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(NO) seen in patients with primary ciliary dyskinesia (PCD) has not been elucidated. A hypothesis has been that the respiratory epithelial cells in these patients do not biosynthesise normal levels of NO. NO is rapidly metabolised to nitrite, which is considered to be a surrogate marker of NO.

Aims: To compare the total nitrite concentrations of differentiated ciliated respiratory epithelial cells from PCD and non-PCD patients at baseline and following stimulation.

Methods: Airway epithelium from PCD and non-PCD patients was obtained by nasal brushing and cultured at air liquid interface (ALI) until differentiated and ciliated. PBS was incubated for 30 minutes on the apical surface of the ALI cultures and total nitrite detected by kit assay (Enzo Life Sciences). Basolateral incubation with 10 mg/ml IL-1 β /IFN γ /TNF α for 18 hours stimulated NO biosynthesis via nitric oxide synthase (NOS) activity.

Results: Total nitrite concentration in differentiated PCD (n=5) and non-PCD (n=7) cultured epithelium was similar both at baseline, 16.1 \pm 1.1 mmol/l and 17.8 \pm 3.2 mmol/l respectively (mean \pm SD, p=0.14) and after stimulation; PCD (n=4) 32.0 \pm 12.7 mmol/l and non-PCD (n=4) 33.2 \pm 11.0 mmol/l respectively (p=0.46).

Conclusion: NO biosynthesis in differentiated ciliated airway epithelium from PCD patients is similar to non-PCD patients at baseline and following NOS stimulation. As nasal NO is increasingly used for screening of PCD, further work assessing the upper airway's role in the low levels of nasal NO seen is warranted. Data collection continues.

P3729

Epithelial ciliary beating analysis in chronic airway diseases

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Human bronchial epithelial cells (HBEC) activation and increased mucus production are constant features of epithelium in chronic airway diseases. However, the mechanisms and involvement of mucociliary component are poorly understood in these diseases. We used an *in vitro* model of bronchial epithelial cell culture in air/liquid interface obtained from bronchial biopsies of asthmatic patients (mild and severe) or COPD patients. We questioned the regulation of mucociliary couple in this system using an original approach combining biology and physics.

Methods: HBEC obtained after processing biopsy were expanded on tissue culture-treated plastic and then, were plated on uncoated nucleopore membranes in air-liquid interface for 21 days to obtain a mucociliary phenotype. For each epithelium re-differentiated *in vitro*, we first measured thickness and ciliary beating frequency (CBF). We used fast videomicroscopy (37°C). The effect of mucus presence on cilia beating was assessed by measuring frequency before and after washes with PBS.

Results: Preliminary results are presented in table 1 and are expressed as median (min-max).

Conclusion: At present we did not find any major change in the velocity of cilia beating according to the airway diseases in our air-liquid interface system. The contribution of mucus should be better investigated.

Epithelium thickness and Ciliary beating frequency obtained in air-liquid interface model

| | Controls (n=2) | Mild Asthmatics (n=5) | Severe Asthmatics (n=4) | COPD (n=3) |
|------------------------|-------------------|--------------------------|----------------------------|---------------|
| Thickness (μ m) | 43 (33–53) | 38 (33–43) | 42 (35–50) | 51 (45–53) |
| CBF (before wash) (Hz) | 16 (12–20) | 17 (15–20) | 19 (16–24) | 14 (13–21) |
| CBF (after wash) (Hz) | 19 (16–21) | 19 (14–21) | 19 (17–21) | 14 (13–21) |

P3730

Air liquid interface culture can alter ciliary beat pattern in epithelium from primary ciliary dyskinesia patients

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Introduction: Primary Ciliary Dyskinesia (PCD) is a rare inherited heterogeneous disorder of cilia, impairing mucociliary clearance. PCD is diagnosed by low nasal nitric oxide concentration, abnormal airway ciliary function with corresponding ultrastructural defects, excepting atypical cases. PCD may be differentiated from secondary dyskinesia by assessing differentiated ciliated epithelium at air liquid interface (ALI).

Aim: To evaluate ciliary beat pattern (CBP) on PCD airway epithelium before and after ALI culture.

Method: Ciliary function of nasal brushing epithelium from 9 PCD patients with ultrastructure defects confirmed by TEM, was analysed (n=10 cell clusters) at 37°C by high-speed video microscopy (100x oil objective). Confluent basal ep-

393. Cell culture and animal models of lung disease

P3727

Pulmonary haptoglobin (pHp) is a scavenger system preventing arterial leakages

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Haptoglobin is a long known molecule. The recently discovered pHp variant is coming into focus in the course of immunoregulatory events in the human lung. Here we describe the fast release of pHp upon different stimuli using a human tissue culture model, cultivated BAL-cells and a cell line (A549). Dependent from the certain cell types we show that pHp get released in different time frames. Whereas the macrophages need up to 36h of stimulation with LPS dexamethasone or IL6, epithelial cells react much faster. We regularly measured reasonable amounts of pHp in the supernatants of human tissue cultures after stimulation periods as short as 5 minutes. Due to the well known anti-inflammatory properties of haptoglobin and the capability to bind and neutralize free hemoglobin, we propose pHp to be a local first line immunoregulatory molecule which could play a crucial role in quickly fixing alveolar damages e.g. due to arterial leakage. The signalling leading to pHp-release need to be illuminated, however, the speed makes it easy to speculate about a system that is largely independent from the common pathways such as TLR-signaling.

P3728

Nitric oxide biosynthesis by primary ciliary dyskinesia respiratory epithelial cells is similar to non-PCD patients

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Introduction: The mechanism for the extremely low levels of nasal nitric oxide

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ithelial cells on 0.3 mg/ml Purecol (Nutacon) coated 12 mm transwell inserts were differentiated over 21 days in 1:1 BEGM:DMEM with SingleQuots (Clonetics, Lonza) and 100 nM retinoic acid. Ciliated ALI membranes were excised and cilia and ciliary function re-examined.

Results: Three ALI cultures (33.3%) retained their CBP compared to the original epithelium: 2/9 completely static cilia; 1/9 static with twitching cilia. Six ALI cultures (66.7%) continued to exhibit abnormal CBP but the phenotype changed following ALI culture: 5/9 static with twitching cilia became completely static on ALI; 1/9 dyskinetic with static cilia became completely static on ALI and additionally dynein arm defects altered from 60 to 98% after culture.

Conclusions: The diagnosis of PCD remained clear before and after ALI culture, however we observe that ciliary function and percentage defect rate may alter in epithelial cell culture at ALI.

P3731

Intrinsic and environmental factors contribute to ciliary dysfunction of human airway epithelial cells in asthma

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Dysfunctional epithelial ciliary function plays a major role in chronic respiratory diseases such as asthma. However the underlying mechanism is not yet clear. We hypothesized that ciliary dysfunction of distal epithelial cells is due to intrinsic and/or environmental factors.

Primary human basal epithelial cell cultures were differentiated *in vitro* (ALI). Following pre-incubation ± antibiotics and exposure to fresh asthmatic sputum for 24h cell strips were collected. Videos of ciliary epithelial side profiles were recorded using a high-speed camera. Ciliary beat frequency (CBF) and beat patterns were assessed. Microbiology was assessed by routine culture & qPCR, reactive oxygen species (ROS) generation by DCFDA assays.

Asthmatic and healthy ALI showed a similar baseline ciliary function. The effect of asthmatic sputum, without antibiotics, on ciliary function in asthmatic ALI is shown below:

| | Healthy (n=5) | Asthmatic (n=6) |
|----------------|---------------|-------------------------|
| CBF (Hz) | 1.9±0.8 | -2.3±1.6* [†] |
| % Normal cilia | -15.0±10.8 | -24.0±6.0* |
| % Static cilia | -2.3±4.8 | 27.3±13.0* [†] |

p<0.05 compared to *no change (Wilcoxon), [†]healthy (t-test).

Bacterial load increased overtime but was not different between groups. ROS generation was increased in asthmatic vs healthy basal cells (LogEC₅₀ -0.5±0.2 vs -1.3±0.2, p=0.05).

Our data suggests that both extrinsic factors i.e. bacterial load and intrinsic abnormalities e.g. ROS handling may contribute to the asthmatic ciliary dysfunction.

P3732

Developing a novel *in vitro* 3D airway model to provide a viable alternative to animal use in asthma research

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Studies into airway disease have been limited to whole animal *in vivo* or single cell-type *in vitro* studies. In asthma, mouse immunological studies have led to our understanding of the Th2 paradigm, but this reveals only a modest proportion of the human condition. *In vitro* primary cells studies provide insight into the underlying signaling mechanisms in airway disease, but tissue cultured-plastic restricts cell growth to a 2D monolayer. Reliance on these traditional platforms is decreasing as current protocols (such as using electrospun matrices) provide a better 3D environment for cells to inhabit *in vitro*.

We are developing a tissue engineered 3D model of an airway bronchiole containing the three main structural cells (epithelial, fibroblast, and smooth muscle) sourced from both healthy and asthmatic patients. Using defined electrospinning parameters we have developed both nano- and micro-fibrous scaffolds to support the cell types within a 3D environment. Once the cell types have been grown on their tailored scaffolds, they are combined to form a simple construct of the bronchiole and held in a flow perfusion bioreactor to provide effective nutrient exchange and metabolic waste removal.

We have optimized a nanofibrous polyethylene tetraphthalate (PET) electrospun scaffold for epithelial cell growth and differentiation. Fibroblast and smooth muscle cells have been successfully cultured on a microfibrillar PET electrospun scaffold. The scaffold system provides a platform to investigate cell-cell interactions within a bi- or tri-culture system. Preliminary data suggest fibroblasts play an important role in epithelial cell responses to airway inflammatory mediators.

P3733

A novel method for harvesting and culturing airway epithelial cells from neonates

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Introduction: The airway epithelium is considered important in the pathogenesis of several respiratory conditions including asthma. While studies of airway epithelial cell (AEC) cultures obtained from adults and children have provided valuable insight into the role of these cells in airway disease, little is known about how neonatal AEC phenotype impacts on respiratory disease in later life. We describe a novel method for culturing nasal AEC within days of birth.

Methods: AEC were sampled from healthy, unsedated infants within 72 hours of birth by gently brushing both nostrils with an interdental brush. Brushes were agitated in specialised AEC growth media and primary cell monolayers grown to confluence before sub-culture. IL-8 concentrations were measured in supernatants from tertiary passage AEC monolayers at rest and after exposure to IL-1β & TNF-α (both at 10ng/ml).

Results: Sampling was acceptable to parents and there were no adverse effects. Primary cultures were successfully established in 74(82%) of 90 neonates sampled. Epithelial lineage of the cells was confirmed by morphological analysis and positive immunostaining for cytokeratin 19 and negative staining for potentially contaminating cell types. Constitutive IL-8 secretion was observed and was upregulated by cytokine stimuli.

Conclusion: We describe a safe, minimally invasive and reproducible method of culturing AEC from neonates suitable for functional cell analysis and amenable to large population based studies. This novel technique offers a unique opportunity to study "naïve" AEC not yet exposed to the confounding effects of environmental pollutants and pathogens and may prove useful in elucidating the early origins of respiratory disease.

P3734

CSE exposure abrogates regulatory effects of the bronchial epithelium on B cell survival and IgA production in a coculture system. A pivotal role of TACI

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Background: Chronic obstructive pulmonary disease (COPD) is associated with chronic airway inflammation and structural remodelling, in particular of the epithelium, which is impaired in its capacity to transport immunoglobulin (Ig) A. Although COPD is mostly related to cigarette smoking, only a minority of smokers develops this disease and factors of cigarette exposure susceptibility remain not clear. Even though peribronchial lymphoid follicles have been described in severe COPD, it remains unknown whether B-cell conditioning is altered in this disease, especially after CSE (Cigarette Smoke Extract) exposure.

Objectives: In this study, we report on CSE exposure data using a coculture model of B cells with human primary bronchial epithelium (re)differentiated *in vitro* in air-liquid interface.

Methods: IgA synthesis was studied following CSE exposure in CD19+ B cells (purified by immunomagnetic sorting from healthy blood donors) after co-culture for 13 days with a bronchial epithelium from severe COPD patients. B cells were also assessed by flow cytometry for cell activation and survival.

Results: In four independent experiments, we observed that IgA production and cell survival were upregulated in B cells cocultured with the bronchial epithelium, as compared to B cells cultured alone. CSE exposure of the epithelium abrogated these effects, and this was associated with the suppression of TACI induction upon co-culture.

Conclusion: These data suggest that a crosstalk exists between B cells and the epithelium with respect to COPD, which could be mediated at least in part through regulation of TACI.

P3735

A reliable and long shelf-life *in vitro* cell model for studying goblet cell metaplasia in the human airways

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Goblet cell metaplasia, induced mainly by TH-2 cytokines like IL-13 and IL-4, is a common feature of several respiratory diseases such as asthma, COPD, and Cystic fibrosis. Recent clinic trials demonstrated that blocking IL-13 signaling seems to be an effective therapeutic strategy. In order to develop more efficient drugs, a reliable and reproducible *in vitro* cell model would be invaluable. Epithelix has developed a high quality *in vitro* cell model of the human airway epithelia (MucilAir) which can be maintained at a functional and homeostatic state for a year, allowing long term tests of drug candidates. In order to create an *in vitro*

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cell model of the airway goblet cell metaplasia, MucilAir was treated with IL-13 at different concentrations, ranging from 0.3 to 30 ng/ml. Using in situ Alcian Blue staining, as well as histological analysis, we demonstrated that MucilAir showed an increased goblet cell density after 14 days of IL-13 treatment, in a dose dependent manner. Furthermore, ELISA analysis revealed a concomitant increase of Eotaxin and Perioxin released in the culture media as a function of IL-13 concentration. Despite some subtle morphological differences of the goblet cells between donors, the results from several different batches of MucilAir are very similar and therefore reproducible. Taken together, MucilAir is a relevant and reliable in vitro cell model for studying Goblet cell metaplasia, and for assessing the efficacy of drug candidates.

P3736

Do characteristic airway epithelial change precede the development of lung fibrosis? Ectopic epithelial marker protein expression in bleomycin induced fibrosis replicates that seen in bronchiolized epithelium in IPF

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The cause Idiopathic Pulmonary Fibrosis (IPF) remains elusive but there is support for the view that epithelial cell damage within the peripheral lung initiates the process that ultimately results in fibrosis. Unfortunately, markers of this process remain elusive due in part to most human studies being performed with tissues from end-stage disease. Ectopic expression of the gel forming mucin, MUC5B was recently described as a specific marker for the bronchiolized epithelium seen in IPF and we have shown that it is co-expressed in this region with LPLUNC1 but not with other airway submucosal gland proteins, including Zinc-alpha2-glycoprotein and Proline-rich protein 4. To shed light on a temporal association of expression of these markers with fibrosis development we studied mice exposed to the pro-fibrotic agent bleomycin (Bleo). MUC5B and LPLUNC1 were co-expressed in a population of goblet cells in the airways of mice within 3-7 days of Bleo exposure, prior to the onset of a fibrosis. Continued expression is seen during the development of fibrosis between 14-21 days post treatment. In contrast, in mice treated with PBS neither protein was seen (due to mouse airways being essentially free of goblet cells). Staining was absent from the fibrotic regions and the lung parenchyma, as is the case in IPF. Our data show that the ectopic expression seen in human IPF is mirrored by that seen in the Bleo mouse model. Furthermore it suggests that these epithelial remodeling events precede the development of lung fibrosis and these can be studied in mice.

P3737

A unique session of aerobic exercise does not decrease pulmonary inflammation in a murine mice model of asthma

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Recent studies have shown that long term exercise training reduces airway inflammation; however, there is a lack of evidences on the effects of a single session of exercise.

Objective: To evaluate the effects of a session of aerobic exercise in the airway inflammation in a murine animal asthma model.

Methods: Thirty two mice were divided in Groups (n=8): Control (CT), Aerobic Training (AT), OVA and OVA+TA. Groups OVA were sensitized by intraperitoneal injections of OVA(50ug/mice in the days 0, 14 and 28) followed by 30 min of 1% OVA inhalation (days 21th, 23th, 25th and 28th). CT and AT groups received saline. Exercise (AT groups) was performed in the day 28th for 60 min at 50% intensity of maximal capacity. Bronchoalveolar fluid (BALF), lung tissue and blood were collected in the day 29th. It was evaluated: total and different cells in BALF, IgE and IgG1 titers, peribronchial eosinophils, and airway remodeling (smooth muscle, collagen and elastic fibers, and mucus expression). Eotaxin, RANTES, VEGF, ICAM, VCAM, IL-1ra, NF-kb and Foxp3 in the airways were also evaluated. Statistical significance was evaluated by two way ANOVA followed by Bonferroni or Newman-Keuls.

Results: OVA increased IgE and IgG1 levels, total and eosinophil cell counting, and all remodeling features (smooth muscle, collagen and elastic fibers, and mucus expression) (p<0.05). In addition, OVA increased the expression of eotaxin, RANTES, VEGF, ICAM, VCAM, NF-kb and Foxp3 (P<0.05). On contrary, a single bout of aerobic training did not changed any of these effects (P>0.05).

Conclusion: A single exercise session seems do not have any anti-inflammatory effect in a murine asthma model.

P3738

Aerobic training inhibits leukocytes production of oxidants, pro-inflammatory and pro-fibrotic factors reducing asthmatic phenotype

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Aerobic training results in beneficial effects to asthmatic patients and also in animal models of asthma. However, the studies have not evaluated yet the effects of aerobic training on the peribronchial leukocytes activation. This study evaluated the effects of 4 weeks of low intensity aerobic training on the expression of inflammatory and fibrotic mediators by peribronchial leukocytes. 32 BALB/c mice divided in 4 experimental groups: Control, Exercise, OVA and OVA+Exercise were sensitized on days 0, 14, 28 and 42 and challenged with aerolized OVA 1% for 3x/week, beginning on 21st day for 4 weeks. Low intensity aerobic treadmill training started on day 22nd for 4 weeks. 72 hours after last OVA challenge mice were assessed to lung inflammation, levels of IL-5 and IL-10 in BAL fluid by ELISA and for quantitative analysis of peribronchial leukocytes activation. The results showed that aerobic training in sensitized animals resulted in significant decrease of total cells and eosinophils in BAL fluid, decreased level of IL-5 and increased level of IL-10 in BAL fluid (p<0.01), decreased expression of IL-4, IL-5, IL-13 (p<0.001), CCL5, CCL10 (p<0.001), ICAM-1 and VCAM-1 (p<0.05), GP91phox and 3-nitrotyrosine (p<0.001), NF-kB (p<0.001), iNOS (p<0.001), while increased the expression of IL-10 (p<0.001). Concerning the airway remodeling, aerobic training in sensitized animals resulted in decreased expression of TGF-beta, IGF-1, VEGF and EGFR (p<0.001). We conclude that aerobic training in sensitized animals results in a direct effect on peribronchial leukocytes by reducing the expression of important factors related with the airway inflammation and remodeling.

P3739

Involvement of oxidative and nitrosative stress in the development of proteolytic pulmonary emphysema

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Our aim was to investigate the participation of oxidative stress in elastase-induced pulmonary emphysema. C57BL/6 mice were submitted to pancreatic porcine elastase (PPE) instillation (0.05U or 0.5U) per mouse (i.t.) to induce pulmonary emphysema. A separated group of mice were treated with aminoguanidine 1% (AMG). Lungs were collected on days 7, 14 and 21 after PPE instillation. Control group was sham-injected. We performed BAL, biochemical analyses of oxidative stress, and lung stereology and morphometry. Emphysema was histologically characterized at 21 days after 0.5 U of PPE, presenting increased alveolar linear intercept and volume density of airspaces in comparison with the control group. TNF-α was elevated at 7 and 14 days after PPE 0.5 U, concomitant with reduction in the IL-10 levels at the same time-points. Myeloperoxidase was elevated in all groups treated with 0.5 U of PPE. A contribution of oxidative stress at early stage of emphysema was observed with increased levels of nitrite, malondialdehyde and superoxide dismutase activity at 7 days after PPE 0.5 U. Glutathione peroxidase activity was increased in all groups treated with 0.5 U of PPE. With iNOS inhibition by AMG 1%, emphysema was attenuated. Furthermore, the proteolytic stimulus by PPE enhanced expression of nitrotyrosine and iNOS, while the group PPE+AMG showed low expression of iNOS and nitrotyrosine. PPE stimulus also induced eNOS expression, but AMG reduced it. Our results suggest a pathway of oxidative and nitrosative stress by nitric oxide production via iNOS expression in pulmonary emphysema.

P3740

The serotonergic receptor subtype 5-HTR1B contributes to the pathogenesis of allergic airway inflammation

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In addition to its well described role in the nervous and cardiovascular system 5-hydroxytryptamine (5-HT, serotonin), has also potent immuno-regulatory properties. Furthermore, elevated levels of free serotonin have been detected in the serum of symptomatic asthmatics. However, the exact serotonergic receptor subtypes involved have not been elucidated, yet. In this study we questioned whether the subtype 5-HTR1B is involved in the pathogenesis of experimental asthma. Expression of 5-HTR1B receptors in lung tissue was analyzed by immunohistochemistry. Allergic airway inflammation was studied in the classical OVA-alum model and in a model of house dust mite (HDM) induced airway inflammation. The experimental induction of allergic airway inflammation led to an increased

5-HTR1B expression in the lungs of animals sensitized to OVA and challenged with OVA-aerosols. 5-HTR1B expression was even higher in animals with chronic asthma. To confirm the in-vivo relevance of this finding, animals sensitized to OVA were treated intratracheally with the selective 5-HTR1B antagonist NAS 181 prior to challenge with OVA aerosols resulting in decreased allergic airway inflammation as depicted by reduced broncho alveolar lavage eosinophils and lymphocytes, by reduced perivascular and peribronchial inflammation, as well as by reduced production of Th2 cytokines by re-stimulated mediastinal lymph node cells. The intratracheal application of NAS 181 had also protective effects in HDM-induced asthma.

Here we provide evidence that 5-HTR1B receptors modulates asthmatic airway inflammation. Therefore, 5-HTR1B receptors might be promising targets for the development of new anti-asthmatic drugs.

P3741

The antimicrobial peptide CRAMP protects against emphysema

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Rationale: COPD is one of the most prevalent causes of death worldwide and is associated with an ongoing destruction of pulmonary structures that finally lead to the development of emphysema. Antimicrobial peptides (AMP) are a part of the innate immune system. The AMP CRAMP is the murine homologue to the human cathelicidin antimicrobial peptide (hCAP-18/LL-37). It plays an important role in angiogenesis, cancer and chemotaxis. This work will show that CRAMP is important for tissue regeneration in an elastase induced model of lung destruction. **Methods:** Lung destruction was initiated by two times intratracheal administration of elastase. Ten days after the last administration lung function was measured. Cytokines in BAL were measured by ELISA. The lungs were resected and fixed for stereological analysis. Lung tissue was also used for immunohistological staining and RNA-extraction.

Results: Elastase treated CRAMP-ko animals had significantly higher neutrophil influx, more IL1- β and TNF- α in their BAL as the controls. 30 days after the first elastase treatment CRAMP-ko had a higher mean linear intercept and a significantly decreased pulmonary system resistance and elastance. MMP-9 expression and activity was increased and the concentration of VEGF was decreased in the elastase treated CRAMP-ko as compared to the elastase treated wildtype mice.

Conclusion: This work shows that the antimicrobial peptide CRAMP has a protective function in a model of elastase induced lung destruction. CRAMP-ko animals showed more inflammation, a higher degree of lung destruction as well as a higher expression and activity of MMP-9. The concentration of VEGF was significantly lower in the elastase treated ko animals.

P3742

Importance of the receptor for advanced glycation end products in the respiratory mechanics

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Background: There is an increasing clinical interest in studying the receptor for advanced glycation end products (RAGE) and its soluble forms in pulmonary diseases. Interestingly, RAGE and its soluble forms are preferentially expressed in alveoli thereby challenging the pathophysiological role of RAGE. As we have already shown the importance of RAGE as an adhesion molecule in alveolar cells, this study aimed to investigate the age-dependent physiological significance of RAGE in respiratory mechanics.

Methods: Lungs of young (<6 month), adult (6-9) and old (>24) RAGE knock out (k.o.) and wild-type mice were analyzed *ex vivo* using the perfused isolated lung system with negative-pressure ventilation at weight-matched constant tidal volume. Elastin expression was assessed by gene array and histochemistry.

Results: Dynamic lung compliance increased gradually with the age in RAGE k.o. as well as in wild-type mice. Independent of age, the lungs of RAGE k.o. mice showed higher compliance than that of the wild-type. In this context, old wild-type and young-adult RAGE k.o. mice had similar lung dynamic compliance. According to the effect of RAGE deficiency on lung compliance, we determined a reduced elastin mRNA and protein expression in the lung tissue of RAGE k.o. mice. However, lack of RAGE had no significant effect on airway resistance and epithelial layer permeability.

Conclusion: Our study suggests the physiological importance of RAGE and/or its soluble forms in mediating an appropriate lung compliance in which its/their impact on the elastin expression might play a critical role.

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Long-term exposure to tobacco smoke on alveolar macrophages phenotype with regard to genetic and age predisposition

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Core role of tobacco smoke as a risk factor in chronic obstructive pulmonary disease (COPD) is proved, but COPD develops not in every smoker.

Objective: To estimate the effect of long-term tobacco smoke on a functional phenotype of alveolar macrophages (A:M) with regard to genetically determined macrophages phenotype while aging.

Methods: Experimental COPD (eCOPD) was simulated in vivo in two genetic mice strains: C57/BL6 (n=40)-predominant M1 phenotype, and Balb/c (n=40)-M2 phenotype. eCOPD groups included 30 mice, control-10 mice. Tobacco smoking lasted for 6 months-2 cigarettes t.i.d. COPD was verified histologically. AM functional phenotype was assessed by nitric oxide production (NO) spectrophotometrically.

Results: COPD was confirmed histologically in both eCOPD groups, changes were more expressed in C57/BL6. There was no significant difference in basal NO production (bNO) in eCOPD groups, but induced NO production (iNO) significantly decreased during 6 months and was much lower in C57/BL6 than in BALB/c: 3.80 ± 0.21 vs 5.53 ± 0.29 mkM ($p < 0.05$). There were no differences in initial bNO in both controls; bNO significantly decreased in both controls during 6 months. Aging decreased iNO in both controls - from 26.40 ± 3.2 to 6.60 ± 0.45 mkM in C57/BL6, and from 19.21 ± 1.20 mkM to 7.57 ± 0.72 mkM in BALB/c, changes were more expressed in C57/BL6.

Conclusions: We elicited genetic predisposition to COPD risk factor - tobacco smoke, associated with M1 macrophages phenotype and age-related transformation of AM phenotype towards antiinflammatory M2, increasing with long-term inhalation of tobacco smoke and more expressed in M1 phenotype.

P3744

Cell therapy with adipose tissue-derived stem/stromal cells for elastase-induced pulmonary emphysema in rats

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Background: Studies demonstrating lung repair by stem cells or growth factors have been reported in animal emphysema. We focused on adipose tissue-derived stromal/stem cells (ASC) for regenerative medicine, since it has a high potential to secrete multiple angiogenic factors and differentiate various kinds of cells.

Aim: To demonstrate the therapeutic impact of ASC transplantation and to elucidate mechanisms of the effects in rat emphysema models.

Methods: ASC were isolated from rat subcutaneous adipose tissue. Emphysema was induced by intratracheal instillation of porcine pancreatic elastase (PPE). One week after PPE, cell transplantation was performed intravenously. One and 2 weeks after transplantation, we assessed pulmonary function and histopathological changes and measurement of chemokine levels in lung tissue.

Results: ASC transplantation restored pulmonary function to near normal levels and enlargement of the alveolar airspaces was also inhibited. Immunohistochemical analysis revealed some transplanted ASC were localized at damaged alveolar spaces. Vascular endothelial growth factor (VEGF) was significantly reduced by PPE. After ASC transplantation, VEGF level was not reduced. Hepatocyte growth factor (HGF) and cytokine-induced neutrophil chemoattractant-1 (CINC-1) levels were significantly higher than PPE.

Conclusion: Transplantation of ASC for emphysema rats improved pulmonary function and inhibit enlargement of the airspaces. Secretion of HGF, VEGF, and CINC-1 by surviving ASC after transplantation may have contributed to lung repair. Cell therapy with ASC may be new therapeutic strategy to improve pulmonary function and inhibit alveolar destruction.

P3745

Differential effects of atorvastatin, pravastatin, rosuvastatin and simvastatin on lungs from mice exposed to cigarette smoke

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Short-term cigarette smoke (CS) exposure leads to acute lung inflammation through its influence on oxidants/antioxidants imbalance, but lately statins have shown anti-inflammatory and antioxidant effects. Therefore, we aimed analyzing the effects of four different statins on the lungs of mice exposed CS. Male C57BL/6 mice were divided into six groups (n=8 each): Mice exposed to the smoke from 12 cigarettes/day/5 days (CS group); exposed to smoke from 12 cigarettes per day for 5

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days plus atorvastatin (10 mg/kg/day; CS+A group), or pravastatin (5 mg/kg/day; CS+P group), or rosuvastatin (5 mg/kg/day; CS+R group) or simvastatin (20 mg/kg/day; CS+S group); control group was sham-smoked. One day after the last CS exposure, mice were sacrificed, the bronchoalveolar lavage fluid (BAL) was performed and the lungs were removed for histological analysis and homogenized for biochemical analyses. Oxidant levels were reduced in CS+S ($p<0.05$); DPPH levels were increased in CS+A, CS+R and CS+S ($p<0.05$); nitrite levels were reduced in CS+P, CS+R and CS+S ($p<0.05$); MCP-1 levels were reduced in CS+R and CS+S ($p<0.01$); hydroperoxides levels were reduced in CS+A, CS+R and CS+S ($p<0.001$); catalase activity was reduced in CS+P ($p<0.01$); SOD activity were reduced in CS+A, CS+P ($p<0.01$) CS+R and CS+S ($p<0.05$) all when compared with CS group. These results suggest that simvastatin is the best treatment for acute lung injury induced by CS due to reduction of inflammatory and oxidant markers.

P3746

Pulmonary function, oxidative stress and inflammatory markers in LPS-induced acute lung injury: Differential effects of atorvastatin, pravastatin and simvastatin

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The present study was designed to determine what statin could attenuate acute lung injury (ALI) induced by lipopolysaccharide (LPS) in C57BL/6 mice. Young male mice (± 23 g) were divided into 5 groups (n=6 each): injected with LPS i.p. (10 mg/kg), LPS plus atorvastatin (10 mg/kg/day; LPS+A group) or pravastatin (5 mg/kg/day; LPS+P group) or simvastatin (20 mg/kg/day; LPS+S group). Control group received saline (i.p.). In a separated group of mice (n=5) the sum of pulmonary resistive and viscoelastic pressures (DeltaPtot) and static elastance (E(st)) were measured. One day later (24 h), the animals were sacrificed, BAL performed and lungs were removed for histopathological analysis and homogenized for biochemical analyses. The amount of leukocytes was lower in LPS+P ($p<0.01$) and LPS+S ($p<0.05$). Cytokine levels of MCP-1 was lower in LPS+P ($p<0.01$) while IL-6 was lower in LPS+P ($p<0.01$) and LPS+S ($p<0.05$). Redox markers (super-oxide dismutase and catalase) were lower in LPS+A ($p<0.01$). Lipid peroxidation (malondialdehyde and hydroperoxides) were lower in all treated groups ($p<0.05$). Myeloperoxidase was lower in LPS+P ($p<0.01$). DeltaPtot and E(st) were significantly higher in the LPS group than in the other groups. Our results suggest that atorvastatin and pravastatin, but not simvastatin, exhibits anti-inflammatory and antioxidant actions in LPS-induced ALI.