e.g. selectins and integrins. In cells trafficking the best known integrins are molecules containing α4 and β2 subunits. We hypothesized that also integrins α1β1 and α2β1 may play a role in asthma pathogenesis.
**Objective, material and methods:** The aim of the study was to assess expression of several very late antigen (VLA) integrin subunits (α1, α2, α4 and β1) on blood CD4 and CD8 T lymphocytes by flow cytometry in 25 adult atopic asthmatics (mild to moderate persistent asthma in a stable clinical condition) and 15 healthy controls.
**Results:** Expression of both α4 and β1 on CD4 T cells was significantly higher in asthma subjects than in controls. The α1 subunit was absent from blood lymphocytes. The α2 chain hardly detected on lymphocytes from healthy subjects was apparently present in asthmatics. Surprisingly, in subjects suffering from asthma for over 4 years (n=15) statistically significant overexpression of α2, α4 and β1 subunits on both CD4 and CD8 T cells was observed as compared to controls.
**Conclusion:** Expression of certain VLA integrin subunits on blood T cells may depend on asthma duration. In long-lasting disease α2, α4 and β1 subunits are overexpressed on both CD4 and CD8 T lymphocytes. The biological role of α2β1 integrin in asthma is unknown. This integrin has been described as a stimulator of collagen accumulation in the airways and in chronic disease may be at least in part, responsible for asthma airway remodelling.

**P1839**

**Different Th1, Th2, Th17 and Treg associated chemokine local pattern expression in lung in a murine model of allergic inflammation**

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We have reported that Th1, Th2, Th17 and Treg cells is differently distributed in the lung during airway allergic inflammation. T helper cells express different chemokine receptors.We hypothesize that Th1, Th2, Th17 and Treg cells distribution in the lung is a result by different local chemokine expression.

Ovalbumin (OVA)-sensitized C57BL/6 mice were exposed intranasally to OVA or PBS on five days and lung tissue was taken 24 h after final allergen exposure. Peribronchial (Pb), perivascular (Pv) and alveoli (A) tissue was selected from lung tissue sections using Laser Microdissection and Pressure Catapulting technology. Total RNA was extracted and each location was pooled from three OVA/OVA mice. Chemokine gene expression was performed using RT-PCR gene array. Tissue from whole lung tissue section of an OVA/PBS mouse was used as control and results were expressed as fold change from control.
Chemokines/ligands for Th1-associated receptors; CCR2 (CCL2, CCL5, CCL7) and CCR5 (CCL3, CCL4, CCL5) were increased mainly in A followed by Pv and Pb tissue, while CXCR3 (CXCL10) was increased in Pv followed by A and Pb tissue. In Th2-associated CCR8 (CCL8) was most highly induced Pv (21.3fold) followed by Pb and A tissue. In Th2/Treg-associated CCR4 (CCL17, CCL22) the pattern was A, Pv, Pb, while in Treg/Th17 CCR7 (CCL19) was increased mainly in Pv then in Pb but not in A tissue.
Allergen exposure increased gene expression of all Th1, Th2, Treg and Th17 associated chemokines in lung. However, the expression pattern shows differences in both tissue distribution and magnitude, arguing for a high complex local milieu that can regulate T-cell subsets distribution.

**P1840**

**T cell specific expression of a short splice variant of the tumor suppressor gene CYLD amplifies the development of allergic airway disease**

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Regulation of transcription factors like NF-κB decides about type and strength of developing inflammatory and immune responses. CYLD acts as a negative regulatory element of NF-κB activation, however the naturally occurring short splice variant $CYLD^{Ex7/8}$ (sCYLD) has positive regulatory properties. An exclusively expression of sCYLD leads to a hyperactive phenotype of dendritic cells (DC) and increased numbers of long living antibody secreting B cells. In the present study we investigated the function of sCYLD on T cells in a murine model of allergic airway disease. Following sensitization and challenge towards the model antigen Ovalbumin (OVA), wild type (WT) and animals with a T cell specific expression of $CYLD^{Ex7/8}$ (CyldFL*CD4cre) were analyzed for the induction of an allergic airway disease. 48 hours following the last challenge animals with exclusively T cells expressing sCYLD demonstrating increased numbers of eosinophils in BAL, enhanced inflammation in the lung and increased numbers of goblet cells in comparison to WT animals. Moreover the T cell specific sCYLD expression leads to increased secretion of total IgE and all classes of antigen specific immunoglobulines were clearly upregulated in comparison to sensitized and challenged WT. These data suggest that CYLD plays an important role in the regulation of T cell responses. Beside the modulation of DC and B cell the short splice variant of CYLD affects also T cells and seems to induce a hyper responsive T cell phenotype. This work provides a new few in T cell regulation and underlines the important role of CYLD and T cells for the induction of allergic responses.

**P1841**

**Apoptosis of peripheral blood lymphocytes in different periods of asthma**

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**Background:** Resistance of lymphocytes to apoptosis can cause decrease of these cells elimination from respiratory ways and play a key role in persistence of inflammation in asthma.
**Objective:** The aim of this study was to reveal the disturbances of blood lymphocytes apoptosis in different periods of asthma.
**Methods:** 25 steroid naïve asthmatic adults with asthma exacerbation and 25 with asthma remission were randomly selected and 20 matched control subjects were included. All were submitted to detailed clinical history and examination, pulmonary function testing. Peripheral blood lymphocytes (PBLs) were stimulated by phytoagglutinin (PHA) and cultured for 72 hours. Apoptosis of PBLs was determined by flow cytometry by evaluating of Annexin V expression for detection of early apoptotic cells (Annexin V positive-PI negative).
**Results:** Subjects with asthma exacerbation had significantly lower values of early apoptotic cells in blood than subjects with asthma remission (20,29±1,69% vs 41,65±2,38%; p<0,001) and controls (20,29±2,38% vs 43,02±2,58%; p<0,001). But there were no differences in values of early apoptotic cells in subjects with asthma remission and controls (41,65±11,91% vs 43,02±11,54%; p>0,05).
**Conclusion:** The value of early apoptotic cells in 72 hours cultures of stimulated by PHA lymphocytes is a sensitive marker for asthma exacerbation and remission and can be predictor for asthma exacerbation.

**P1842**

**Mechanism of long-lasting suppression against Th2 immune response in the lung by a novel antedrug TLR7 agonist**

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**Rationale:** To identify the mechanisms by which a novel antedrug Toll like receptor 7 (TLR7) agonist, AZ12441970, mediates the long-lasting suppression of Th2 immune responses.
**Methods:** Long-lasting efficacy of AZ12441970 against bronchoalveolar lavage (BAL) eosinophilia was investigated utilizing Ovalbumin (OVA)/Alum-sensitised mice or OVA-specific Th2 cells adoptively transferred into mice. The mechanism by which AZ12441970 mediated long-lasting efficacy was investigated utilizing T cell-deficient nude mice and several knock-out (KO) mice including type I IFN receptor KO mice, B cell KO mice and rag2 KO mice lacking B and T cells.
**Results:** Weekly lung doses of AZ12441970 for 6 weeks significantly inhibited BAL eosinophilia for at least 6 weeks following cessation of dosing. The long-lasting efficacy observed with the compound was largely abolished in type I IFN receptor KO mice, rag2 KO mice or nude mice, but maintained in B cell KO mice. T cells were shown to be involved in the mechanism of efficacy in a T cell reconstituted nude mouse model of allergic airways inflammation. Nose only exposure of AZ12441970 also resulted in long-term efficacy in the lung.
**Conclusions:** We show that intranasal dosing of AZ12441970 to achieve lung exposure in mice induces a long-lasting efficacy against Th2 immune responses in the lung. The efficacy in the lung is also achieved by nose only exposure of the TLR7 agonist. Furthermore, our mechanism studies demonstrate that the long-lasting inhibition of the Th2 immune responses with the compound is IFN-α- and T-cell-dependent. These data suggest that novel antedrug TLR7 agonists may have potential in the treatment of allergic disease.

**P1843**

**Repeated intranasal TLR7 stimulation reduces allergen responsiveness in allergic rhinitis**

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**Background:** The TLR7 agonist AZD8848 was developed as a treatment for allergic airway disease with the rationale of suppressing allergic (Th2-like) immune responses by triggering innate immune responses through TLRs. Intranasal administration of AZD8848 has demonstrated protection against allergen challenge in animal models of asthma and allergic rhinitis. AZD8848 is rapidly hydrolysed *in vivo* to a metabolite that is over 100-fold less active, to minimise the risk of undesirable systemic effects.
**Methods:** Five once-weekly intranasal doses of AZD8848 (60 μg) were given in a randomised, placebo-controlled, double-blind, parallel group clinical trial to patients with allergic rhinitis (AR) off season (NCT00770003). Nasal symptoms were recorded during a 7-day nasal allergen challenge period starting 24 hours post last dose of AZD8848 to assess clinical effect. Biomarkers of TLR7 activation and plasma exudation were monitored in plasma or nasal lavage. Safety, tolerability and pharmacokinetics were also monitored.
**Results:** Successful stimulation of TLR7 was indicated by reversible reductions in blood lymphocytes and increased plasma levels of IL-1Ra 24 hrs after administration of AZD8848. Some patients reported transient flu-like symptoms, as expected based on mechanism of action. AZD8848 reduced total nasal symptoms of allergic rhinitis as recorded 10 minutes after allergen challenge on days 5, 6, 7 and 8 after the last dose of AZD8848. Levels of $\alpha_2$-macroglobulin in nasal lavage were also reduced by AZD8848.
**Conclusion:** Repeated intranasal administration of AZD8848 activated TLR7 and this resulted in a sustained reduction in responsiveness to allergen in patients with AR.

**P1844**

**Farm dust decreases Th2 driven allergic airway inflammation in mice: A role for airway TLR2 and TLR4?**

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Exposure to farm dust is known to decrease allergic inflammation in an ovalbumin

mouse model for asthma. However, the underlying mechanisms of this protection are not known. With the focus on the role of epithelium in this process, we investigated whether farm dust also protects against allergic airway inflammation in a more clinically relevant house dust mite (HDM) asthma mouse model.

Dust from different farms in Germany was collected and pooled. BALB/c mice were exposed intranasally 4 times/week for 5 weeks to PBS or farm dust (1 mg/ml, 50 μl/day), followed by PBS or HDM (2.5 mg/ml, 10 μl/day). IgE levels, inflammatory cells, cytokines (CC chemokine ligand (CCL)20, thymic stromal lymphopoietin (TSLP) and Th2) and Toll-like receptor (TLR)2 and TLR4 expression on epithelial cells were assessed.

Farm dust+HDM exposed mice had lower levels of HDM-specific IgE in serum, eosinophilic inflammation, and Th2 cytokines in lungs than HDM exposed mice. Expression of TLR2 and 4 in epithelium, and CCL20 and TSLP levels in lung tissue were lower in farm dust exposed than HDM exposed mice. Additionally, we observed less spindle shaped CD68+ cells beneath the epithelial basement membrane in farm dust exposed mice.

Lower expression of TLR2 and 4 in epithelium after farm dust exposure may explain the lower levels epithelial cell-derived cytokines CCL20 and TSLP. These lower CCL20 and TSLP levels could have prevented the recruitment of antigen presenting cells as evidenced by the spindle shaped CD68+ cells, as well as the skewing towards a Th2 response. Hence, lower expression of TLR2 and 4 may contribute to the observed protection against HDM-induced allergic inflammation in farm dust-exposed mice.