Cigarette smoking inhibits LTA4H aminopeptidase activity and contributes to chronic neutrophilic airway inflammation and COPD

James Wells, James Blalock, Philip O'Reilly, Medicine; Division of Pulmonary, Allergy, and Critical Care, University of Alabama Birmingham, Birmingham, AL, United States

This study investigated the role of CD387, a neutrophil-activating enzyme, in chronic neutrophilic airway inflammation in COPD. Neutrophils were isolated from induced sputum and were incubated with lipopolysaccharide (LPS) or CD387. The results showed that CD387 increased the expression of interleukin-8 (IL-8) and decreased the expression of eotaxin-1, a chemoattractant for eosinophils. The findings suggest that CD387 may contribute to the chronic neutrophilic inflammation in COPD.

P3821

Conclusions: This data indicate that there are qualitative and quantitative alterations in 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 underling mesenchymal cells which may result in fibrotic deposition.

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P3826
Roflumilast potently mediates neutrophil migration in COPD patients
Amy Turner, Peter Fenwick, Peter Barnes, Louise Donnelly. Airway Disease, National Heart and Lung Institute, London, United Kingdom
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 [32P]PI phosphorimager. Both RNO and rolipram completely inhibited migration of neutrophils from healthy subjects towards CXCL1 (3nM) with EC50 values of 0.18±0.03 nM and 2.8±0.5 nM, respectively. A similar response was not observed from patients with COPD (EC50 RNO: 0.74±0.89 nM; rolipram 42±14.9 nM, n=3). Having established that cell migration was attenuated by PDE4 inhibitors, the effects of these compounds on CXCL1 stimulated [Ca2+]i was investigated. RNO (1μM) inhibited the peak of [Ca2+]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 80.3±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 [Ca2+]i, and this could contribute to the anti-inflammatory effects of roflumilast in the treatment of COPD.

P3827
Apopcynin reduces H2O2 and NO2 concentrations in exhaled breath condensate of COPD patients
Joanna Stefanka1, Agata Sarniak2, Anna Wlodarczyk3, Milena Sokolowska1, Maria Czeniew Domiec1, Dariusz Nowak2, Rafal Pawliczak1. 1Department of Pulmonology, National Institute for Tuberculosis and Lung Diseases, Rabka, Poland, 2Department of Pneumonology, National Institute for Tuberculosis and Lung Diseases, Lublin, Poland, 3Department of Clinical Immunology, Medical University of Lodz, Lodz, Poland
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 has been regarded as a potential cause. Apocynin is an agent that inhibits activation of an enzyme responsible for ROS generation and thus probably, alleviates 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 H2O2, NO2, and NO 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 ± 0.59 μM vs. 0.41 μM ± 0.58 μM respectively, p<0.05) Interestingly, apocynin caused decrease of NO2 concentration 30, 60 and 120 minutes after apocynin inhalation (3.9 μM ± 4.5 μM vs. 3.8 μM ± 4.5 μM and 3.7 μM ± 4.4 μM respectively, p<0.05) comparing to placebo, but did not cause any significant changes in concentration of NO, 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.
Cigarette smoke exposure in mice leads to a loss of reversible cysteine oxidations

This work was supported by ARMIA and AstraZeneca.

Our results show that NKG2D expression and IFN-γ hypoxia can restore the loss of alveolar surface area caused by tobacco smoke.

We have previously shown that hypoxia can induce sustained particles in 2.5 h/day followed by 3 months of sustained hypoxia (SH)

Results: Mice exposed to tobacco smoke had a trend towards a lower 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.


P3831
Role of highly reactive aldehydes in cigarette smoke induced airway inflammation

Agnieszka Troszok1, Marco van der Toorn1, Delaram Rezayat 1, Renee Graa 1, Harolde Brun2, Dirk-Jan Slees2, Antoon van Oosterhout 1, 1Pathology and Medical Biology, University Medical Centre Groningen, Groningen, Netherlands; 2Pulmonary Diseases, University Medical Centre Groningen, Groningen, Netherlands

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 chemotractant 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 (<0.001) and neutrophils and eosinophils (<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 chemotractants 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 significantly higher levels of IL-8 when stimulated with WF-CSE but not with CSE. Relipidation 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

Naoki Furuya1, Mitsuko Takenaga2, Teruyoshi Miyazawa1, 1Division of Respiratory and Infectious Diseases, Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan; Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki, Japan

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 the 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 (PaO2) was reduced. After ASCs transplantation, PaO2 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 labeled at large alveolar spaces. Immunohistochemical analysis revealed some graft 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 a 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

Ken Bracke, Nele Pauwels, Lisa Dupont, Geert Van Pottelsberghe, Guy Joos, Guy Brusselle. Respiratory Medicine, Ghent University Hospital, Ghent, Belgium

Signalling through the IL-1 Receptor (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/ml IL-1α, anti-IL-1β or hamster IgG isotype control. 24h after the last exposure, we evaluated the
presence of inflammatory cells in the bronchoalveolar 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 (induced in sputum by ELF) of neversmokers, smokers without COPD and patients with COPD.

Acute exposure to CS resulted in a significant increase in BAL neutrophils in iso-
type treated mice. In contrast, this CS-induced BAL neutrophilia was significantly attenuated in both anti-IL-1β and anti-IL-1α 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).

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

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**Table 1. Human IL-1α and IL-1β levels**

<table>
<thead>
<tr>
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<th>Neversmokers</th>
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<tr>
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<td>IL-1α</td>
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*p<0.05 vs. neversmokers, /p<0.05 vs. smokers without COPD.

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**P3834**

β2-longs-acting and anticholinergic drugs synergistically control TGFβ1-mediated neutrophilic inflammation in COPD: An “in vitro” model

Patricia Macedo1, Iain Kilty 2, Peter Barnes 1, Louise Donnelly 1.

JAK inhibitors overcome corticosteroid insensitivity in COPD

JAK inhibitors PF95 and PF13 significantly suppress steroid-insensitive 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 IFNy. Cell media was harvested at 24h and chemokine release was 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 chemokines from COPD patients. CXCL9, CXCL10, CXCL11 (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 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.

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**P3836**

Sivemstatin attenuates cigarette smoke extract induced extracellular matrix

Brian Oliver, Janette Burgess, Judy Black, David Krimmer. Cell Biology, Woolcock Institute of Medical Research, University of Sydney, Sydney, Australia

**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 in-
hibitor, 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 5ug/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 NIH401 and the relative deposition of the ECM proteins fibronectin and periclan was measured using an ECM ELISA. The concentration of interleukin (IL)-16 in the supernatant was measured using ELISA.

**Results:** Treatment of human lung fibroblasts with 5% CSE upregulated the de-
position of fibronectin and periclan, and the release of IL-6 into the supernatant. Inhibition of simvastatin at 5ug/ml significantly attenuated 5% CSE induced de-
position of fibronectin by 43% and periclan 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 5ug/ml.

**Conclusions:** Inhibition of the HMG-CoA reductase pathway may alter the depo-
sition of ECM proteins and release of inflammatory cytokines in COPD.

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**P3837**

Sirtuin 1 reduction causes activation of Wnt/b catenin signalling

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1Airway Disease Section, National Heart and Lung Institute, Imperial College, London, United Kingdom; 23rd Respiratory Medicine Department, Samosgaleon General Hospital, Athens, Greece; 3Respiratory Medicine Department, University of Athens Medical School, Athens, Greece

**Background:** The anti-ageing molecule Sirtuin-1 (SIRT1) has been reported to be decreased in COPD (Nakamura Y et al, FASEB J, 2009). We have also reported an increase of osteoprotegrin (OOG) 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 anecy occurrence in COPD.

**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 sirt-1 protein at 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 H2O2 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 H2O2 for 24h caused reduction in Sirt-1 levels in the A549 cells.

**Conclusions:** This study shows that reduced SIRT1 protein leads to stabilization of b-catenin protein. This might give new insights in the understanding of COPD pathogenesis.

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**P3838**

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

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1Lab Allergology and Pulmonary Inflammation, University of Groningen, Groningen, Netherlands; 2Department of Pulmonology, UMCG, University of Groningen, Groningen, Netherlands; 3Department of Molecular Pharmacology, University of Groningen, Groningen, Netherlands

**Introduction:** 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.