478. The smoking gun in COPD biology

P4580
Murine lung airway fibroblasts drive fibrosis through STAT4 signaling after cigarette smoke exposure
Tilie Hackett, Steven Zhou, Joanne Wright, Andrew Churg. Pathology, University of British Columbia, Vancouver, BC, Canada

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 Ia1 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
Andrea Forkert, Nikica Mise, Oliver Eickelberg. Comprehensive Pneumology Center, HMGU, Munich, Germany

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
Results: 98 COPD patients and 29 non-COPD healthy controls were analyzed. GOLD stages and biochemical 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 clearance levels.

P4584

Cigarette smoke induces a distinct fibrotic signature in bronchial epithelial cells

Brian Oliver, David Krimmer, Ling Chen, Janette Burgess, Judith Black, QG. Ge.

The Woolcock Institute of Medical Research & The Discipline of Pharmacology, University of Sydney, NSW, Australia

Rationale: We have previously shown cigarette smoke extract (CSE) induces the production of the extracellular matrix proteins fibronectin and perlecain 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 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 3ng/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: TGFβ1 upregulated 49 different fibroses 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, TGFβ1 upregulated a variety of ECM proteins (eg collagen I, IV-VIII, fibronectin); integrins (eg integrin α2, γ6), and degradative enzymes (eg matrix metalloproteinase (MMP) 1-3, 9-13, 9-16). In contrast, CSE upregulated only two collagens (XI and XIV) and MMP9.

Conclusion: As CSE selectively upregulated a subset of TGFβ-induced genes, elucidation of the specific mechanisms involved is likely to give novel insight into the pathophysiology of COPD.
**P4587**

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

**Jing An**, Yong-Chun Shen, Tao Wang, Ting Yang, Fu-Qiang Wen. Department of Respiratory Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan, China.

**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:** Cells with or without exposure to cigarette smoke were intraperitoneally injected with resveratrol (5,10,20mg/kg). TNF-α levels in BALF were determined by ELISA. Total glutathione, SOD and H2O2 levels in rat lungs were detected. Human alveolar epithelial cells (A549) were exposed to cigarette smoke extract (CSE, 50%), 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 upregulation was significantly reduced 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 H2O2 was decreased by resveratrol (<p>0.05).

**Conclusion:** 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.

**P4588**

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

**Lipa Boldo1, Daria Iagove1, Agnese Kissina1, Sergey Iagove1, Gunta Stradza1, Normunds Jurka2, Uldis Kopeika2, Maris Bukovskis1.**

1Department of Pathology, University of Latvia, Riga, Latvia; 2Institute of Experimental and Clinical Medicine, University of Latvia, Riga, Latvia.

**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±0.2,789 vs 3.328±2.94, <p>0.007).

In COPD patient’s IS FPRL-1 positive polynuclear cells (PPC) were in greater amount compared to healthy controls (8,802±2,789 vs 2,94; p=0,007). Also, in COPD patients a count of FPRL-1 positive mononuclear cells (MNC) in IS was increased compared to healthy controls (2,563±1,711 cells/mm³ vs 0,655±0,5,22 cells/mm³; p=0,031).

Correlation between FPRL-1 PPC and LTB4 concentration in IS (r=0,628; p=0.05).

**Conclusions:** Increased LTB4/LXA4 indicate a disbalance of inflammatory media tors 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 patient’s IS might be a mechanism of inflammation adaption that is initiated by LTB4.
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).

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).

Methods: Wild-type, GPs-1 overexpressing and knockout mice were exposed to chronic term cigarette smoke. Inflammatory responses and phosphatase activities were determined from lung tissue.

Results: Over-expression of GPs-1 protected CS-induced inflammation and airway enlargement. Knockdown of GPs-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 (Tyrl307).

Conclusion: Therefore, PP2A is redox regulated by GPs-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
Nicola Sinden 1, Robert Stockley. Lung Function and Sleep Department, Queen Elizabeth Hospital, Birmingham, United Kingdom

Introduction: In COPD an imbalance is thought to exist between the activity of proteinases & their inhibitors. Neutrophil elastase (NE) has been studied in detail

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 activities were determined from lung tissue.

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).

Conclusion: When serum 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
Velim Stratescu 1, Jordan Peters 2, Sonya Galcheva 3, Marinka Peneva 1, 1 Clinic of Pulmonology and Allergology, University Hospital “St. Marina”, Varna; 3 Dept. of Internal Diseases, Military Medical Academy-Naval Hospital, Varna, Bulgaria

Introduction: 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.

Methods: 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.

Results: Oxidative stress was measured by erythrocyte activities of SOD and GPXs. 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=0.24, p<0.001 for CRP) and negatively with FEV1% (r=-0.187, p<0.05 and r=-0.219, p=0.001 resp.).

Conclusion: 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
César Gutiérrez 1, Carmen Calero 1, Elena Arellano 1, Ana Blanca 1, 1 Unidad Médico Quirúrgica de Enfermedades Respiratorias, Hospital Universitario Virgen del Rocío, Sevilla, Spain; 2 Instituto de Bioquímica de Sevilla (IBIS), Hospital Universitario Virgen del Rocío, Sevilla, Spain

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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.

### P4997

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

Alvaro Giron-Martinez1,2, Terron-Exposito Raul1,2, Sandra Perez-Rial1,2, Nicolas Gonzalez-Mangado1,2, Peces-Barba German1,2. 1Pulmonology Exp Lab, IIS-FJD, Madrid, Spain; 2PCI-COPD, Group 4, CIBERES, Madrid, Spain

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.