## 478. The smoking gun in COPD biology

**P4580**
**Murine lung airway fibroblasts drive fibrosis through STAT4 signaling after cigarette smoke exposure**
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Cigarette smoke-induced emphysema and small airway remodeling (SAR) are the anatomic bases of chronic obstructive lung disease (COPD), but the pathogenesis of these changes is unclear and current treatments for COPD are minimally effective. We exposed wild type (WT) and STAT4-/- mice to cigarette smoke for 6 months and found that STAT4-/- mice are protected against smoke-induced small airway remodeling but not emphysema. Unexpectedly, we observed that STAT4 is expressed in cultured murine wild type (WT) lung parenchyma-derived and airway-derived fibroblasts, but to a much greater extent in the latter. The same phenomenon was seen in cultured human parenchymal and airway fibroblasts. WT airway fibroblasts proliferated faster than STAT4-/- airway fibroblasts, whereas there was no difference between strains for parenchymal fibroblasts. IL-12 is up-regulated in human and mouse lungs after smoke exposure, and treatment with IL-12 caused phosphorylation of STAT4 in WT airway fibroblasts. Exposure of WT airway, but not parenchymal, fibroblasts to IL-12 caused increased expression of collagen 1α1 and TGFβ, factors involved in SAR, whereas STAT4-/- fibroblasts were unresponsive to IL-12. STAT4 thus controls proliferation and matrix production in airway but not parenchymal fibroblasts, and smoke-induced IL-12 can drive small airway remodeling via STAT4 signaling. These findings suggest that treatment with clinically available anti IL-12p40 drugs might provide a new completely approach to preventing SAR in cigarette smokers.

**P4581**
**LSC 2012 Abstract – TGFβ1 compensates cigarette smoke induced disruption of tight junctions in the bronchial epithelium**
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**Rationale:** The airway epithelium protects the body from inhaled insults, such as smoke, or allergens. The integrity of this epithelial barrier is crucial for bronchial homeostasis. COPD and asthma have been associated with defective airway barrier

function. Tight junctions (TJ) represent the major junctional components, determining the permeability of an epithelial sheet. Cigarette smoke extract (CSE) has been described to impair TJ integrity. The aim of this study was to investigate if CSE also promotes EMT in human bronchial epithelial cells (HBEC), thereby contributing to small airway diseases.
**Methods and results:** Normal HBECs (16HBE) underwent EMT-like processes in response to TGFβ1 treatment, as characterized by elevated mesenchymal markers (FN1, SNAI1, or ZEB1). Epithelial junctional markers (e.g. CDH1, JAM1, or ZO1) were upregulated or unaltered upon TGFβ1 stimulation. 16HBE cells do not change morphological or migrational upon TGFβ1 treatment, as demonstrated by IF or live imaging. CSE downregulated TJ-associated protein expression and destabilized TJ, as observed by IF. Combined long-term treatment (7 days) of 16HBE cells with TGFβ1 and CSE resulted in restored mRNA and protein levels of TJ proteins. This was accompanied by altered MAP kinase signaling.
**Conclusion:** TGFβ1 and CSE resulted in EMT-like changes in HBECs. CSE induced TJ disruption, which was compensated by TGFβ1 through modified signaling processes. Thus, TGFβ1 could serve as a protective factor for bronchial epithelial cell homeostasis.

**P4582**
**Antioxidant tetrapeptide UPF1 exerts an immediate effect on cigarette smoke-altered metabolic state of human bronchial epithelial cells**
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We evaluated the protective capacity of UPF-1 (4-methoxy-L-tyrosinyl-γ-L-glutamyl-L-cysteinyl-glycine) against cigarette smoke condensate (CSC)-induced alterations in metabolic profile of human bronchial epithelial cells (HBEC).
HBEC were exposed to 10 μg/mL CSC for 1h, followed by treatment with 0-10 μM UPF1 or 2 mM N-acetylcysteine (NAC) for 1-12 h. Cell lysates were analysed on a Q-Trap 3200 mass spectrometer to obtain full spectra between mass-to-charge (m/z) ratios 50-1700 Da. Principal component analysis, partial least squares regression analysis and t-test were used.
Levels of many compounds (e.g. 226, 310, 408 Da) were significantly ($p<0.001$) elevated in response to CSC. Exposure to CSC caused a rapid and significant shift of the HBEC metabolic state visible both in positive and, to a lesser extent, in negative ionization mode, followed by a delayed return to the state close to that of untreated cells not earlier than by 12 h. Instead, addition of 10 μM UPF1 was able to return the metabolic state already by 1 h to the state, which was present in lone CSC-stimulated cells just after 6 h. By 1 h, 1 μM UPF re-established the metabolic state to that what was evident at 3 h of CSC-exposure without UPF1, showing a concentration-dependent effect of UPF1. In contrast, NAC reverted the CSC-affected metabolic state of the HBEC to near-normal not earlier than by 12 h.
Compared to NAC, the novel antioxidant tetrapeptide UPF1 acts effectively towards restoring the metabolic status in HBEC by eliminating the immediate effect of CSC. The results may speed up the design of drugs that facilitate prevention of COPD.
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**P4583**
**COPD patients with more severe GOLD stages have higher serum cystatin C levels**
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**Introduction:** Creatinine is widely used as a biomarker of kidney function but may be inaccurate at detecting mild renal impairment since creatinine levels may vary with muscle mass and protein intake. Advanced COPD is characterized by wasting of skeletal muscles and imbalances in protein metabolism.
Cystatin C may be used as a muscle independent biomarker of kidney function. Recently, it has been studied as a predictor of cardiovascular diseases and emphysema development.
**Aim:** The aim of this study was to evaluate the relationships between COPD severity and serum cystatin C levels in prospective study.
**Materials and methods:** One center longitudinal prospective study in COPD patients was designed. Consecutive COPD patients treated in respiratory department and healthy health care workers were studied. Serum levels of cystatin C, creatinine and urea were measured and glomerular filtration rate (GFR) was assessed.

| Patients/Controls | No. of pts | Age | Creatinine | GFR | Cystatin C | Urea |
|---|---|---|---|---|---|---|
| Healthy controls | 29 | 60,4±7,7 | 0,90±0,15 | 97,2±28,6 | 0,962±0,207* | 35,1±6,2 |
| COPD stage I | 12 | 62,6±7,0 | 0,84±0,16 | 97,5±28,0 | 0,989±0,124* | 33,0±7,1 |
| COPD stage II | 34 | 65,7±7,2 | 0,95±0,25 | 81,7±31,8 | 1,021±0,222* | 34,7±7,7* |
| COPD stage III | 13 | 66,6±9,9 | 0,95±0,22 | 77,7±34,5 | 1,048±0,209* | 40,1±9,1 |
| COPD stage IV | 39 | 67,0±10,1* | 0,85±0,23 | 80,7±57,4 | 1,234±0,371* | 43,4±18,9* |
| All | 127 | 64,8±8,7 | 0,89±0,22 | 85,3±41,2 | 1,073±0,286 | 38,0±12,9 |

**Results:** 98 COPD patients and 29 non-COPD healthy controls were analyzed. GOLD stages and biochemistry parameters are shown in the table (*$p<0.05$) Results are means ±SD
**Conclusions:** COPD patients with more severe GOLD stages have higher serum cystatin C levels but not creatinine or calculated creatinine clearence levels.

**P4584**
**Cigarette smoke induces a distinct fibrotic signature in bronchial epithelial cells**
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**Rationale:** We have previously shown cigarette smoke extract (CSE) induces the production of the extracellular matrix proteins fibronectin and perlecan in human lung fibroblasts (Krimmer et al AJRCMB 2011). The proteins were also induced by transforming growth factor β (TGFβ); however the signal transduction pathways activated by CSE were different to those used by TGFβ. These differences lead us to speculate that if we were to look at an increased number of molecules involved in the development of fibrosis, we would find differential upregulation by TGFβ and CSE.
**Aim:** To compare fibrosis related genes upregulated by TGFβ or CSE in primary human bronchial epithelial (HBE) cells.
**Methods:** HBE cells were grown to confluence in BEGM in the absence or presence of 5ng/ml of TGFβ1 or 5% CSE. Total RNA were collected after 72 hours. The expression of fibrosis related genes was measured by real time PCR. In total we examined 85 fibrosis related genes. A cut off of a minimum of 1.5 fold induction was used to indicate upregulation of a gene.
**Results:** TGFβ upregulated 49 different fibrosis associated genes, CSE upregulated only 16, and of all the upregulated genes, 4 were uniquely upregulated by CSE (catenin β1, extracellular matrix protein 1, and TIMP 1 and 2). As expected, TGFβ upregulated a variety of ECM proteins (eg collagen I, IV-VIII, fibronectin); integrins (eg integrin α2, α4-6), and degradative enzymes (eg matrix metalloproteinase (MMP) 1-3, 9-16). In contrast, CSE upregulated only two collagens (XI and XIV) and MMP9.
**Conclusion:** As CSE selectively upregulated a subset of TGF-β induced genes, elucidation of the specific mechanisms involved is likely to give novel insight into the pathophysiology of COPD.

**P4585**
**Epidermal growth factor (EGF) and its receptors (ERBBs) in smokers with and without COPD: An index of epithelial repair imbalance?**
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**Background:** The EGF and its receptors are expressed in the lung and orchestrate the epithelial repair process. We hypothesized that EGF pathways are involved in COPD pathogenesis.
**Aim:** To study EGF and ERBB receptors mRNA expression in lung tissue of smokers with or without COPD and control non-smokers.
**Methods:** Lung tissue specimens from 45 male subjects were studied: A) 10 patients with mild COPD (GOLD stage I), B) 10 patients with moderate COPD (GOLD stage II), C) 15 non-COPD smokers with normal lung function, and D) 10 non-smokers, serving as the control group. Quantitative Real-Time PCR experiments were carried out for EGF and receptors ERBB1, ERBB2, ERBB3, ERBB4, using beta-actin housekeeping gene as internal control.
**Results:** All study groups were matched for age. Moderate COPD patients had higher ERBB1 (EGFR), ERBB2 and ERBB3 mRNA levels compared to the other three groups. On the contrary, while moderate-COPD ERBB4 mRNA levels were statistically increased when compared to non-smokers (P=0.041), they did not differ when compared to mild-COPD patients (P=0.19) or to non-COPD smokers (P=0.18). Finally, EGF expression was at least 2-fold increased in moderate- and mild-COPD patients and non-COPD smokers, when compared to non-smokers, a finding which was statistically significant only between moderate-COPD patients and non-smokers (P=0.010).
**Conclusions:** EGF was over-expressed in all three smoking' study groups. However, its receptors (especially ERBB1-3), were elevated only in moderate-COPD patients. This could reflect the epithelial "repair" or "remodeling" process exerting by the EGF signaling pathway as COPD progresses.

**P4586**
**Effects of α1-antitrypsin on neutrophil extracellular traps formation**
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Neutrophils belong to the innate immune response and are essential for elimination of invading pathogens. Apart from phagocytosis and secretion of anti-microbials, neutrophils are also capable of producing neutrophil extracellular traps (NETs) to kill pathogens extracellularly (NETosis). Neutrophil elastase (NE) is a critical initiator of NETosis and also is one of the main components of NETs. The acute

phase protein $\alpha_1$-Antitrypsin (AAT) is a potent inhibitor for NE released from the activated neutrophils. Therefore, we asked a question if AAT inhibits NETosis?
We induced NETosis in neutrophils isolated from healthy donors by applying phorbol myristate acetate (PMA, 10 ng/ml) alone or together with purified AAT protein (1 mg/ml). To our surprise, AAT did not inhibit NETs formation but make the structures less adherent to the surface. Remarkably, using anti-AAT antibodies we detected AAT in the NETs either separately or in co-localization with elastase. In the next set of experiments, we isolated neutrophils from emphysema patient with inherited ZZ (Glu342 Lys) AAT deficiency before and after AAT augmentation therapy. In response to PMA, neutrophils isolated before augmentation therapy formed NETs similar to those observed in healthy donors. However, after augmentation therapy, NETs contained large cell aggregates some of which were detached from the specimen. Again, exogenous AAT did not inhibit NETs.
We suggest that an increased risk for development of chronic obstructive pulmonary disease (COPD) in subjects with inherited AAT deficiency results from both-increased elastase activity and uncontrolled NETosis.

**P4587**

**Resveratrol attenuates cigarette smoke induced oxidative stress: Possible involvement of SIRT1**

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**Objectives:** Cigarette smoke is known to cause oxidative stress in alveolar epithelial cells. In this study, we investigated the effects of resveratrol, a phytoalexin produced by some spermatophytes, on oxidative stress induced by cigarette smoke in airway epithelial cells.
**Methods:** Rats with or without exposure to cigarette smoke were intraperitonealy injected with resveratrol (5,10,20mg/kg·d). TNF-α levels in BALF were determined by ELISA. Total glutathione, SOD and $H_2O_2$ levels in rat lungs were detected. Human alveolar epithelial cells (A549) were exposed to cigarette smoke extract (CSE), with or without pretreatment of resveratrol (40μM). The activity of ROS was detected and the expression of SIRT1 protein was evaluated with western blotting.
**Results:** Cigarette smoke exposure significantly increased TNF-α expression in BALF and this upregulaion was significantly attenuated by resveratrol ($p<0.05$). Meanwhile, the treatment of resveratrol increased the expression of glutathione and SOD in lung homogenates, which were attenuated by cigarette smoke exposure ($p<0.05$). The expression of $H_2O_2$ was decreased by resveratrol($p<0.05$). Exposure of A549 cells to CSE resulted in the elevated ROS expression, which was inhibited by resveratrol. Meanwhile, SIRT1 protein levels were activated by the pre-treatment of resveratrol.
**Conclusions:** These results suggest that resveratrol attenuated oxidative stress induced by cigarette smoke. The anti-oxidant effect might act through the expression of SIRT1 proteins.

**P4588**

**Chronic obstructive pulmonary disease is characterized with suppressed lipoxin A4 and increased lipoxin receptor expression in lungs**

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Persistent inflammation of COPD could be influenced by disorders of arachidonic acid metabolism when synthesis of leukotrienes does not switch to lipoxin generation, and thereby promotes further inflammation process.
**Aim:** To estimate concentration of lipoxin A4 (LXA4), leukotriene B4 (LtB4), as well as expression of LXA4 receptor (FPRL-1) in induced sputum (IS) of COPD patients and healthy controls.
**Materials and methods:** 17 COPD patients and 7 healthy controls. LXA4 and LtB4 concentration in IS was assessed with ELISA. FPRL-1 expression was detected immunocytochemically.
**Results:** Concentration of LXA4 in COPD patient's IS was decreased compared to healthy controls (0,909±0,43 ng/ml vs 2,198±1,189 ng/ml; p=0,009). Ratio LtB4/LXA4 in COPD patients was three times greater compared to healthy persons (8,884±2,789 vs 3,328±2,94; p=0,0071).
In COPD patient's IS FPRL-1 positive polinuclear cells (PPC) were in greater amount compared to healthy controls (8,802±5,758 cells/mm$^2$ vs 2,123±2,232 cells/mm$^2$; p=0,0109). Also, in COPD patients a count of FPRL-1 positive mononuclear cells (PMC) in IS was increased compared to healthy controls (2,563±1,711 cells/mm$^2$ vs 0,655±0,522 cells/mm$^2$; p=0,0311).
Correlation between FPRL-1 PPC and LtB4 concentration in IS (r=0,628; p=0,0013).
**Conclusions:** Increased LtB4/LXA4 indicate a disbalance of inflammatory mediators in COPD patients that could be one of the causes of inflammation persistence. In turn, increased LXA4 receptor expression and its correlation with LtB4 concentration in COPD patients could denote mechanism of inflammation adaption that is initiated by LtB4.

**P4589**

**Immunoregulation and FoxP3 expression in the bronchial mucosa of stable COPD patients**

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**Background:** T regulatory cells ($T_{reg}$) control the immune responses at sites of inflammation. The differentiation and function of $T_{reg}$ cells require the transcription factor forkhead box p3 (FoxP3). Conflicting results have been reported in central and peripheral airways of COPD patients regarding numbers of FoxP3+ cells populating the airways (Isajevs S, Eur Respir J 2009;33:61).
**Objectives:** To investigate the expression of T-regulatory molecules in the bronchi of patients with severe/very severe (FEV1% pred:35±9; n=19), mild/moderate (FEV1% pred:66±14; n=13) stable COPD, control smokers (FEV1% pred:104±13; n=12) and non-smokers (FEV1% pred:116±14; n=11).
**Methods:** Expression of FoxP3 was measured in the bronchial mucosa using immunohistochemistry in cryostat sections.
**Results:** Immunopositivity for FoxP3 was similar in the bronchial epithelium (cells/mm) (median(range): 1.1(0.5-2) vs 1.2(0.5-1.7) vs 1.1(0.5-2) vs 1.2(0.2-2), Kruskal Wallis (KW), p=0.948, and submucosa (cells/mm$^2$) (86(32-177) vs 105(23-155) vs 92(12-187) vs 86(19-306), p=0.990, in control non smokers, control smokers, mild/moderate and severe/very severe COPD, respectively.
**Conclusion:** We show no significant differences in the numbers of FoxP3+ cells populating the bronchial mucosa in COPD patients and control groups, suggesting that immunomodulation of inflammation is not influenced by FoxP3 in the bronchi of patients with COPD.

**P4590**

**Altered proliferation of alveolar epithelial cells is involved in progressive elastase-induced emphysema**

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**Rationale:** Chronic obstructive pulmonary disease (COPD) is characterized amongst others by development of emphysema. We showed that a single application of porcine pancreatic elastase (PPE) causes a severe progressing emphysema-like phenotype in C57BL/6 mice. Since the development of emphysema is apparently not completed after PPE application, we aimed to identify possible key mechanisms that drive this process even at late time points.
**Methods:** Female C57BL/6 mice received a single oropharyngeal application of PPE or PBS, and lung function, histology and gene expression were analyzed on days 2, 28, 56, and 162. Fibroblasts of PPE treated mice were characterized analyzing mitochondrial membrane potential. Furthermore, LA-4 lung epithelial cells were treated with PPE and proliferative and apoptotic characteristics were measured using gene expression or wound healing assays.
**Results:** PPE treated C57BL/6 mice develop progressive airway enlargement and impairment of pulmonary function during 23 weeks of analysis. Q-PCR revealed elevated expression of apoptosis markers, reduced proliferation and increased expression of matrix components. Lung fibroblasts of PPE treated mice show reduced proliferation and an altered mitochondrial membrane potential. Reduced proliferation was also found in PPE treated LA-4 cells.
**Conclusion:** Diminished proliferation in PPE treated lung epithelial cell lines as well as in lungs and primary fibroblasts of PPE treated mice could explain the persistent progression of PPE induced emphysema in mice.

**P4591**

**Different pathology of pulmonary arterioles in centrilobular and panlobular emphysema**

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Two distinct pathological phenotypes have been described in patients with emphysema: Centrilobular Emphysema (CLE) and Panlobular Emphysema (PLE), with distinct clinical and functional characteristics. A different involvement of small airways and parenchyma has been described in CLE and PLE, but the involvement

of pulmonary arterioles has never been analyzed. Therefore, we evaluated remodelling of pulmonary arterioles in surgical samples from 26 patients with CLE, 18 with PLE, 7 smokers without emphysema (SNE) and 8 non smokers (NS). By image analysis we measured total arterial wall, intima, media and adventitial thickness. Furthermore, since mast cells (MCs) are potentially involved in vascular remodeling, we quantified the infiltration of tryptase+ MCs in arterioles. Subjects with CLE have a higher total wall thickness compared to those with PLE (median [range] 61 [21-198] vs 46 [17-143] μm; p<0,001) and NS (51 [20-123]; p<0,05). In particular, thickness of the intima was greater in subjects with CLE than in those with PLE (9 [2-66] vs 6 [2-41]; p<0,05), as was thickness of the media (19[7-94] vs 16 [7-63]; p<0,005). Finally subjects with CLE have a higher number of MCs in the adventitial layer than those with PLE (200 [0-1200] cells/mm$^2$ vs 111 [0-1000]; p<0,001). In conclusion, our study demonstrates that pulmonary arterioles show a different pathology in CLE and PLE, suggesting that the mechanism responsible could be different in the two forms of emphysema.

**P4592**

**Anti-oxidants modulate protein phosphatase 2A (PP2A) activity to counteract cigarette smoke generated reactive oxygen species**

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**Introduction:** Phosphatases are redox regulated and play important roles in inflammatory signaling. Protein phosphatase 2A (PP2A) activity can be modified by reactive oxygen species (ROS) produced by numerous stimuli, including cigarette smoke (CS).
**Aims and objectives:** We examined whether the alteration of an anti-oxidant, glutathione peroxidase-1 (GPx-1), in mice could affect phosphatase response against CS and impact on CS-induced emphysema.
**Methods:** Wild-type, GPx-1 overexpressing and knockout mice were exposed to short and long term cigarette smoke. Inflammatory responses and phosphatase activities were determined from lung tissue.
**Results:** Over-expression of GPx-1 protected CS-induced inflammation and airway enlargement. Knockdown of GPx-1 resulted in an exaggerated emphysema phenotype, correlating with low PP2A activity levels. Induction of PP2A activity coincided with increased protein tyrosine phosphatase 1B (PTP1B) activity and subsequent tyrosine dephosphorylation of the catalytic subunit of PP2A (Tyr307).
**Conclusions:** Therefore, PP2A is redox regulated by GPx-1 and contributes to the inflammatory and proteolytic responses that occur in CS driven diseases. Targeting the PP2A pathway may be effective means of treating COPD and other diseases where inflammation plays a central role.

**P4593**

**Proteinase 3 activity is present in sputum from subjects with alpha-1-antitrypsin deficiency (AATD) and COPD**

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**Introduction:** In COPD an imbalance is thought to exist between the activity of proteinases & their inhibitors. Neutrophil elastase (NE) has been studied in detail but proteinase 3 (PR3) has not. PR3 causes features of emphysema in animals & is more abundant in neutrophils than NE suggesting it may also play a role.
**Aims:** To investigate the importance of PR3 in AATD & COPD.
**Methods:** Spontaneous sputum was collected from 28 AATD & 24 usual COPD patients who were clinically stable, and from 15 of the COPD patients during an exacerbation. Patients had demographic data collected, lung function tests & quality of life scores. The sol-phase of sputum was analysed for NE & PR3 activities using specific substrates. The concentration of these proteinases was also measured by ELISA together with airway inhibitors & markers of neutrophilic inflammation.
**Results:** Most sputum samples had no detectable NE activity, but PR3 activity was present & higher than NE activity (AATD p=0.0043, COPD p=0.0052).
PR3 activity was higher in AATD patients compared to COPD patients (p=0.0023) & was higher during exacerbations than in stable state in COPD patients (p=0.037).
PR3 activity showed positive correlations with markers of neutrophilic inflammation & quality of life score. No correlations were found with lung function.
The main airway inhibitor of NE is secretory leukoproteinase inhibitor which does not inhibit PR3. The data suggests that concentrations of PR3 exceed the inhibitory capacity especially in AATD, but NE does not.
**Conclusion:** Active PR3 is present in sputum from stable patients with AATD & COPD and is greater than active NE indicating it may play a role in the pathophysiology of airways disease.

**P4594**

**Proteinase 3 and its potential role in emphysema**

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**Introduction:** Proteinase 3 (PR3) is an elastin-degrading proteinase similar to neutrophil elastase (NE). PR3 is the most abundant serine proteinase in neutrophils, causes emphysema in animal models & induces mucus secretion in humans. Its main circulating inhibitors are alpha-1-antitrypsin (AAT) & alpha-2-macroglobulin (A2M). Its role in respiratory disease has not been studied in detail.
**Aims:** 1) To determine how efficiently PR3 activity is inhibited by healthy (PiM), AAT deficient (PiZ) & AAT variant (PiFZ, PiIZ) sera.
2) To study partitioning of PR3 between its 2 serum inhibitors.
**Methods:** Serum from subjects with different AAT phenotypes (PiM, PiZ, PiFZ, PiIZ) was taken & AAT concentration measured. Increasing molar ratios of serum AAT to PR3 were incubated & residual PR3 activity measured. Experiments were reproduced using comparable mixtures of pure AAT & A2M. Association rate constants (Kass) of PR3 & NE with AAT variants were determined using methylamine-treated serum (A2M inactivated).
**Results:** Increasing the molar ratio of serum AAT to PR3 increasingly inhibited PR3 activity, however even in AAT excess some residual PR3 activity remained which was greater in PiZ serum compared to PiM serum. These results were reproduced using mixtures of pure AAT & A2M suggesting that PR3 bound to A2M remains catalytically active. The Kass values for AAT variants with PR3 & NE are shown in table 1.

Table 1

| AAT variant | Kass with NE (25°C, $M^{-1}$ $s^{-1}$) | Kass with PR3 (25°C, $M^{-1}$ $s^{-1}$) |
|---|---|---|
| PiM | $1.4\times10^7$ | $9.6\times10^5$ |
| PiIZ | $9.9\times10^6$ | $1.1\times10^6$ |
| PiFZ | $7.2\times10^6$ | $1.7\times10^6$ |
| PiZ | $7.3\times10^6$ | $1.5\times10^6$ |

**Conclusion:** When serine proteinases are released from neutrophils, NE is more likely to be inhibited by AAT than PR3 (due to the lower Kass values) & binding of PR3 to A2M retains its activity.

**P4595**

**Increasing oxidative stress and inflammation in patients with exacerbated chronic obstructive pulmonary disease (COPD) and their association with lung function**

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Oxidative stress and chronic inflammation are the milestones in pathogenesis of COPD. It is assumed that their increase is associated with a worse lung function and frequent exacerbations.
Our aim was to assess changes in antioxidant enzymes superoxide-dismutase (SOD) and glutathione-peroxidase (GPx) activities and inflammatory markers in patients with exacerbated COPD, stable COPD and healthy controls and their association with airway obstruction.
We performed a cross-sectional study among 244 participants (mean age 60.5±9.5 years) divided into 3 groups: 76 were with exacerbated COPD (group 1), 65 were with stable COPD (group 2) and 103 were matched healthy subjects (group 3). We performed a questionnaire to define pack years, spirometry and biochemical tests. Oxidative stress was measured by erythrocyte activities of SOD and GPx.
Compared to the control group, patients from group 1 had significantly lower SOD and GPx activities (p<0.0001), with an increasing trend between groups. ESR and CRP activities were significantly higher in patients with exacerbated COPD (p<0.0001). The inflammatory markers correlated positively with pack years (r=0.15, p<0.05 for ESR and r=0.242, p<0.0001 for CRP) and negatively with FEV1% (r=-0.427, p<0.0001 for ESR and r= -0.259, p<0.0001 for CRP). Using multiple linear regression we found that SOD and GPx were significantly influenced by FEV1% (β=0.187, p<0.05 and β=0.219, p<0.0001 resp.).
The present study suggests that there is an increased oxidative stress and inflammation in patients with COPD in exacerbation and there is a relationship with the severity of the disease.

**P4596**

**Evaluation of dendritic cell population in chronic obstructive pulmonary disease**

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Chronic obstructive pulmonary disease (COPD) is characterized by an increase

in CD8+ T cells within the central and peripheral airways suggesting an antigen-specific adaptive immune response. It usually requires the assistance of antigen-presenting cells (APCs), like dendritic cells (DCs), that recognize, process, and present the processed antigen to naive lymphocytes. In human airway tissue three different pulmonary DC subsets have been described: type 1 myeloid DCs (BDCA1), type 2 myeloid DCs (BDCA3), and plasmacytoid DCs (BDCA2).
The objective of this study was to assess dendritic cells in the airway tissue in patients with COPD.
In this case-control study, a total of 31 lung biopsies were obtained from a group of patients undergoing major lung resection because of suspected neoplasm. There were 16 COPD patients and 15 controls. Flow cytometry method was used to quantify the number and subsets of DCs in lung parenchymal.
There was no significant difference in baseline characteristics between the two groups. We observed a lower proportion of BDCA1 as well as BDCA2 cells in COPD patients than in control patients (10% and 18% respectively). The number of BDCA3 cells was 37% lower in the control group.
We found a higher concentration of mature DCs identified by CD40/CD80/CD83/CD86 expression in the COPD group. The results of this study support the role of dendritic cells in the systemic inflammation in COPD. Pulmonary DCs migration in COPD seems to be accompanied by DCs maturation.

**P4597**

**Liver growth factor (LGF) reverts experimental lung emphysema induced by tobacco smoke in mice**

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The liver growth factor (LGF) is a hepatic mitogen able to modulate the remodeling induced in several diseases, as hypertension in the arteries or cirrhosis in the liver. Considering the therapeutic effect of the LGF on the experimental COPD model induced by CdCl2 (Arch. Bronconeumol. 2010; 46 (1:20-26), we aimed to study the regenerative effects of this factor over the experimental COPD tobacco induced model.
15 mice C57BL/6j were exposed to cigarette smoke or sham smoke during 6 month, twice a day and five days a week. After last exposition the treated group received 1.7 μg of LGF i.p. in two administrations (1/wk).Variables of lung morphometry (mean linear intercept –LM-), lung function (maximal volume at 30 cmH2O –Vmax-) and in vivo fluorescence molecular imaging (FMI) with the MMPsense680 probe that detects activation of matrix metalloproteases (MMPs-4-3-9-13) were analyzed in this study.
The tobacco smoke group developed lung emphysema as estimated by the LM value (41.88±0.64 μ vs 32.46±0.46 μ in the control group. $p<0.01$) and the Vmax (1.391±0.15 ml vs 1.287±0.149 ml. $p<0.05$). The LGF treated group significantly improved both values reaching the control values (LM 32.04±0.35 μ. $p<0.01$; Vmax 1.308±0.149 ml. $p<0.05$). In vivo FMI of MMPs activation showed a significative increasing in the tobacco smoke group (1.83±0.1 AU from respective control. $P<0.01$). After LGF treatment, in vivo FMI was significantly reduced also reaching the control level ($p<0.01$).
In conclusion, experimental lung emphysema developed by chronic exposition to cigarette smoke in C57BL/6j was reverted by LGF treatment, as estimated by MMPs activity, lung morphometry and lung function.