365. Asthma: basic science and clinical studies

P3260
Increased sputum IL-17 and neutrophils in asthmatic patients after climbing to extreme altitude
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Introduction: A group of 18 well-controlled asthmatic patients were evaluated before/during/after an expedition to the Aconcagua mountain (6900m).
Methods: Symptoms, spirometry and FeNO values were obtained before, during and after the climb (up to 5600 m of altitude). Pre BD FEV1 was measured and cells from the lower airways were obtained by sputum induction before and after the expedition. mRNA was isolated, cDNA was prepared and RT-PCR was used to measure sputum mRNA quantities as described.
Results: During the expedition there was a decrease in FEV1 and FVC which reached a maximum at altitude of 4300 m. The pre BD FEV1 (% predicted) was significantly decreased when measured after the climbing expedition as compared to baseline measurements before the expedition (6.6%, p=0.004). FeNO values were not different between the different time points (p=0.17). Decreased asthma control was observed after the expedition as assessed by asthma control questionnaire (p=0.002). Sputum neutrophil percentages were significantly higher after expedition compared to before the expedition (p=0.04). Concomitantly, sputum IL-17 mRNA was increased after the expedition as compared to before expedition (p=0.007). A correlation of serum Clara Cell protein 16 and sputum IL-17mRNA was found (Spearman r=0.57; p=0.008).
Conclusion: Asthma patients have slightly worse lung function and asthma control after climbing to extreme altitude. Increased signs of neutrophilic inflammation were found in the airways as represented by higher sputum IL-17 mRNA and neutrophils.

P3261
Assessment of airway neutrophil activation in adult non-eosinophilic asthma
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Background: It is now evident that there are different pathophysiologies underlying different asthma phenotypes. The neutrophil has been proposed to be a principal cell type involved in non-eosinophilic asthma (NEA).
Aims: To investigate different aspects of neutrophil activation in NEA.
Methods: 24 adult asthmatics (9 eosinophilic asthma (EA>2% sputum eosinophils), 15 NEA) and 18 healthy controls were recruited by advertisement and successfully underwent clinical assessment, spirometry and sputum induction. Sputum was analysed for neutrophil number (differential cell count), levels of neutrophil-associated soluble mediators (myeloperoxidase (MPO), IL-8 and MMP-9, by ELISA) and neutrophil expression of CD11b (flow cytometry).
Results: Only one asthmatic individual in our study was neutrophilic (>61% neutrophils). EA was generally associated with a more severe clinical phenotype and poorer control (40% poorly controlled) than NEA (8%). There was no statistically significant difference in percentage of sputum neutrophils, neutrophil expression of CD11b, or sputum levels of MPO, IL-8 or MMP-9, between EA, NEA or control subjects. However, we observed that neutrophil percentage increased with age regardless of disease status (R_S=0.71, p<0.0001), and this increase in neutrophils was associated with higher levels of IL-8 (R_S=0.36, p=0.02), and MMP-9 (R_S=0.55, p=0.0002).
Conclusions: Our results show no difference in neutrophil activation in NEA compared to either EA or healthy controls. They also support previous data showing an increase in neutrophil number with aging in both asthma and healthy controls, and suggest that age must be appropriately controlled for when defining a neutrophilic asthma phenotype.

P3262
Implication of interleukin-18 in airway remodelling in refractory asthma
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Interleukin (IL)-18 is suggested to contribute to the pathophysiology of asthma by modulating airway inflammation. However, the involvement of IL-18 on chronic airway inflammation and airway remodelling which are characterizing refractory asthma, has not been assessed. Aim of this study was to investigate IL-18 levels in refractory asthma and its relation to eosinophilic airway inflammation and remodelling.
IL-18 levels were measured in sputum supernatants obtained from mild asthmatic patients (33 smokers and 32 non smokers), patients with refractory asthma (n=32) and healthy subjects (17 smokers and 17 non-smokers). Eosinophilic airway inflammation was assessed by measuring ECP, eosinophil counts in sputum and AHR to methacholine. Airway remodelling was assessed by measuring IL-13, VEGF and transforming growth factor (TGF)-b1 in induced sputum.
Patients with refractory asthma and smoking asthmatics had significantly lower IL-18 levels in sputum compared to non smoking asthmatics (p=0.004 and p=0.049, respectively). No correlation was found between IL-18, ECP, eosinophils and AHR in patients with refractory asthma, although the correlations in mild smoking and non smoking asthmatics were documented (r=-0.636, p=0.026 for PD20meth, and r=-0.495, p=0.043 for FEV1% pred, in non smoking asthmatics, and r=0.765, p=0.006 for PD20meth and r=0.768, p<=0.001, in smoking asthmatics). Significant correlations were found between IL-18 levels and (TGF)-b1, and IL-13 (r=0.803, p<0.001, and r=0.640, p=0.10, respectively).
These findings suggest that in refractory asthma IL-18 is possibly involved in chronic airway inflammation and airway remodelling through an eosinophil independent pathway.

P3263
Effect of adenosine receptors stimulation on generation of $CD1a^{low}CD14^{+}CD209^{+}$ cells in patients with bronchial asthma
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Background: Increased concentration of adenosine have been found in bronchoalveolar lavage, blood and exhaled breath condensate of patients with BA (Spicuzza *et al*, Eur J Pharmacol. 2006;533:77-88). Adenosine skews dendritic cell (DC's) differentiation toward cell population characterized by $CD1a^{low}CD14^{+}CD209^{+}$ antigenic phenotype and high expression of proinflammatory cytokines (Novitskiy *et al*, Blood 2008;112:1822-31).
Aim: To investigate effects of adenosine receptors (AdoR's) stimulation during initial time period of DC's differentiation from peripheral blood (PB) monocytes in patients with bronchial asthma.
Methods: PB monocytes were received from 32 subjects diagnosed with BA and 19 healthy volunteers and cultured in DC-differentiation medium in the absence or presence of AdoR's agonist NECA (30μM) for 38 hours. Cell surface expression of CD1a, CD14 and CD209 was analyzed by FACS.
Results: We have found that stimulation of AdoR's results in higher yield of $CD1a^{low}CD14^{+}CD209^{+}$ cells in patients with BA compare to healthy volunteers (8.22% ±1.72% and 3.43%±0.83%, in BA patients and healthy volunteers respectively; p<0,05). According to number of $CD1a^{low}CD14^{+}CD209^{+}$ cells generated after treatment with NECA we documented the presence of two groups of individuals characterized by high and low response to AdoR's stimulation. We identified that 50% of individuals with BA and 16% of healthy volunteers demonstrated high responsiveness to NECA.
Conclusion: Our study indicates the role of adenosine regulation of DC's differentiation in BA. The heterogeneity in responses to AdoR's stimulation may be used as a basis for individual BA treatment development.

P3264
Role of target-cells sensitivity to corticosteroids in cold bronchial hyperresponsiveness development
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Background: There are no data concerning the correlation of intracellular absorp-

tion of corticosteroids and cold bronchial hyperresponsiveness (CBH) in patients with bronchial asthma (BA).
Aim: To define the role of target-cells sensitivity to corticosteroids in cold bronchial hyperresponsiveness development in BA.
Methods: 44 patients with BA were recruited. The mean level of asthma control was 16.8±0.1 points (Asthma Control Test). The absorption of cortisol by blood lymphocytes (ACL) in the standard test "in vitro" with hydrocortisone before 3-minute isocapnic cold air (-20°C) hyperventilation (ICAH) was studied. CBH was diagnosed by the drop of FEV_1 after ICAH (ΔFEV_1)≥10% from initial value.
Results: All the patients were divided into two groups: the 1st (28 persons) was with constant values of hormone absorption by lymphocytes, the 2nd (16 persons) had lower values. ACL values were $(0.701\pm0.054)\times10^{-4}$ mkg/1000 cells and $(0.527\pm0.038)\times10^{-4}$ mkg/1000 cells, respectively ($p<0.01$). CBH was found out in 22 patients of the 1st group and in 6 patients of the 2nd one ($\chi^2=7.42$; $p<0.01$). In the 1st group there was the biggest part of patients (87%) with a high degree of CBH ($\Delta FEV_1>18.5\%$) and a mean level of CBH (ΔFEV_1 within the range of 14.2-18.4%). The 2nd group had only low values of CBH (ΔFEV_1 within the range of 10.0-14.1%). The risk of high degree of CBH in patients of the 2nd group was three times higher than in the 1st group: chances ratio was 3.45; 95% confidence interval 1.50-7.64 (p=0.0005).
Conclusion: CBH in BA patients is associated with the decrease of transmembrane penetration of glucocorticoids into target cells.

P3265
LSC 2011 Abstract: Effect of inhaled apocynin on reactive oxygen species concentrations in exhaled breath condensate of asthmatics
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Reactive oxygen species (ROS) have a strong impact on homeostasis and are thought to play an important role in inflammation in asthma. The sources of oxidative stress in patients with chronic inflammatory lung diseases derive mainly from increased amounts of ROS and reactive nitrogen species (NOS), generated by airway cells. Apocynin is and agent which blocks NADPH oxidase - the enzyme, responsible for ROS production. The anti-inflammatory activity of apocynin has been demonstrated in a variety of cells and animal models of inflammation. Therefore, considering apocynin activities, we investigated the effect of nebulized apocynin in 14 nonsmoking asthmatics, in placebo-controlled, *cross-over* design study. Effects of apocynin have been checked 30, 60 and 120 minutes after nebulization by collecting exhaled breath condensate (EBC) samples. Additionally, we investigated safety parameters.
Apocynin significantly decreased H_2O_2 concentration in EBC in comparison to placebo after 60 and 120 min. (0.29 μM *vs*. 0.44 μM, and 0.27 μM *vs*. 0.4 μM, respectively). Moreover, apocynin significantly reduced NO_2^- concentration 30 and 60 min after nebulization (2.75 μM *vs*. 4.65 μM, and 2.5 μM *vs*. 4.05 μM, respectively) in comparison to placebo. Finally, apocynin caused a significant decrease of NO_3^- concentration in EBC after 60 and 120 min after administration, comparing to placebo (5.34 μM *vs*. 8.2 μM (60 min), and 5.3 μM *vs*. 8 μM, (120 min) respectively). No influence of apocynin on safety parameters, and no adverse effects has been observed.
These data suggest that using apocynin might be a promising solution to alleviate inflammatory process, and probably, symptoms of inflammatory diseases.

P3266
Quantitative proteomics on bronchial biopsies from asthma and COPD: Effects of budesonide treatment
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The global proteome of individual bronchial biopsy material from asthma and COPD patients has not been fully ascertained.
The aim was to determine if mechanisms of disease and responses to treatment can be detected in biopsies from patients with asthma and COPD, using a quantitative proteomics technology.
Endobronchial biopsies, pre and post treatment, were taken from patients with asthma (n=12) and COPD (n=11), as well as non-smoking (n=3) and smoking (n=2) healthy controls. Patients were randomised to double blind treatment with either placebo or budesonide (800 μg daily for 3 months). Quantitative proteomics technology was used to identify and quantify biopsy proteins. Pathways analysis was conducted to identify global proteome differences.
A total of 1937 proteins were identified from all subjects. Proteome differences in asthma and COPD and changes in response to treatment could be observed. Analysis of the global proteome of COPD revealed that the top protein network contained the function of connective tissue disorder and that metabolic pathways were most relevant. In comparison, the top network in asthma contained dermatological functions, with the pathways being more actin-based. Changes in the proteome could also be observed in response to treatment. The increased relevance of cellular biological processes and decrease of oxidative stress can be seen for both asthma and COPD. Of particular interest, hepatic fibrosis was more associated with the COPD proteome and is altered with budesonide treatment.
These results show that proteome differences can be detected using quantitative proteomics technology on clinically relevant biopsies from individual patients.

P3267
The interplay of LXA4 and glucocorticoid receptor-based mechanisms in airway inflammation of childhood asthma
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Lipoxins (LXs) are biologically active eicosanoid possessing anti-inflammatory properties. Lipoxin A4 (LXA4) signaling blocks asthmatic responses in human and experimental model system. There are evidences that respiratory diseases, including severe asthma, display defective generation of lipoxin signals. To assess the role of pro- and anti-inflammatory mediators in airway homeostasis of asthmatic children, we examined the levels of LXA4 and leukotriene (LT) B4, a potent neutrophil chemoattractant, in the induced sputum supernatants (ISs), from intermittent (IA) and moderate (MA) asthmatic children treated with high doses of inhaled corticosteroids (ICS), and control children (C). In order to address whether LXA4 and glucocorticoids have overlapping role in the resolution of inflammation, we evaluated the effect of LXA4 treatment on glucocorticoid receptor (GR) phosphorylation, in the presence or absence of LXA4 receptor (FPRL-1/ALXR) blocking peptide, in peripheral blood neutrophils (PBN) from C, using both Western Blot and Flow cytometry analyses.
We found that LXA4 was higher in ISs from IA compared with MA and C, while LTB4 was significantly higher in IA and MA than in C. In MA, LXA4 and LTB4 were inversely correlated. In addition, we showed that LXA4 induced GR phosphorylation (Ser211) via ALXR in PBN from C, since the use of the receptor functional blocking peptide counteracted the effect of LXA4.
Our findings provide evidences for the hypothesis that a defective generation of anti-inflammatory LXA4, associated with an increased LTB4 production, may be involved in a reduced ability of the ongoing ICS therapy in the control of airway inflammation in children with MA.

P3268
LSC 2011 Abstract: An expression survey of toll-like receptors (TLRs) and nod-like receptors (NLRs) in allergic asthma
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Allergic disorders, such as asthma, are symptomatic reactions of the immune system to common and innocuous environmental antigens. These inflammatory disorders are caused by aberrant immune regulation in which various signalling receptors are involved. Pathogen recognition receptors like the TLRs and NLRs families of receptors are one of the key components of the innate immune system. The function of these receptors has been linked with susceptibility towards the development of allergic diseases, including asthma, making the TLRs and NLRs good targets for novel effective therapies of allergic diseases. In this study the mRNA expression levels of different TLRs and NLRs in the lung tissue in mild and severe mouse models of allergic asthma were measured by q-PCR. In addition, broncho-alveolar lavage fluid (BALF) was collected and cell numbers analysed. In the mild and severe asthma models different TLR and NLR mRNA expression profiles are observed. In the severe asthma model, a higher cell influx in BALF is seen. Moreover, a significant correlation is found between the mRNA expression of TLR3, TLR6 and TLR9 and the total cell number in the BALF.

P3269
Mesenchymal stem cells down-regulate inflammation but not airway hyperresponsiveness in experimental asthma
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Introduction: Mesenchymal stem cells (MSCs) have immunomodulatory properties. MSCs may have a potential to down-regulate airway inflammation in asthma, but may as well serve as building blocks for unwanted airway remodeling. Here we tested the effect of MSCs in experimental asthma.

Methods: MSCs from peritoneal adipose tissue of donor mice were transduced to express green fluorescent protein (GFP). Experimental asthma was induced in recipient mice by repeated intranasal instillation of house dust mite (HDM) extract. These mice subsequently received 3×10^5 MSCs intravenously (HDM-MSC group). Control groups consisted of HDM-instilled miced that received MSC vehicle (HDM-noMSC group), or PBS-instilled mice that received MSCs (PBS-MSC group). Airway responsiveness to methacholine, bronchoalveolar lavage (BAL) leukocytes and the presence of GFP^+ MSCs in the lung were analyzed 3 days after the MSC or vehicle injection.
Results: HDM-MSC mice showed: (i) unchanged airway hyperresponsiveness to methacholine compared with HDM-noMSC mice; (ii) BAL leukocyte counts decreased to the PBS-MSC group baseline; and (iii) GFP^+ MSCs present in lung parenchyma but not in the conductive airway wall.
Conclusion: In experimental asthma, a single MSC infusion effectively decreases airway inflammation but leaves airway hyperresponsiveness untouched. MSCs infused for therapeutic aims may not home to the airway wall. Whether clinicaly relevant outcomes in terms of lung function can be expected from MSC therapy in severe asthma needs further studies.
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P3270
Aerobic exercise reduces allergic airway inflammation and remodeling by deactivating airway epithelial cells and leukocytes
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The regular practice of aerobic exercise (AE) has been shown to reduce chronic allergic airway inflammation and remodeling (CAAIR), but the mechanisms involved remains poorly understood. In the present study we investigated if 4 weeks of AE deactivates airway epithelium and peribronchial leukocytes in a model of CAAIR. Thirty-two animals were divided in control, aerobic exercise, ovalbumin and ovalbumin+aerobic exercise groups. Mice sensitized (10ug/mouse, days 0, 14, 28 and 42) and challenged with ovalbumin (3x/week; 21st until 50th day), were submitted to AE (21st until 50th day, 60min/session; 5x/week). The results demonstrated that AE in OVA-sensitized mice significantly reduced eosinophils in BAL and in airway wall and also the accumulation of collagen fibers on airway wall. This anti-inflammatory and anti-fibrotic response induced by AE in sensitized animals was followed by reduced expression of the followings markers by *airway epithelial cells and also by peribronchial leukocytes:* IL-4, IL-5, IL-13, CCL11, CCL5, ICAM-1, VCAM-1, iNOS, NF-kB, GP91phox, 3-nitrotyrosine, 8-Isoprostane, IGF-1, EGFr, VEGF, TGF-beta, MMP-12 and TIMP-2 ($p<0.01$). AE in sensitized animals also increased the expression of anti-inflammatory cytokine IL-10 by leukocytes and airway epithelial cells. Taken together, these results demonstrate that the beneficial effects of AE on allergic airway inflammation and remodeling is a multifactorial response involving deactivation of airway epithelial cells and also of leukocytes, by inhibition of Th2 response, chemokines, adhesion molecules, oxidative and nitrosative stress, and also on the growth factors and matrix metalloproteinases.

P3271
Upregulated expression of interleukin-33 and alternative activation of macrophages in a model of an acute exacerbation of asthma
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The role of alveolar macrophages (AM) in the pathogenesis of an acute exacerbation of asthma is poorly understood. In a clinically relevant mouse model, we have shown that AM are activated to secrete pro-inflammatory cytokines, and that these activated AM can stimulate primed CD4+ T cells to secrete Th2 cytokines (Am J Pathol 2010; 177:1657). We sought to further characterise the nature of these activated macrophages and the mechanism of activation. Female BALB/c mice were systemically sensitised to ovalbumin (OVA) and received chronic low-level challenge with aerosolised OVA for 4 weeks. Following this, mice received a single moderate-level challenge to induce airway inflammation simulating an acute exacerbation. AM and tissues were collected 4 hours later. Control groups included naive animals, and mice that only received either chronic challenge or a single moderate-level challenge. AM from an acute exacerbation exhibited significantly enhanced expression of markers of alternative activation, including mRNA for arginase-1, FIZZ1, Ym1 and eotaxin-2. These AM also exhibited elevated expression of cell surface proteins associated with antigen presentation to T cells, including CD86 and MHC class II. In parallel, expression of mRNA for IL-33 in the airway wall was strikingly increased, with evidence of enhanced immunoreactivity for IL-33 in the cytoplasm of airway epithelial cells and plasma cells within the airway wall. Collectively, these data imply an important role for alternatively activated AM in the pathogenesis of an acute exacerbation of asthma, and suggest that IL-33 may contribute to the activation of these cells.

P3272
Naturally occurring CD4+ CD25+ FoxP3+ Treg cells are related to the absence of antigen induced airway obstruction in a guinea pig asthma model
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Some allergic asthma individuals did not display airway obstruction after an anaphylactic challenge (dAOAC); however, the mechanism involved in this process is unknown, it has been suggested that regulatory T cells (Tregs) may be involved. We developed a model of airway obstruction-induced by antigenic challenge in guinea pig (GP) to elucidate the potential role of Tregs. In our model, the sensitized GP are intermittently challenged with the antigen (ovalbumin, OA; applied every 10 days). We found two groups of GP ones that showed dAOAC and ones that did not. Then, we evaluated the antigen-induced airway obstruction and hyperresponsiveness (HR) to histamine and identified the population of Tregs in the bronchoalveolar lavage (BAL) in GP after three and six OA challenges. Controls received saline solution instead of OA. From the first challenge, GP that dAOAC showed a transient response and developed of HR at third and sixth challenges. In GP that did not dAOAC HR was observed at third but not at sixth challenge. The total number of cells that produced TGF-β1 and IL-10 in BAL were similar to controls in all groups. In GP that dAOAC, CD4+CD25+Foxp3+ T cells (nTreg) numbers were reduced in BAL in comparison to controls and GP that did not dAOAC. The number of inducible regulatory T cells (iTreg) that produced IL-10 was similar to controls in all groups, while iTregs that produced TGF-β1 increased only in the group of GP that did not dAOAC on the sixth challenge. We suggests that nTregs might play an important role in suppression of dAOAC and that probably iTregs that produce TGF-β1 are involved in the attenuation of HR in this asthma model.

P3273
Overproduction of IL-18 in the lungs induces IL-13 and IFN-g producing CD4+ T cell in the lungs, and results in airway hyperresponsiveness in Balb/c mice
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We newly established Balb/c background IL-18 transgenic (TG) mice using the human surfactant protein C promoter to drive expression of mature mouse IL-18 cDNA in the lungs. After sensitization on days 0 and 5 with ovalbumin (OVA), mice were challenged with OVA aerosol on day 19. Pulmonary inflammations and airway hyperresponsiveness (AHR) were examined on day 20. We previously reported that constitutive mouse mature IL-18 overproduction in the lungs of C57BL/6 mice induces emphysema (Am J Respir Crit Care Med 2007, 176:49-62). In contrast to C57BL/6 IL-18 TG mice, emphysematous changes were not observed in the lungs of Balb/c IL-18 TG mice. AHR to inhaled acetylcholine were not induced in non-treated Balb/c IL-18 TG mice. However, AHR and airway inflammation accompanied with CD4+ T cells, eosinophils and neutrophils were significantly and greatly increased in OVA-sensitized/challenged Balb/c IL-18 TG mice, when compared with OVA-sensitized/challenged WT Balb/c mice. Moreover, levels of IFN-g and IL-13 in the bronchoalveolar lavage fluid of OVA-sensitized/challenged Balb/c IL-18 TG mice were significantly elevated when compared with OVA-sensitized/challenged WT Balb/c mice. We used anti-CD4 mab or Balb/c IL-18 TG/IL-13 KO mice to examine the role of CD4+ T cells and IL-13 in this Balb/c IL-18 TG mouse. Anti-CD4 mab decrease the level of IL-13 and IFN-g. AHR and pulmonary inflammations were decreased in anti-CD4 mab or Balb/c IL-18 TG/IL-13 KO mice. This study suggested that overproducing IL-18 proteins in the lungs of Balb/c mice induce IL-13 and CD4+ T cells which may involve in the pathogenesis of asthma.

P3274
The role of bone-marrow derived adult stem cells in asthmatic airway remodeling
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Background and objective: Asthmatic airway remodeling is an abnormal injury/repair process of small airway on the basis of chronic inflammation, in which the quantities of multiple lung parenchyma cells dramatically increase. However, the origin of these proliferative cells is still not clearly elucidated. The aim of the study is to examine whether BM-derived adult stem cells are responsible for the massive proliferative cells in asthmatic airway remodeling.
Methods: Adult mice were durably engrafted with BM isolated from GFP transgenic mice. Using GFP BM-chimera mice, OVA-induced chronic asthma model were established. The distribution of BM-derived GFP^+ cells in the lung of chronic asthma mice was detected by fluorescence microscopy. The phenotype of BM-derived GFP^+ cells in the lung was analyzed by flow cytometry.
Results: BM-chimera mice were successfully constructed, with no detectable ra-

dioactive inflammation observed. Using BM-chimera mice, we established a mouse model of chronic asthma characterized by significant increment of the thickness of airway subepithelial base membrane and smooth muscle layers. OVA treatment caused many GFP^+ cells to appear in the sites of small airway inflammation. The extravascular localization of some GFP^+ cells and their morphology was not consistent with leukocytes. Flow-cytometric analysis revealed a significant increase in Col I^+GFP^+ cells and a-SMA^+GFP^+ cells in OVA-treated GFP BM-chimera mice. **Conclusions:** Considerable Col I-producing cells and a-SMA-producing cells originated from bone marrow in the lung tissues of OVA-induced chronic asthma mice and bone-marrow derived adult stem cells are at least partly responsible for asthmatic airway remodeling.

P3275

Efficient gene transduction of adipose tissue-derived mesenchymal stem cells (MSCs) for asthma research

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Background: MSCs may offer therapeutic potential for asthma due to their immunomodulatory properties and host tolerability. MSCs may however be involved in airway remodeling. Solid preclinical development is therefore indispensable prior to clinical trial attempts. We aimed at establishing a technique for MSC genetic engineering and in vivo tracking in experimental asthma.
Methods: A gene transfer retroviral vector to induce permanent green fluorescent protein (GFP) expression on MSCs was generated. MSC cultures were produced from abdominal adipose tissue of adult mice, as per established procedures (MesenCult Kit, StemCell Technologies). GFP expression was assessed by flow cytometry and fluorescent microscopy. The MSC phenotype was verified by cell surface marker analysis, and by differentiation in adipogenic and osteogenic commercial media followed by oil-red and alizarin-red staining.
Results: Passage-4 cells targeted with a single retroviral hit at a multiplicity of infection of 1 preserved 99.4% cell viability and yielded 82.6% transduced cells highly expressing GFP. The cells showed a Sca-$1^+CD44^+CD14^-CD45^-CD11b^-$ MSC phenotype, and differentiated into adipocyte and osteocyte lineages in conditioned media.
Conclusions: Adipose tissue derived MSCs can be efficiently transduced with retroviral vectors for permanent gene expression. This provides a tool for in vivo MSC tracking to remodeling airways, and for exploring pathogenic mechanisms and therapeutic avenues through MSC genetic modifications.
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Analysis of $P2Y_{12}$ receptor responsiveness to cysteinyl leukotrienes

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Leukotriene E_4 (LTE_4), the most stable of the cysteinyl leukotrienes (cysLT), binds poorly to classical type 1 and 2 cysLT receptors although it may potently induce bronchial constriction, airway hyperresponsiveness and inflammatory cell influx to the lungs of asthmatic individuals. Evidence of the presence of a previously unidentified LTE_4 receptor has also been provided in $CysLT_1^-/CysLT_2^-$ double knock out mice. A recent study has suggested that purinergic receptor $P2Y_{12}$ is required for LTE_4 mediated pulmonary inflammation in a mouse model of asthma. The aim of the study was to characterise the responsiveness of human $P2Y_{12}$ to cysteinyl leukotrienes. A model of human $CysLT_1$, $CysLT_2$ and $P2Y_{12}$ transiently overexpressed in HEK293 cells was used and responsiveness to different agonists was measured using intracellular calcium and cAMP assays. The responsiveness of human $P2Y_{12}$ stably overexpressed in CHO cells was also analysed using a β-arrestin recruitment assay. CysLTs induced concentration dependent calcium mobilisation in cells overexpressing $CysLT_1$ and $CysLT_2$ but failed to induce any calcium response in cells expressing $P2Y_{12}$ or $P2Y_{12}+G_\alpha16$. In contrast, a selective $P2Y_{12}$ agonist 2-MeSADP, induced specific calcium flux in cell expressing $P2Y_{12}+G_\alpha16$. Similarly, specific response to 2-MeSADP, but not to cysLTs was also observed in cells expressing $P2Y_{12}$ when intracellular cAMP and β-arrestin signalling was analysed. These results suggest that LTE_4 as well as other cysLTs may not activate intracellular signalling acting through human $P2Y_{12}$ and another LTE_4 specific receptor has yet to be identified.