153. Basic and translational studies in COPD

P1423

Cigarette smoke upregulates IL-8/CXCL8 expression by augmenting mRNA stability *via* p38 MAPK/MK2 signalling in normal human pulmonary cells

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Interleukin-8 (IL-8/CXCL8) is an important neutrophil chemoattractant known to be elevated in the airways of cigarette smokers and in patients with chronic obstructive pulmonary disease (COPD), a syndrome associated with chronic cigarette smoking. We examined the acute effect of aqueous cigarette smoke extract (CSE) on IL-8 expression in normal human bronchial smooth muscle cells (HBSMC) and alveolar macrophages. CSE upregulates IL-8 mRNA levels in a concentration and time-dependent manner and such an effect was accompanied by IL-8 secretion into the extracellular medium. CSE-evoked elevation of IL-8 mRNA was mimicked by its component acrolein at concentrations (3-30μM) found in CSE. Both CSE and acrolein induced p38 mitogen-activated protein kinase (MAPK) phosphorylation which was accompanied by the phosphorylation of MAPK-activated kinase 2 (MK2), a known downstream substrate of the p38 MAPK. In both HBSMC and human alveolar macrophages, pharmacological inhibition of p38 MAPK or MK2 strongly accelerated the decay of IL-8 mRNA levels upon stimulation with CSE or acrolein and subsequent blockade of mRNA neosynthesis with actinomycin D. Conversely, pharmacological inhibition of extracellular-signal-regulated kinases 1/2 (ERK1/2) signalling did not affect mRNA stability but inhibited both CSE- and acrolein-induced steady-state levels of IL-8 mRNA, suggesting a transcriptional effect. In sum, p38 MAPK/MK2 signalling appear to be an important post-transcriptional mechanism underlying CSE-induced IL-8 mRNA upregulation.

P1424

Pharmacological inhibition of epidermal growth factor receptor modulates cigarette smoke-induced CXCL8 release but not VEGF release

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Cigarette smoke is the most important risk factor for the development of Chronic Obstructive Pulmonary Disease. A large body of evidence exists indicating that cigarette smoke is able to activate the Epidermal Growth Factor Receptor. We have previously established that aqueous cigarette smoke extract (CSE) stimulates CXCL8 and VEGF release from normal human lung fibroblasts (NHLF) and airways smooth muscle cells (ASMC) and that these effects are mediated by the α,β-unsaturated aldehydes contained in the CSE such as acrolein. Here we examined the effect of pharmacological inhibition of EGFR on CXCL8 and VEGF release induced by CSE and acrolein. We found that the EGFR inhibitors AG1478, gefitinib and PD153035 inhibit CSE- and acrolein-induced CXCL8 release in both NHLF and ASMC, but do not affect neither TNFα-induced CXCL8 release nor CSE-induced VEGF release. We have previously shown that CSE-evoked CXCL8 and VEGF release was accompanied by a rapid p38 MAPK phosphorylation mimicked by acrolein. Because p38 MAPK phosphorylation is one of the possible downstream pathway activated by EGFR, we examined the effects of EGFR inhibitors on p38 MAPK phosphorylation. We observed that all three EGFR inhibitors failed in modifying p38 MAPK phosphorylation evoked by CSE. In sum, pharmacological inhibition of EGFR suggests that EGFR is involved in CXCL8 release through a p38 MAPK independent mechanism. Given the pivotal role of CXCL8 and VEGF as neutrophil chemoattractant and angiogenic factor respectively, this study sheds light on the different mechanisms through which cigarette smoke can orchestrate inflammation and vascular remodeling in the lung.

P1425

Cigarette smoke suppresses mast cell maturation and cytokine release independent of TLR4 signaling

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Chronic obstructive pulmonary disease (COPD) is a multicomponent disease characterized by emphysema and/or chronic bronchitis. COPD is mostly associated with cigarette smoking. Many inflammatory cells are present in the airways of patients with COPD. Cigarette smoke contains over 4,700 chemical compounds, including free radicals and LPS (a Toll Like Receptor 4 agonist) at concentrations which may contribute to the pathogenesis of diseases like COPD. Toll-like receptors (TLRs) are an integral part of the innate immune system and these receptors recognize conserved pathogen-associated molecular patterns. We have previously shown that cigarette smoke medium (CSM) can stimulate several inflammatory cells via TLR4 and that CSM reduces the degranulation of bone marrow-derived mast cells (BMMCs). Moreover, CSM causes the release of chemokines but reduces IgE/antigen-induced degranulation and cytokine release. Interestingly, CSM had no effect on the surface expression of the IgE receptor (FcRI), but did reduce Syk kinase signaling. In the current study, the effect of CSM on mast cells maturation and function was investigated during a prolonged time period of 3 weeks. Co-culturing of BMMC with CSM during the last week suppressed the number of granules, degranulation and the release of Th2 and Th1 cytokines. Moreover, the surface expression of c-Kit and FcRI receptors were decreased. Interestingly, these effects were not observed with LPS. Thus, we conclude that CSM differentially affects mast cells dependent upon the duration of exposure and that these effects are TLR4-independent.

P1426

Regulation of immunoproteasomes by cigarette smoke

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Cigarette smoke (CS), as a major source for oxidative stress in the lungs, is the main cause of chronic obstructive pulmonary disease (COPD). Patients suffering from COPD are more susceptible to viral infections resulting in acute exacerbations. Viral infections induce expression of immunoproteasomes (IP) via IFNγ-signaling. This specialized form of proteasome is destined to improve antigen presentation in infected cells and to efficiently degrade oxidatively damaged proteins. The role of IPs in COPD pathology is unknown.
Expression levels of IP-subunits LMP2 and LMP7 were evaluated in wildtype (wt) as well as $LMP2^{-/-}$ and $LMP7^{-/-}$ deficient mice in whole lung homogenates. Of note, we observed pronounced expression of IP-subunits in wt lungs compared to other organs. Immunohistochemical analysis of lung sections revealed that IP positive staining was observed in cells adjacent to airways, but also in alveolar regions. To study regulation of IP *in vitro*, we analyzed expression of IP in different lung cell lines. IFNγ induced pronounced expression of IP in both lung epithelial and fibroblasts cells, as detected by qRT-PCR and western blotting. We then investigated regulation of IP by CS. A549 human lung epithelial cells were treated with extracts of CS for 24h up to 12 days, and expression of IP was investigated on RNA and protein level. Long-term treatment of cells with CS-extract resulted in downregulation of basal IP expression. *In vivo*, wt mice exposed to CS for 3 days also showed significant downregulation of IP on the protein level. Diminished expression of IP due to smoke exposure may affect antigen processing of viral proteins and thus add on severity and delayed resolution of viral infections in COPD exacerbations.

P1427

Effect of cigarette smoke on peripheral blood Th17 (PBTh17) cells from COPD patients. Role of acetylcholine

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PBTh17 cells producing IL-17A and IL-22, as well as Acetylcholine (ACh) are involved in the systemic inflammation of several autoimmune and inflammatory diseases, including COPD.
We investigated the effect of cigarette smoke extract (CSE) and the role of ACh on the expression of RORgt, IL-17A and IL-22 in PBTh17 cells during systemic inflammation in COPD.
ACh, IL-17A, IL-22 and RORgt levels as well as the colocalization of ACh with IL-17A, IL-22 and RORgt were evaluated (flowcytometry) in PBT-lymphocytes (PBT) from COPD patients (n=16), healthy smokers (HS) (n=12) and healthy control subjects (HC) (n=13). Furthermore, PBT cells from COPD (n=6) patients and PBT cells from HC (n=6) stimulated in vitro with CSE (10%) were cultured for 72 hrs in the presence or absence of Tiotropium (Spiriva®) (20 nM) and Olodaterol (1 nM) alone or in combination.
The colocalization of ACh with IL-17A, IL-22 and RORgt was significantly increased in PBT cells from COPD patients when compared to HC and HS subjects as well as in PBT cells from HC stimulated with CSE when compared with unstimulated cells. Tiotropium and Olodaterol alone reduced the increased levels of colocalization between ACh with IL-17A, IL-22 and RORgt in cultured PBT-cells from COPD patients and in PBT-cells from HC subjects stimulated with CSE.
We suggests that cigarette smoke might be able to increase the levels of ACh in PBT cells promoting the switch into PBTh17 cells which produce IL-17A and IL-22 during the systemic inflammation in COPD. The use of anticholinergics as Tiotropium and long-acting β2-agonists as Olodaterol might prevent these events.
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P1428

Role of interferon-γ in tumor necrosis factor-α-mediated increase of lung microvascular endothelial cells

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Background: Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease in which tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) are overexpressed and have been suggested to play pathogenic roles. The effect of these agents on angiogenesis in the lung tissues of COPD is unknown.
Objective: To examine the effects of these mediators on lung endothelial cells (ECs).
Methods: *Nrf2* knockout mice were exposed to cigarette smoke (CS) for 4 weeks, and the down-regulated genes referring to vascularity in the whole lung were identified by microarray analysis. To confirm the protein levels, which were indicated in the microarray data, co-cultivation of lung fibroblasts with ECs in the presence of TNF-α and IFN-γ was performed, thereafter ECs were submitted to an examination of protein levels expression using immunoblotting or immunocytochemistry.
Results: Microarray analysis data have shown that the mRNA expression of angiomotin-like protein 1 (AmotL1) decreased in response to CS when compared to no exposure to CS. TNF-α enhanced vascular endothelial growth factor (VEGF) production by cultured lung fibroblasts, however, vascularity was decreased when treated with IFN-γ. In addition, IFN-γ induced tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors on ECs and attenuated the expression of AmotL1 localized to endothelial cell-cell junctions.
Conclusions: These results suggest that IFN-γ acts as anti-angiogenesis by regulating the expression of TRAIL receptors and AmotL1 on ECs, which were induced by the enhanced VEGF production by TNF-α-stimulated lung fibroblasts.

P1429

Second hand smoke exposure impairs CD39 expression and function in the lung

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Chronic second hand smoke (SHS) exposure is the main risk factor for non-smokers to develop chronic obstructive pulmonary disease COPD. The mechanisms behind the chronic inflammation and lung destruction are not completely understood. In response to injury vascular and blood cells release ATP that is implicated in regulating cellular and immune responses. Plasma membrane ecto-nucleotidase CD39 hydrolyzes ATP to ADP and AMP. Ecto-5'nucleotidase CD73 hydrolyzes AMP to adenosine. Here we investigated the contribution of CD39/73 expressing inflammatory cells in lung remodeling in response to SHS exposure.
Sprague Dawley rats were exposed to SHS in a smoking chamber (total particulate matter levels 115 mg/m^3). The expression levels of CD39 and CD73 in the inflammatory cells and lung tissue were determined by flow cytometry, real time PCR and Western blot analysis.
Chronic second hand smoke exposure resulted in the development of emphysema in rats as measured by MLI (87.2±1.5 mm after 2 month of exposure versus 73.4±1.1 mm in room air exposed controls). The CD39 expression was significantly downregulated in the whole lung tissue on mRNA and protein levels. Moreover, CD39 expression was decreased in the lung tissue from COPD patients. Cigarette smoke extract in vitro almost abolished CD39 and CD73 expression in the alveolar macrophages and vascular endothelial cells.
Second hand smoke exposure impairs ectonucletidase expression in the lungs and leads to accumulation of extracellular ATP that confers increased proinflammatory responses leading to the development of emphysema.
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P1430

TLR4 up-regulation and reduced Foxp3 expression in mechanically ventilated smokers with obstructive chronic bronchitis

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Chronic bronchitis (CB) is a risk factor in chronic obstructive pulmonary disease (COPD) for accelerated lung function decline and increased mortality. The lung and systemic inflammatory and immunological profile of COPD patients with CB which acutely experience respiratory failure upon a disease exacerbation is unknown. In this study, we explored the expression of Foxp3 by western blot analysis, TLR4 by immunocytochemistry and the concentrations of IP-10 and IL-8 by ELISA in the mini-bronchoalveolar lavages (mini-BAL) and in the peripheral blood (PB) of patients with respiratory failure requiring intubation and mechanical ventilation. The recruited subjects were separated into three different groups: smokers with CB and COPD (COPD, n=18), smokers with CB but without COPD (S, n=8) and patients without CB and without COPD (C, n=10).
In mini-BAL of COPD group, Foxp3 and IP-10 were significantly reduced while TLR4 was significantly increased in comparison to C. TLR4 was also increased in mini-BAL of S. In COPD peripheral blood, Foxp3 was reduced in comparison to C but not significant differences were observed for TLR4 and for IP-10. No significant differences were observed for IL-8 concentrations in the mini-BAL and in the blood of the recruited patients. The mini-BAL TLR4 expression correlated with the Clinical Infective Pulmonary Score.
In conclusion, in COPD patients with respiratory failure, lung and systemic reduced immune regulatory events (low Foxp3 expression) and lung increased innate immunity responses (high TLR4 expression) may contribute to the increased inflammatory events leading to respiratory failure.

P1431

Lectins improve efferocytosis via changes to cytoskeletal remodeling: Relevance to COPD

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We have shown that the defective ability of alveolar macrophages (AM) to phagocytose apoptotic cells (efferocytosis) in COPD could be therapeutically improved using mannose binding lectin (MBL) although the exact mechanisms are unknown. A further lectin, galectin-3, is also known to regulate macrophage phenotype and function, via interaction with its receptor CD98 (an 'M2' mediator). We hypothesized that defective expression of MBL and galectin/CD98 would be associated with defective efferocytosis in COPD via effects on cytoskeletal remodeling and macrophage phenotype.
Galectin-3 was measured by ELISA in BAL from controls, smokers and current/ex-smokers with COPD. CD98 was measured on AM using flow cytometry. We assessed the effects of MBL and galectin-3 on efferocytosis, CD98, actin polymerisation, rac activation, and the involvement of PI3K (using β-actin probing and wortmannin inhibition) *in vitro* using human AM.
A significant decrease in galectin-3 was observed in BAL from smokers and COPD subjects vs controls. Galectin 3 and MBL increased efferocytosis via an increase in active GTP bound Rac1. This was confirmed with β-actin probing and the role of PI3k was confirmed using wortmannin inhibition. The increased efferocytosis was associated with increases in available glutathione and expression of CD98.
We provide evidence for a role of airway lectins in the failed efferocytosis in COPD, supporting their further investigation as potential macrophage-targeted therapies.

P1432

Enhanced cytotoxic function of NK and NKT-like cells associated with decreased CD94 (Kp43) in the airway in COPD

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NK and NKT-like cells represent a small but important proportion of effector lymphocytes that we have previously shown to be a major source of pro-inflammatory cytokines and granzymes[1]. We hypothesized that NK and NKT-like cells would be increased in the airway in COPD and that this would be accompanied by a reduction in expression of the inhibitory receptor CD94 (Kp43) and increased expression of the cytotoxic mediators granzyme B and perforin.
We measured NK and NK-like T-cells and their expression of CD94 in the blood of patients with COPD (n=61), smokers (16) and healthy controls (25) and BAL from a cohort of subjects. We further assessed activation by expression of CD69 and cytotoxic potential by production of granzymes A and B and using a cytotoxicity assay.
In *blood* from COPD subjects, there were no significant changes in NK or NKT-like cell numbers or expression of granzyme A or cytotoxic potential vs controls. There was however, increased expression of granzyme B and decreased expression of CD94 by both cell types vs controls.
In the *airway* in COPD, NK and NKT-like numbers were increased, associated with increased NK cytotoxicity, increased expression of granzyme B and decreased expression of the inhibitory receptor CD94.
Treatment strategies that target NK and NKT-like cells, their cytotoxicity and production of inflammatory mediators in the airway may improve COPD morbidity.

P1433

The role of IL -17 and lymphoid follicles in the pathogenesis of COPD

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Chronic Obstructive Pulmonary Disease (COPD) is one of the most prevalent respiratory diseases in the world. There is no definitive treatment to arrest the progressive loss of lung function characteristic of COPD, partly due to a lack of understanding of the underlying cellular pathophysiological mechanisms. In individuals with severe COPD, there is an accumulation of adaptive immune cells as well as an increase in the frequency of lymphoid follicles in the lung; however, the role of lymphoid follicles in driving the disease and the factors that promote their formation are largely unknown. In addition, individuals with COPD exhibit elevated levels of IL-17, a cytokine that is associated with autoimmunity and was recently shown to promote lymphoid neogenesis. We have modeled different

severities of COPD by incremental instillation of LPS and elastase in mice. We observed an increase in the levels of IL-17A following repeated challenges, which coincided with the progressive drop in lung function as well as the appearance of lymphoid follicles. IL-17A production was in part triggered by the engagement of Toll Like Receptor 3 implicating a role for an endogenous danger signal in COPD. Neutralization of IL-17A at specific times after the initiation of disease ameliorated the impaired lung function and affected B Cell and macrophage activation states. Our data indicates that IL-17A is involved in COPD progression by induction of lymphoid follicles and regulation of both innate and adaptive immunity.

P1434

Expansion of alveolar-lymphoid interfaces in lungs of patients with COPD

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Rationale: Although adaptive immune responses are critical for combating distal airway infections in COPD lungs, the structural basis for alveolar antigen uptake has remained poorly investigated. This study investigates the interface between alveolar lumen and the adaptive immune system at different severities of COPD.
Methods: Lung resections (n=31) from mild (GOLD I), moderate-severe (GOLD II-III), and very severe (GOLD IV) COPD were subjected to detailed histological assessment of components of the adaptive immune system in distal lung. Never-smokers and non-COPD smokers served as controls (n=15).
Results: In COPD, both numbers and mean size of lymphoid aggregates were increased in small airways, pulmonary vessels and the alveolar parenchyma. Irrespective of anatomical localization, the vast majority (88%) of the aggregates had direct contact with alveolar luminal spaces (37% of the aggregate perimeter). In advanced COPD, the epithelium at alveolar-lymphoid interfaces had transformed into a significantly higher columnar phenotype (p=0.02) that, apart from expressing immune-regulatory molecules, contained increased numbers of langerin-positive dendritic cells (p=0.02). Also, the total alveolar-lymphoid interface and interface-associated dendritic cells were increased (p=0.02 and p=0.002, respectively).
Conclusions: The progression of COPD is linked with an expansion and remodeling of alveolar lumen-lymphoid interfaces. These alterations, which predict an increased capacity to respond to alveolar antigens, correlated with lung function parameters and may thus contribute to the aggravated inflammation in COPD lungs.

P1435

Diverse and altered distribution patterns of TLR5 and TLR7 in the distal lung of COPD patients

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Activation of the innate immune system in the distal lung is a hallmark of COPD. Toll-like receptors (TLRs) trigger innate immune responses to pathogens. Although COPD patients are susceptible to infections, the distribution patterns of TLRs at different stages of disease have remained poorly studied. The present study characterizes TLR5 and TLR7 in the distal lung of COPD patients.
Methods: GOLD I (n=6), GOLD II-III (n=13), GOLD IV patients (n=8), and controls (never smokers and smokers) (n=13) were enrolled in this study. Immunohistochemical staining was used to identify TLR5 and TLR7 positive cells.
Results: TLR5 immunoreactivity was identified in sub-epithelial glands, airway smooth muscle, CD68+ macrophages, CD138+ plasma cells, CD208+ type II pneumocytes and the small airway epithelium. In control subjects and mild COPD epithelial TLR5 had a foremost apical distribution. In contrast, in advanced COPD the distribution of epithelial TLR5 shifted into a distinct basolateral expression that was higher in GOLD IV (p=0.002) compared to controls. TLR7 displayed a peri-nuclear expression in the small airway epithelium irrespective of the study group. However, the total epithelial TLR7-immunoreactivity was upregulated in GOLD IV-patients compared to never smokers (p=0.009). TLR7 expression was also detected in S100B+ nerve cells, CD68+ macrophages, B- and T-lymphocytes, and CD56+ NK cells.
Conclusion: Both epithelial TLR5 and TLR7 are upregulated in advanced COPD. Whether the altered expression reflects a natural adaption to the increased pathogen burden in advanced COPD or is part of a dysfunctional immune-regulation in COPD remains to be determined.

P1436

Metabolomic fingerprinting in the identification of biomarkers in COPD patients

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Introduction: Metabolomics fingerprinting is able to achieve the identification of novel biomarkers the comprehensive characterization of the entire metabolome of a disease. The main objective is to use it to understand the pathological basis underlying COPD and its relationship to the severity and phenotypic characteristics.
Methods: Observational case-control study involving COPD patients and controls without COPD or cardiovascular history. COPD patients were grouped in chronic bronchitis and emphysema, and at different stages of GOLD. Different platforms are required to capture all metabolites in one sample of plasma and separation techniques such as gas chromatography coupled to mass spectrometry (GC-Q-MS).
Results: This study involved 48 participants; 32 COPD patients (22 men, 10 women) and 20 healthy controls (7 men, 9 women). Mean age: 58,3 and 56,6 respectively. Mean FEV1 value was 64,3%. We can not establish significant differences between COPD and controls in the total population. In women, we can differentiate COPD from the controls by metabolites such as glycolic acid, although its origin is uncertain and could be related to medication. This metabolite, in women, also helps to differentiate emphysema (and smokers) from controls, emphysema and chronic bronchitis from controls and smokers from non smokers. With oxalic acid we can differentiate chronic bronchitis (and smokers) from controls in women, and emphysema from chronic bronchitis in men with COPD (and former smokers). Besides, in this last group, we can differentiate GOLD I from GOLD II with myristic acid.
Conclusion: Using metabolomic fingerprinting in plasma we could identify markers and differentiate phenotypes and early stages in COPD.

P1437

Comparable matrix alterations in the alveolar and small airway wall of COPD patients

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Rationale: Remodeling in COPD is considered twofold with thickening of the wall of airways <2mm on one hand and destruction of alveolar walls on the other. However, we hypothesize that matrix alterations in both alveolar and small airway (SA) walls of COPD patients show more similarity.
Methods: Lung tissue sections of 8 smoking controls and 10 moderate to severe COPD patients were stained for elastin by Weigert's Resorcin-Fuchsin and collagen 1, 2 and 3 by Picrosirius red. In addition, hyaluronan, a glycosaminoglycan matrix component, was stained with a hyaluronan binding protein. All stainings were corrected for total surface area and data expressed as mean % of stained area ± SD.
Results: Elastin was significantly decreased in COPD in both alveolar (27.7% ± 5.3 vs. 17.9% ± 3.3, $p<0.01$) and SA walls (24.2% ± 4.4 vs. 15.2% ± 2.7, $p<0.01$). Both collagen in alveolar (11.6% ± 5.4 vs. 25.9% ± 10.4, $p<0.01$) and SA walls (11.8% ± 2.3 vs. 23.2% ± 6.7, $p<0.01$) and hyaluronan in alveolar (11.7% ± 3.2 vs. 19.8% ± 3.5, $p<0.01$) and SA walls (12.9% ± 5.4 vs. 25.6% ± 10.0, $p<0.05$) increased significantly. Alveolar and SA wall matrix components correlated significantly: elastin ($r=0.644$, $p<0.01$), collagen ($r=0.741$, $p<0.01$) and hyaluronan ($r=0.626$, $p<0.05$). Furthermore matrix compounds were significantly related with FEV_1: alveolar elastin ($r=0.742$, $p<0.01$), SA elastin ($r=0.824$, $p<0.01$), alveolar collagen ($r=-0.755$, $p<0.01$), SA collagen ($r=-0.675$, $p<0.01$), alveolar hyaluronan ($r=-0.775$, $p<0.01$) and SA hyaluronan ($r=-0.538$, $p<0.05$).
Conclusion: These results indicate that remodeling in the alveolar and SA wall in COPD show marked similarities and both relate to FEV_1.

P1438

Corticosteroid insensitivity in airway smooth muscle cells of severe asthma and COPD: Modulation by IFN-γ

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Background: Patients with severe asthma or COPD respond poorly to corticosteroids (CS). IFN-γ impairs CS response in airway smooth muscle cells (ASMCs) of healthy subjects.
Aims and objectives: Compare cytokine-induced chemokines and CS response in ASMCs of severe asthma or COPD. Investigate the effect of IFN-γ on CS response.
Methods: ASMCs of healthy subjects (12), non-severe (NSA; 10) and severe asthma (SA; 10), smokers (8) and COPD (8), were obtained via endobronchial biopsy. At passage 4-5, cells were pretreated with dexamethasone (Dex; 10^{-10}-10^{-6} M; 2 hr) and stimulated with TNF-α/IFN-γ (10 ng/mL each; 24 hr). CCL11 and CXCL8 release and mRNA were assessed by ELISA and qRT-PCR, p65 NF-κB promoter recruitment by ChIP, and p38 MAPK activity by Western Blot.
Results: Baseline and TNFα-induced CCL11 release/mRNA were increased in NSA, and CXCL8 increased at baseline in smokers and COPD. Dex suppression of induced chemokines was impaired in SA, smokers and COPD. IFN-γ inhibited i) induced CCL11 and CXCL8; ii) p65 recruitment to chemokine promoters, and iii) Dex suppression of induced chemokines in the healthy. In SA, IFN-γ further reduced suppression of only CXCL8. Paradoxically, IFN-γ improved the suppressive effect of Dex on CXCL8 in smokers and COPD. p38 MAPK activity was raised in SA, and inhibition of p38 restored corticosteroid sensitivity. However, IFN-γ did not modulate induced p38 activity.

Conclusions: ASMCs of SA, smokers and COPD display CS insensitivity. IFN-γ impairs the suppressive effect of Dex on CXCL8 in the healthy and asthmatics but improves it in smokers and COPD, suggesting differential mechanisms underlying CS insensitivity in SA and smokers/COPD.

P1439

Inhaled corticosteroids (ICS) attenuates epithelial mesenchymal transition (EMT) in COPD: A key to understanding long term benefits?

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Introduction: We recently published that EMT is an active process in COPD airways. Our knowledge about the effects of ICS on this process in COPD is very limited.

Objective: To assess the effects of ICS on EMT in endobronchial biopsies (ebb) from COPD patients.

Methods: A double-blinded, randomized, placebo-controlled study assessed the effects of inhaled fluticasone propionate (FP; 500μg twice daily) on EMT in 34 COPD patients. Ebb were assessed for EMT related reticular basement membrane (Rbm) fragmentation and immunostained for the EMT signatures S100A4 (a fibroblast epitope), matrix-metalloproteinase-9 (MMP-9) and epithelial activation marker, epidermal growth factor receptor (EGFR).

Results:

Comparison at baseline and after treatment (FP, n=23 and placebo, n=11)

Markers	Before (FP)	After (FP)	Before (Placebo)	After (Placebo)
% Rbm fragmentation	19.1 (0.2-42.8)*	2.6 (0-88.6)†	24.0 (6.6-100)	26.9 (2.5-48.5)
S100A4 positive cells in BE per mm of Rbm	25.8 (2.4-55.3)*	12.3 (0.6-24.9)†	19.8 (2.9-31.6)	17.4 (10.3-35.5)
S100A4 positive cells in Rbm per mm of Rbm	44.4 (15.3-92.6)*	20.8 (2.6-60.7)†	23.1 (14-82.9)	29.3 (3.6-48.1)
MMP-9 positive cells in Rbm clefts per mm of Rbm	0.6 (0-22.4)*	0 (0-10.6)†	1.1 (0-4.1)	1.3 (0-2.7)
EGFR % epithelium	34 (14.6-59.5)*	5.8 (2.6-43.8)†	14.4 (3.6-38.2)	10.3 (1.3-39.1)

Data expressed as medians and ranges. *No significant difference at baseline. †Significant difference after treatment with FP ($p<0.03$).

Conclusions: This is the first study to report that ICS have potent anti-EMT effects in COPD. This may be a mechanistic link between ICS treatment and long term reduction in smoking-related lung cancer seen in COPD.