406. COPD: clinical studies and animal cell models

P3820

Altered composition of airway basement membranes in COPD

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Rationale: The basement membrane provides a chemical and mechanical structure which is crucial for the mucosal homeostasis in the airways. Thickening of lamina reticularis is a common feature in asthma but the situation in COPD is less clear. Our hypothesis was that there are quantitative and qualitative alterations in airway basement membranes in COPD that influence the integrity of epithelial and mesenchymal cells.
Methods: Lung tissue sections from COPD patients and control subjects were subjected to H & E and picrosirius-RED staining. Sections were also immunostained using antibodies against the small proteoglycans: decorin and biglycan. Fibroblasts were isolated from lung explants from COPD patients (n=8) (GOLD stage IV) and biopsies from control subjects (n=9) and proteoglycan production were examined *in vitro.*
Results: Hematoxylin/Eosin-staining showed an unevenly distributed thickening of lamina reticularis in bronchial and bronchiolar airways from COPD patients but not from control subjects. The thickened areas stained positive for picrosirius RED, which shows the presence of fibrillar collagens (collagen I and III). This was accompanied with increased immuno-positivity for the small leucin-rich proteoglycans decorin and biglycan. Fibroblasts isolated from bronchial specimen from COPD patients had decreased production of the basement membrane stabilizing proteoglycan perlecan ($p<0.05$).
Conclusions: This data indicate that there are qualitative and quantitative alterations of airway basement membranes in COPD. We suggest that the observed alterations are indicators of areas with active remodeling which influence the phenotype of epithelial cells and underlying mesenchymal cells which may result in fibrotic deposition.

P3821

Cigarette smoking inhibits LTA_4H aminopeptidase activity and contributes to chronic neutrophilic airway inflammation and COPD

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Proline-glycine-proline (PGP) acts as a potent neutrophil chemokine and marker for COPD and substrate for the triaminopeptidase (TAP) site of LTA_4H, a bifunctional enzyme made by neutrophils. Normally, LTA_4H degrades PGP and leads to resolution of inflammation but cigarette smoke selectively inhibits TAP activity, preventing PGP breakdown and promoting neutrophil accumulation *in vitro* and in animal models. We hypothesized that neutrophils would be elevated and TAP activity would be diminished in serum and induced sputum (IS) from smokers (CS) compared to non-smokers (NS).
IS was collected, immunoprecipitated (IP), Western blotted (WB), and underwent separate ELISAs specific for LTA_4H and myeloperoxidase (MPO). Serum samples were collected and treated with bestatin, an inhibitor of TAP activity, or normal saline. TAP activity was determined by PGP breakdown, as measured by mass spectrometry.
Smoking causes increased airway neutrophils as detected by MPO levels. Sputum LTA_4H amounts are higher in CS than NS, as measured by WB band intensity and ELISA, even though the TAP activity is similar based on PGP degradation. Consequently, the IP-WB and ELISA along with the TAP assay demonstrate the profound inhibitory effect and impact of cigarette smoke on TAP activity in the airways of CS. The addition of bestatin to sera suggests that TAP activity is systemically inhibited by cigarette smoke.
Smoking inactivates TAP activity in the airways of humans. The effects of smoking seem to be systemic since TAP activity appears to be inhibited in sera from CS as compared to NS. These findings may provide a new link between smoking, chronic neutrophilic inflammation, and COPD.

P3822

Expression patterns of IL-1 receptor 1 in COPD lungs

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Rationale: Recent research in murine models suggests an important role of Interleukin-1 receptor 1 (IL-1R1) signalling in the pathogenesis of COPD. Yet, the features of IL-1R1 expression in human COPD patients remain poorly investigated. This study characterizes the expression of IL-1R1 in lung tissues from COPD patients and control subjects.
Methods: Lung resections were obtained from 27 COPD patients; GOLD I (n=6), GOLD II-III (n=13) and GOLD IV (n=8). Never smoking controls (n=7) and smoking controls (n=6) served as controls. IL-1R1 expression was studied by immunohistochemistry and computerized image analysis. A double staining immunofluorescence protocol visualized IL-1R1 expressing cell populations.
Results: The expression of IL-1R1 in small airway epithelium was significantly higher in GOLD IV vs. GOLD II-III patients ($p=0.03$). In the small airway lamina propria, there was no overall statistical difference between the groups ($p=0.12$), but there was a tendency towards a higher IL-1R1 expression in GOLD IV vs. GOLD II-III patients (uncorrected $p=0.02$). In the endothelium, IL-1R1 expression was similar between the groups (overall $p=0.8$). IL-1R1 was found to be expressed on multiple leukocyte populations such as T-cells, dendritic cells, macrophages and NK-cells.
Conclusions: Our data suggest that at baseline conditions IL-1R1 is expressed in the airway epithelium, endothelial cells, and on multiple subepithelial leukocyte populations. Severe stages of COPD appear to be associated with increased IL-1R1 expression in small airway epithelium and in infiltrating cells in the lamina propria, a phenomenon that may participate in the abnormal immune reactions in advanced COPD.

P3823

Expression of epidermal growth factor receptor (EGFR) in the bronchial epithelium of patients with chronic obstructive pulmonary disease (COPD)

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COPD is a major health problem that is caused predominantly by smoking. In COPD, airway epithelium undergoes alterations which are partially attributed to activation of EGFR. Our aim was to study the expression of EGFR in bronchial epithelium of COPD patients and smokers in comparison to normal bronchial epithelium, to investigate its role in pathogenesis of COPD and in the epithelial alterations that characterize this disease.

Methods: We studied 50 subjects (3 groups): group I (10 non-smoking controls), group II (10 apparently healthy smokers), and group III (30 COPD patients) who was subdivided into subgroup IIIA (COPD current smokers) and IIIB (COPD ex-smokers). Bronchoscopic biopsies were subjected to immunohistochemical staining for EGFR.
Results: Histological epithelial alterations were significantly exaggerated in group III compared to group II with no differences between subgroups IIIA and IIIB. EGFR expression was significantly higher in group II and III than group I. Group III showed significantly higher EGFR expression than group II. Subgroup IIIB had significant higher EGFR expression than IIIA. EGFR had a significant positive correlations with goblet cell hyperplasia and a significant negative correlation with FEV_1 in groups II and III and a significant positive correlation with smoking index in group II.
Conclusion: EGFR plays an important role in epithelial changes in bronchial epithelium in COPD through active smoking, and it has a significant role in regulating mucus production in airway epithelium. Disruption of its cascade may provide a mechanism of airway inflammation by its effect on goblet cells and mucus secretion.

P3824
Expression profiling of Th17 associated molecules in bronchoalveolar cells from patients with chronic obstructive pulmonary disease (COPD)
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Recently, the presence of inflammatory cells expressing IL-17A has been reported in the small airway subepithelium obtained from COPD patients (Eustace A et al. Chest. 2010) which, with further evidence (reviewed in Alcorn JF et al. Annu Rev Physiol. 2010), suggests that Th17 immune response may participate in COPD pathomechanisms. However, to date there has been no data about the presence of IL-17A and Th17 associated cytokines in bronchoalveolar (BAL) cells from COPD patients.
We therefore explored the mRNA expression of key cytokines (IL-17A, IL-17F, IL-21, IL-22 and IL-23) associated with Th 17 immune response in BAL cells from COPD patients (n=27; GOLD stage I=4, stage II=18, stage III-IV=5) and the control subjects (n=25) using quantitative RT-PCR (PSMB2 as a denominator).
Expression profiling did not reveal differences in mRNA expression of IL-17A and other products of Th17 cells (IL-17F, IL-21 and IL-22) between COPD patients and the control subjects ($p>0.05$). However, an activator of Th17 cells, a cytokine IL-23, was upregulated in BAL cells from COPD patients (median of relative expression IL-23/PSMB2: patients, 0.07; controls, 0.02; $p=0.001$).
In conclusion, we show here that IL-23 transcripts are present in unseparated BAL cells from COPD patients. Characterisation of the cell source and of possible functional significance of IL-23 for Th17 mediated immune response will be subject of further investigations.
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P3825
Enhanced expression of IL-18 receptor but not soluble form of IL18R in chronic obstructive pulmonary disease (COPD)
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We have recently reported that a significant correlation exists between serum levels of IL-18 and pulmonary functions in COPD patients (Imaoka, ERJ 2008). These results suggest that IL-18R expression can be over-expressed in the lungs of COPD. More recently, we isolated and characterized the soluble human IL-18Ra complex (sIL-18Ra) from human serum. We also found that serum levels of the complex in RA patients were significantly higher than in healthy controls (submitting). In this study, we examined whether the expression of IL-18Ra proteins is increased in the lungs obtained from very severe COPD patients who underwent for lung volume reduction surgery. We also examined the serum levels of sIL-18Ra in non-smokers, smokers and COPD patients. We used immunohistochemical techniques to examine the expression of IL-18Ra in the lungs of very severe COPD (GOLD Stage IV) (n = 16), smokers (n = 13), and non-smokers (n = 13). IL-18Ra proteins were strongly expressed especially in alveolar macrophages, mononuclear cells, and both the bronchiolar and alveolar epithelia in the lungs of COPD, but not smokers nor non-smokers. Interestingly, serum levels of sIL-18Ra in 100 COPD (GOLD stage I [n=28], II [n=34], III [n=22], and IV [n=16]), 36 smokers and 51 non-smokers were 41.6±8.1, 53.1±17.5, and 58.6±11.8 ng/ml, respectively. Interestingly,serum levels of sIL-18Ra in COPD (n=100) were significantly ($P<0.001$) lower than seen in non-smokers (n=51). Overexpression of IL-18Ra proteins in the lungs may be involved in the pathogenesis of COPD.

P3826
Roflumilast potently inhibits CXCL1 mediated neutrophil migration in COPD patients
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Chronic obstructive pulmonary disease (COPD) is associated with glucocorticosteroid insensitive inflammation. Neutrophils from COPD patients migrate more in response to CXCL1 than cells from control subjects. Roflumilast, a PDE4 inhibitor recently approved for COPD in the EU, reduces neutrophil numbers in sputum from COPD patients. The mechanism of this anti-inflammatory effect is not clear, but may be due to inhibition of neutrophil migration. Thus this study investigated the effects of roflumilast N-oxide (RNO), the active metabolite of roflumilast, on neutrophil migration in comparison with another PDE4 inhibitor, rolipram.
Neutrophils were isolated from whole blood samples from healthy subjects and COPD patients. Chemotaxis was measured using 48-well Boyden chambers and $[Ca^{2+}]_i$ by fluorimetry.
Both RNO and rolipram completely inhibited migration of neutrophils from healthy subjects towards CXCL1 (3nM) with EC_{50} values of 0.18±0.13 nM and 2.8±0.51 nM, n=4, respectively. A similar response was seen with neutrophils obtained from patients with COPD (EC_{50} RNO: 0.74±0.89 nM; rolipram 42±45.9 nM, n=3). Having established that cell migration was attenuated by PDE4 inhibitors, the effects of these compounds on CXCL1 stimulated $[Ca^{2+}]_i$ was investigated. RNO (1μM) inhibited the peak of $[Ca^{2+}]_i$ and area under the curve (AUC) by 83.0±2.6% and 90.3±2.1%, n=6. Rolipram (1μM) also inhibited these responses by 80.7±3.2% and 86.3±2.1%, n=6.
These data indicate that oral PDE4 inhibitors may have added anti-inflammatory effects by reducing CXCL1 stimulated neutrophil migration into the lung via regulation of $[Ca^{2+}]_i$ and this could contribute to the anti-inflammatory effects of roflumilast in the treatment of COPD.

P3827
Apocynin reduces H_2O_2 and NO_2^- concentrations in exhaled breath condensate of COPD patients
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It is well known that COPD is a preventable and treatable disease, characterized by not fully reversible airflow limitation. In individuals with COPD, there is a marked exacerbation of the inflammatory response, which increases with the progression of the disease. The molecular mechanism of exacerbations remains unknown; however, prolonged oxidative stress have been reported as potentially responsible for the exacerbation. Apocynin is an agent that inhibits activation of an enzyme responsible for ROS generation and thus – probably, alleviate inflammatory process.
Therefore, we investigated the effect of nebulized apocynin in fourteen COPD patients in placebo-controlled, cross-over design study. Exhaled breath condensate was collected in three timepoints (30, 60 and 120 min.) after apocynin/placebo application and H_2O_2 NO_2^- and NO_3^- concentrations have been evaluated. Moreover, safety parameters have been controlled throughout the study.
Apocynin reduced hydrogen peroxide concentration in exhaled breath condensate 60 and 120 minutes after apocynin nebulization comparing to placebo (0.43 μM vs. 0.59 μM and 0.41 μM vs. 0.58 μM respectively, $p<0.05$). Interestingly, apocynin caused decrease of NO_2^- concentration 30, 60 and 120 minutes after apocynin inhalation (3.9 μM vs. 4.5 μM, 3.8 μM vs. 4.5 μM and 3.7 μM vs. 4,4 μM respectively, $p<0.05$) comparing to placebo, but did not cause any significant changes in concentration of NO_3^- in any timepoint ($p>0.05$). No influence of apocynin on safety parameters, and no adverse effects has been observed.
Our findings suggest that apocynin might be a promising agent to soothe locally inflammatory process and improve life quality of patients suffering from COPD.

P3828
Modulation of NK receptors by budesonide and/or formoterol in healthy volunteers and COPD patients
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Rationale: Natural killer (NK) cells function is regulated by multiple cell-surface receptors. Nowadays little is known about the involvement and function of NK cells in COPD. Recently a role in pulmonary immunity has been ascribed to NK cells and several in vitro studies have shown corticosteroid-induced inhibition of NK cell-mediated cytotoxicity.
Methods: NK cells were isolated from peripheral blood of healthy volunteers and COPD patients. Cells were cultured for 20 hours in 96-well plates with IL-2 (100 I.U./ml)+IL-12 (2,5 ng/ml), in the absence or in the presence of budesonide (B) (1μM and 10nM) and formoterol (F) (30 and 0,3nM) alone or in combination.

Cells were analyzed by flow cytometry and supernatants were used to investigate the production of IFN-γ by ELISA technique.
Results: We found no difference in expression of NK cell receptors analyzed in resting conditions both in healthy volunteers and patients. When cells were stimulated over night with cytokines and treated with drugs, only NKG2D receptor resulted modulated. Its expression was significantly reduced by budesonide alone and in combination, irrespectively of the dose, in COPD patients ($p<0.05$ and $p<0.01$, respectively). IFN-γ production induced by stimulation with IL2+IL12 was higher in healthy volunteers than in patients ($p<0.05$), but it was decreased in a highly significant way ($p<0.01$) by all treatments in both groups.
Conclusion: Our results show that NKG2D expression and IFN-γ secretion are modulated by treatment with budesonide, both alone and in combination with formoterol, in COPD patients. This might be of relevance in the treatment of diseases such as COPD.
This work was supported by ARMIA and AstraZeneca.

P3829
Cigarette smoke exposure in mice leads to a loss of reversible cysteine oxidations PSSG and PSNO in lung
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It is established that cigarette smoke causes irreversible oxidations in lung tissue. However, its impact on reversible and physiologically relevant redox-dependent protein modifications remains to be investigated. In this study the effect of cigarette smoke exposure in vivo was investigated on the covalent binding of glutathione to protein thiols, known as S-glutathionylation (PSSG), which can be reversed by the enzyme glutaredoxin 1 (Grx1). Also, protein S-nitrosylation (PSNO) which is the modification of protein thiols by NO and which is reversed by the enzyme ADH5 was investigated.
Both PSSG and PSNO levels in lung tissue were markedly attenuated after four weeks of cigarette smoke exposure in mice. This coincided with an attenuation of protein free thiol levels and an increase in protein carbonyl levels. Grx1 mRNA expression and activity were attenuated as well, whereas no alterations in expression or activity of ADH5 were observed.
Taken together, cigarette smoke exposure decreases reversible cysteine oxidations PSSG and PSNO, which does not result in an increase in free thiols. These alterations are likely not the result of differences in regulatory enzymes, but of oxidative stress.

P3830
Hypoxic exposure after smoke cessation restores alveolar surface area in mice
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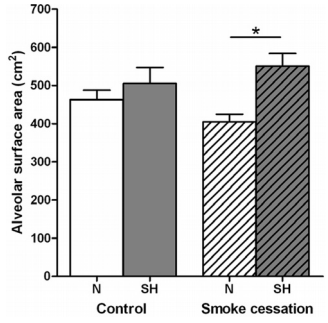
Introduction: Chronic obstructive pulmonary disease (COPD), which mainly results from chronic exposure to tobacco smoke, is a major public health burden with no effective treatment. We have previously shown that hypoxia can induce lung growth in adult mice [1]. The aim of this study is to investigate if sustained hypoxia can restore the loss of alveolar surface area caused by tobacco smoke.
Methods: We exposed C57BL/6J mice to 6 months of tobacco smoke (250 mg/m^3 suspended particles in 2.5 h/day) followed by 3 months of sustained hypoxia (SH) with an FiO_2 of 10% and appropriate control groups (no smoke/normoxia (N)). Pulmonary function was measured in anesthetized mice. Following euthanasia lungs were processed for morphometry, mean airspace chord length (Lm) was measured and alveolar surface area was calculated.
Results: Mice exposed to tobacco smoke had a trend towards a lower alveolar surface area compared to control mice even 3 months after smoke cessation ($p=0.10$). SH increased total lung capacity, whereas Lm was unchanged. SH significantly increased the alveolar surface area in mice previously exposed to smoke ($p=0.008$, Fig. 1), but not in control mice.



Conclusions: Sustained hypoxia after cessation of tobacco smoking restores alveolar surface area in mice. Future studies of the underlying mechanisms might lead to potential therapies.
Reference:
[1] Reinke et al, Am J Physiol Lung Cell Mol Physiol 2011, 300(2):L266-73.

P3831
Role of highly reactive aldehydes in cigarette smoke induced airway inflammation
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Cigarette smoke (CS) is the most important cause of COPD, which is associated with chronic neutrophilic airway inflammation. We hypothesize, that the presence of highly reactive α,β-unsaturated aldehydes in CS is a crucial factor for neutrophilic airway inflammation.
BALB/c mice were exposed to CS, water filtered CS (WF-CS) or air. Levels of CO and aldehydes were measured in CS and WF-CS. Six hrs after the last CS exposure, cell differentials and cytokine levels were measured in lung tissue and bronchoalveolar lavage fluid (BALF). Beas-2b cells (epithelial cell line) were exposed to cigarette smoke extract (CSE) or water filtered cigarette smoke extract (WF-CSE) with and without acrolein. The neutrophil chemoattractant IL-8 was measured after 24 hrs.
CO levels were not different between CS and WF-CS but aldehyde levels were strongly decreased in WF-CS compared to CS. The numbers of neutrophils in BALF ($p<0.001$) and neutrophils and eosinophils ($p<0.05$) in lung tissue were significantly increased in the CS-exposed but not in WF-CS-exposed mice compared to air control. Levels of neutrophil and eosinophil chemoattractants e.g. KC, MCP-1, MIP-1α and IL-5 were significantly increased in lung tissue from CS-exposed mice compared to WF-CS-exposed mice. Beas-2b cells produced significant levels of IL-8 upon stimulation with CSE but not with WF-CSE. Repletion of WF-CSE with the aldehyde acrolein restored the IL-8 production by Beas-2b cells whereas acrolein in culture medium did not. It can be concluded that highly reactive aldehydes present in cigarette smoke appear to play a crucial role in CS-induced IL-8 production and neutrophilic airway inflammation.

P3832
Effects of adipose tissue-derived stromal/stem cells transplantation on elastase-induced pulmonary emphysema in rats
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Many studies demonstrating lung repair by means of stem/progenitor cells or growth factors have been reported in animal emphysema models. We focused on adipose tissue-derived stromal/stem cells (ASCs) for regenerative medicine, since it has a high potential to secrete multiple angiogenic factors and differentiate various kinds of cells. To demonstrate the therapeutic impact of ASCs transplantation and to elucidate mechanisms of the effects in rat emphysema models. ASCs were isolated from Wistar rat inguinal subcutaneous adipose tissue. Emphysema was induced by intratracheal instillation of porcine pancreatic elastase (PPE). One week after induction of PPE, cell transplantation was performed intravenously. At 1 and 2 weeks after transplantation, we assessed arterial blood analysis and histopathological changes and measurement of chemokine levels. After PPE induction, arterial oxygen pressure (PaO_2) was reduced. After ASCs transplantation, PaO_2 significantly improved. In addition, transplantation significantly prevented the enlargement of alveolar airspace elicited by PPE. PPE gradually reduced the levels of endogenous hepatocyte growth factor (HGF) in lung tissue. ASCs augmented HGF level was significantly higher than PPE alone. ASCs from GFP transgenic rats were localized at damaged alveolar spaces. Immunohistochemical analysis revealed some grafted ASCs were expressed CD31 or SP-C at 1w after transplantation. The results showed that transplantation of ASCs for emphysema rats improved gas exchange and inhibit enlargement of the airspaces. Transplantation of ASCs may be new therapeutic strategy to improve pulmonary function and inhibit alveolar destruction in COPD.

P3833
Role of IL-1α and IL-1β in cigarette smoke-induced pulmonary inflammation and COPD
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Signalling through the IL-1 Receptor-I (IL-1RI) is critical in the pulmonary inflammation induced by cigarette smoke (CS). Although several reports implicate IL-1β in the pathogenesis of COPD, it remains unclear whether IL-1α or IL-1β is the predominant ligand for IL-1RI in CS-induced inflammation.
C57BL/6 mice were exposed to air or CS for 3 days (acute exposure) and were treated on day 1 and 3 with an intravenous injection of 40μg anti-IL-1α, anti-IL-1β or hamster IgG isotype control. 24h after the last exposure, we evaluated the

presence of inflammatory cells in the bronchoalveoalar lavage (BAL) fluid. In a translational study, we measured the levels of IL-1α and IL-1β mRNA (in total lung tissue by RT-PCR) and protein (in induced sputum by ELISA) of never smokers, smokers without COPD and patients with COPD.
Acute exposure to CS resulted in a significant increase in BAL neutrophils in isotype treated mice. In contrast, this CS-induced BAL neutrophilia was significantly attenuated in both anti-IL-1α and anti-IL-1β treated mice. Interestingly, we found increased mRNA and protein levels of IL-1α and IL-1β in lung tissue and induced sputum of patients with COPD, compared to never-smokers (Table 1).

Table 1. Human IL-1α and IL-1β levels

	Never-smokers	Smokers without COPD	Smokers with COPD
Lung mRNA	n=10	n=18	n=29
IL-1α	0.87±0.13	2.08±0.37*	2.57±0.34*
IL-1β	0.82±0.09	1.46±0.35	1.69±0.27
Sputum protein (pg/ml)	n=15	n=20	n=18
IL-1α	25.98±2.78	40.93±8.91	98.61±24.78
IL-1β	137.35±38.58	147.45±29.82	667.23±190.50*§

*p<0.05 vs. never-smokers; §p<0.05 vs. smokers without COPD.

These results suggest that not only IL-1β, but also IL-1α should be considered important in CS-induced inflammation.

P3834

β_2 long-acting and anticholinergic drugs synergistically control TGFβ1-mediated neutrophilic inflammation in COPD: An *"in vitro"* model

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TGFβ1 is involved in airway inflammation in COPD and in the regulation of muscarinic receptors (mAChRs) expression. We quantified TGFβ1 and Acetylcholine (ACh) concentrations in induced sputum supernatants (ISs) from 10 Healthy Controls (HC), 14 Healthy Smokers (HS) and 16 COPDs. We tested: 1) ISs from HC, HS and COPD on neutrophil adhesion to human bronchial epithelial cells (16HBE) in the presence or absence of an anti-TGFβ1ab or Olodaterol (β2-adrenoceptor agonist) and Tiotropium (Spiriva®) alone or in combination; 2) ISs from COPD on mAChRs expression in 16HBE in the presence or absence of anti-TGFβ1-antibody and Olodaterol; 3) hrTGFβ1on neutrophil adhesion and mChRs and ChAT expression in 16HBE. We showed that: 1) TGFβ1 and ACh concentrations are increased in ISs from COPD in comparison with HC and HS; 2) ISs from COPD caused higher levels of neutrophil adhesion to 16HBE than ISs from HC and HS and this was significantly reduced by TGFβ1 depletion and by the addition of Olodaterol or Tiotropium alone with a synergistic effect when the drugs were used in combination; 3) mAChR2, mAChR3 and ChAT expression was increased in 16HBE stimulated with ISSs from COPD and are reduced by TGFβ1 depletion while not by Olodaterol; 4) in vitro experiments we confirmed that hrTGFβ1 increases mAChR2, mAChR3 and ChAT expression. These findings suggest that TGFβ1 and mAChRs promote neutrophil adhesion to bronchial epithelial cells during airway inflammation in COPD. Olodaterol and Tiotropium used in combination might synergistically control this proinflammatory event in COPD.
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P3835

JAK inhibitors overcome corticosteroid insensitivity in COPD

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Corticosteroid-insensitive CXCR3 chemokines (CXCL9, -10 and -11) are thought to drive the $CD8^+$ inflammatory infiltrate in chronic obstructive pulmonary disease (COPD). These chemokines are elevated in COPD airways. This study investigated whether JAK inhibitors, PF95 and PF13, suppress CXCR3 chemokine release from macrophage lineage cells.
Peripheral blood mononuclear cells (PBMC) and monocyte-derived macrophages (MDM) were isolated from non-smokers (NS), smokers (S) and COPD patients. Cells were pre-treated with either JAK inhibitor or dexamethasone (DEX) prior to stimulation with IFNγ. Cell media was harvested at 24h and chemokine release measured by ELISA.
Compared to DEX, compound PF 95 significantly suppressed CXCL9, CXCL10 and CXCL11 ($p<0.05$) from PBMC from NS and COPD patients. PF13 significantly suppressed all 3 CXCR3 chemokines in PBMC from COPD patients, CXCL9 and CXCL10 ($p<0.001$) from PBMC from NS and CXCL10 release from MDM from COPD patients ($p=0.01$) (Table 1). However, there were no differences in the response to JAK inhibition between subject groups.
JAK inhibitors PF95 and PF13 significantly suppress steroid-insensitive CXCR3 chemokines in COPD cells and may have benefit as a novel anti-inflammatory treatment.

Table 1. Inhibition (%) of CXCR3 chemokine release by JAK inhibitors PF95 and PF13

	NS		S		COPD		
	PF95	PF13	PF95	PF13	PF95	PF13	DEX
PBMC	n=5	n=4	n=6	n=4	n=7	n=6	n=5
CXCL9	72±7	76±5	36±8	59±15	59±14	69±10	16±8
CXCL10	87±4	89±5	91±4	91±4	77±8	69±10	7±4
CXCL11	52±7	44±13	30±4	27±80	43±7	39±8	5±1
MDM	n=3	n=4	n=2	n=4	n=5	n=5	n=5
CXCL9	59±20	52±8	70±20	56±14	80±5	74±11	53±13
CXCL10	49±26	55±19	63±8	63±8	86±3	83±8	7±7
CXCL11	39±19	11±6	28±13	37±3	48±12	62±10	14±14

Mean % inhibition ± S.E; n=number of subjects.

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Simvastatin attenuates cigarette smoke extract induced extracellular matrix

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Introduction: Production of extracellular matrix (ECM) proteins is increased in the small airway walls of patients with chronic obstructive pulmonary disease (COPD). Current pharmacotherapies are unable to reduce or attenuate ECM in vivo. The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, simvastatin, is emerging as a new potential therapy for COPD. In this study we investigated if simvastatin could affect cigarette smoke extract (CSE) induced ECM and cytokine production from human lung fibroblasts.
Methods: Primary human lung fibroblasts were stimulated with 5% CSE in the presence of 0.5 and 5μg/ml simvastatin, or relevant concentrations of vehicle control, for 72 hours. Supernatants were collected and assessed for pro-inflammatory cytokine and matrix metalloproteinase (MMP) release. Cells were removed by addition of NH4OH and the relative deposition of the ECM proteins fibronectin and perlecan was analysed using an ECM ELISA. The concentration of interleukin (IL)-6 in the supernatant was measured using ELISA.
Results: Treatment of human lung fibroblasts with 5% CSE upregulated the deposition of fibronectin and perlecan, and the release of IL-6 into the supernatant. Addition of simvastatin at 5μg/ml significantly attenuated 5% CSE induced deposition of fibronectin by 43% and perlecan by 41.5% ($p<0.05$, n=6). 5% CSE induced IL-6 release was also attenuated by 31% with the addition of simvastatin at 5μg/ml.
Conclusions: Inhibition of the HMG-CoA reductase pathway may alter the deposition of ECM proteins and release of inflammatory cytokines in COPD.

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Sirtuin 1 reduction causes activation of Wnt/b catenin signalling

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Background: The anti-ageing molecule Sirtuin-1 (SIRT1) has been reported to be decreased in COPD (Nakamaru Y et al, FASEB J, 2009). We have also reported an increase of osteoprotegerin (OPG) in COPD (To M et.al, *CHEST* 2011), which is b-catenin dependent. Our hypothesis is that the reduction of SIRT1 induced by oxidative stress may lead to increased b-catenin activation.
Methods: U937 (human monocytic cell line), A549 (human alveolar epithelial cell line) and BEAS2B (human bronchial epithelial cell line) were grown until 70% confluent, starved for 24h and incubated with 2uM of sirtinol at 4 different time points (i.e. 1h, 4h, 8h, and 24h). The levels of b-catenin were measured in nuclear and cytoplasmic extracts by Western Blotting. Cells were also treated with H_2O_2 for 24 hrs and sirt-1 protein level was also evaluated.
Results: The level of b-catenin was higher in A549 and BEAS2B cells than U937 cells at baseline. In all three cell lines, nuclear b-catenin levels increased at 8h after treatment with 2uM of sirtinol by approximately 52% for the U937, 38% for the A549 and 25% for the BEAS2B cells. No difference was detected in cytoplasmic b-catenin levels in any cell line. Incubation with H_2O_2 for 24h caused reduction in Sirt-1 levels in the A549 cells.
Conclusions: This study shows that reduced SIRT1 protein leads to stabilization of β-catenin protein. This might give new insights in the understanding of COPD pathogenesis.

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Role of aberrant Wnt signaling in the lung epithelial response to cigarette smoke in COPD

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It is still unclear how exposure to cigarette smoke, the major risk factor for COPD,

leads to different phenotypes of COPD, i.e. inflammation and remodelling with increased extracellular matrix (ECM) deposition in the airways and loss of ECM in the parenchyma. The Wnt signaling pathway is known to regulate inflammation, tissue repair and remodelling. We aimed to determine whether dysregulation of Wnt genes may contribute to COPD development. We studied the effects of cigarette smoke extract (CSE, 5%) on expression of a variety of Wnt ligands, Wnt receptors (frizzled, Fzd) and Wnt target genes, including IL-8, MMP-2/9 and fibronectin (qPCR, ELISA, immunodetection) in alveolar A549 and human bronchial epithelial 16HBE cells. Furthermore, we compared expression of Wnt genes in primary bronchial epithelium from COPD patients, healthy smokers and non-smokers. CSE induced Wnt4, Wnt7b and Fzd8 mRNA expression in A549 cells, without significant changes in the other detected genes, i.e. Wnt5a,b, Fzd1 and 2 and MMP-2. In contrast, CSE significantly decreased Wnt4 and Fzd2, and increased Wnt5b mRNA in 16HBE cells. Interestingly, expression of Wnt4, but none of the other detected genes, was significantly higher in COPD than in healthy smoker epithelium. Therefore, we were interested in potential autocrine effects of Wnt4. We observed that recombinant Wnt4 significantly increased IL-8 production in 16HBE cells. Thus, inefficient downregulation of Wnt4 mRNA expression in response to cigarette smoke may have important consequences for the development of airway inflammation in COPD. Furthermore, our results suggest that CSE differentially affects Wnt gene expression in alveolar and bronchial epithelium.