Interleukin-8 (IL-8/CXCL8) is an important neutrophil chemoattractant known to be elevated in the airways of cigarette smokers and in patients with chronic obstructive pulmonary disease (COPD), a syndrome associated with chronic cigarette smoking. A large body of evidence exists indicating that cigarette smoke is able to activate the Epidermal Growth Factor Receptor. We have previously established that aequous cigarette smoke extract (CSE) stimulates CXCL8 and VEGF release from human normal lung fibroblasts (HNF) and airways smooth muscle cells (ASMC) and that these effects are mediated by the α,β-unsaturated aldehydes contained in the CSE such as acrolein. Here we examined the effect of pharmacological inhibition of EGFR on CXCL8 and VEGF release induced by CSE and acrolein. We found that the EGFR inhibitors AG1478, gefitinib and PD153035 inhibit CSE- and acrolein-induced CXCL8 release in both HNF and ASMC, but do not affect neither TNF-induced CXCL8 release nor CSE- and VEGF release. We have previously shown that CSE-evoked CXCL8 and VEGF release was accompanied by a rapid p38 MAPK phosphorylation mimicked by acrolein. Because p38 MAPK phosphorylation is one of the possible downstream pathways activated by EGFR, we examined the effects of EGFR inhibitors on p38 MAPK phosphorylation. We observed that all three EGFR inhibitors failed in modifying p38 MAPK phosphorylation evoked by CSE. In sum, pharmacological inhibition of EGFR suggests that EGFR is involved in CXCL8 release through a p38 MAPK independent mechanism. Given the pivotal role of CXCL8 and VEGF as neutrophil chemoattractant and angiogenic factor respectively, this study sheds light on the different mechanisms through which cigarette smoke can orchestrate inflammation and vascular remodeling in the lung.

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

Giusy Daniela Allbano, Giusy Daniela Allbano1, Caterina Di Sano1, Angela Giorgia Volpi1, Silke Meiners1
1Department of Pharmacology and Toxicology, Chiesi Farmaceutici S.p.A., Parma, Italy; 2Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.

We have previously shown that cigarette smoke extract (CSE) modulates and human alveolar macrophages, pharmacological inhibition of p38 MAPK or EGFR signaling which was accompanied by the phosphorylation of MAPK-activated kinase 2 (MK2), a known downstream target of the p38 MAPK. In both HBMSC and human alveolar macrophages, pharmacological inhibition of p38 MAPK or MK2 inhibited the decay of IL-8 mRNA levels upon stimulation with CSE or acrolein and subsequent blockade of mRNA neosynthesis with actinomycin D. Conversely, pharmacological inhibition of extracellular-signal-regulated kinases 1/2 (ERK1/2) signalling did not affect mRNA stability but inhibited both CSE- and acrolein-induced steady-state levels of IL-8 mRNA, suggesting a transcriptional effect. In sum, p38 MAPK/MK2 signalling appear to be an important post-transcriptional mechanism underlying CSE-induced IL-8 mRNA upregulation.

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

Regulation of immunoproteasomes by cigarette smoke

Bona Kellei1, Ali Önder Yıldırım2, Oliver Eckel2, Serena Bertolini1, Ilona Keller1, Gessica Marchini1, Dave Singh2, Fabrizio Facchinetti2, Carla Silva, Gert Folkerts, Johan Garssen, Frank Redegeld.
1IBIM, CNR, Palermo, Italy; 2Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.

We investigated the effect of cigarette smoke extract (CSE) on peripheral blood Th17 (PBTh17) cells from COPD patients. Role of acetylcholine

Mirella Protta1, Grazi Daniela Albano1, Caterina Di Sano1, Angela Giorgia Volpi1, Anna Bonanno1, Michael Paul Pieper1, Mark Gjomarkaj1, 1IBIM, CNR, Palermo, Italy; 2Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.
PBT17 cells produced IL-17A and IL-22, as well as Acetylcholine (ACH) are involved in the systemic inflammation of several autoimmune and inflammatory diseases, including COPD. We investigated the effect of cigarette smoke extract (CSE) and the role of ACH on the expression of RORγt, IL-17A and IL-22 in PBT17 cells during systemic inflammation in COPD.

ACH, IL-17A, IL-22 and RORγt levels as well as the colocalization of ACH with IL-17A, IL-22 and RORγt were evaluated (flowcytometry) in PBT-lymphocytes (PBT) from COPD patients (n=16), healthy smokers (HS) (n=12) and healthy control subjects (HC) (n=13). Furthermore, PBT cells from COPD (n=6) patients and PBT cells from HC (n=6) were stimulated in vitro with CSE (1%) were cultured for 72 hrs in the presence or absence of Tiotropium (Spira?) (20 nM) and Olodaterol (1 nM) alone or in combination. The colocalization of ACH with IL-17A, IL-22 and RORγt was significantly increased in PBT cells from COPD patients when compared to HC and HS subjects as well as in PBT cells from HC stimulated with CSE when compared with un-stimulated cells. Tiotropium and Olodaterol alone reduced the increased levels of colocalization between ACH with IL-17A, IL-22 and RORγt in cystic fibrosis patients from COPD patients in PBT cells from HC subjects stimulated with CSE. We suggests that cigarette smoke might be able to increase the levels of ACH in PBT cells promoting the shift into PBT17 cells which produce IL-17A and IL-22 during the systemic inflammation in COPD. The use of anti-smoking or anti-inflammatory drugs as Tiotropium and long-acting ß2-agonists as Olodaterol might prevent these events. Funded by: Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.
P1428 Role of interferon-y in tumor necrosis factor-a-mediated increase of lung microvascular endothelial cells
Yoshio Nakajima, Yutaka Nakamura, Naomi Suzuki, Yu Utsumi, Nobuhito Sasaki, Kohei Yamashita. Division of Pulmonary Medicine, Allergy, and Rheumatology, Iwate Medical University School of Medicine, Morioka, Japan

Background: Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease in which tumor necrosis factor-a (TNF-a) and interferon-y (IFN-y) are expressed and have been suggested to play pathogenic roles. The effect of these agents on angiogenesis in the lung tissues of COPD is unknown.

Objective: To examine the effects of these mediators on lung endothelial cells (ECs).

Methods: Nr2f knock-out mice were exposed to cigarette smoke (CS) for 4 weeks, and the down-regulated genes referring to vascularization in the whole lung were identified by microarray analysis. To confirm the protein levels, which were indicated in the microarray data, co-culture of lung fibroblasts with ECs in the presence of TNF-a and IFN-y was performed, thereafter ECs were submitted to an examination of protein levels expression using immunoblotting or immunocytochemistry.

Results: Microarray analysis data have shown that the mRNA expression of angiogenin-like protein 1 (Amlot1) decreased in response to CS when compared to no exposure to CS. TNF-a enhanced vascular endothelial growth factor (VEGF) production by cultured lung fibroblasts, however, vascularization was decreased when treated with IFN-y. In addition, IFN-y induced tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors on ECs and attenuated the expression of Amlot1 localized to endothelial cell-cell junctions.

Conclusions: These results suggest that IFN-y acts as anti-angiogenesis by regulating the expression of TRAIL receptors and Amlot1 on ECs, which were induced by the enhanced VEGF production by TNF-a-stimulated lung fibroblasts.

P1429 Second hand smoke exposure impairs CD93 expression and function in the lung
Adelheid Krater1, Jonas Saly1, Jean Sévigny2, Martin Zumora1, Laima Tarasiewicz-Stewart3. 1Medicine, University of Colorado, Aurora, CO; 2United States; 3Microbiology, Infectious Diseases and Immunology, University of Quebec, Canada

Chronic second hand smoke (SHS) exposure is the main risk factor for non-smokers to develop chronic obstructive pulmonary disease COPD. The mechanisms behind the chronic inflammation and lung destruction are not completely understood. In response to injury vascular and blood cells release ATP that is implicated in regulating endothelial responses. Plasma membrane ecto-nucleotidases CD39 and CD73 hydrolyze ATP and ADP. Ecto-5’-nucleotidase CD73 hydrolyzes AMP to adenosine. Here we investigated the contribution of CD39/73 expressing inflammatory cells in lung remodeling in response to SHS exposure.

Sprague Dawley rats were exposed to SHS in a smoking chamber (total particulate matter levels 115 mg/m3). The expression levels of CD39 and CD73 in the inflammatory cells and lung tissue were determined by flow cytometry, real time PCR and Western blot analysis.

Chronic second hand smoke exposure resulted in the development of emphysema in rats as measured by MLI (87.2 ± 1.6 mm after 2 month of exposure versus 73.4 ± 1.1 mm after 2 month of exposure controls). The CD39 expression was significantly downregulated in the whole lung tissue on mRNA and protein levels. Moreover, CD39 decreased was increased in the lung tissue from COPD patients. Cigarette smoke extract in vitro almost abolished CD39 and CD73 expression in the chronic inflammation and lung destructive cells. Second hand smoke exposure impairs ectonucleotidase expression in the lungs and leads to accumulation of extracellular ATP that causes increased proinflammatory responses leading to the development of emphysema.

Funded by AHA 073538NS, 11GNT7520002, FAMMRI CIA 072053, Emphysema Research Fund and Bixler Family Foundation.

P1430 TLR4 up-regulation and reduced Foxp3 expression in mechanically ventilated smokers with obstructive chronic bronchitis
Elisabetta Pace1, Maria Ferrari1, Antonino Garratano2, Chiara Cipollina1, Mark Gjomarkaj1,2,3. 1IBIM, CNR, Palermo, Italy; 2DARE, Università degli Studi di Palermo, Italy; 3RIMED, RIMED, Palermo, Italy

Chronic bronchitis (CB) is a risk factor in chronic obstructive pulmonary disease (COPD) for accelerated lung function decline and increased mortality. The lung and systemic inflammatory and immunological profile of COPD patients with CB which acutely experience respiratory failure upon a disease exacerbation is unknown. In this study we explored the expression of Foxp3 by western blot analysis, TLR4 by immunocytochemistry and the concentrations of IP-10 and IL-8 by ELISA in the mini-bronchoalveolar lavages (mini-BAL) and in the peripheral blood (PB) of patients with respiratory failure requiring intubation and mechanical ventilation. The recruited subjects were separated into three different groups: smokers with CB and COPD (COPD, n=18), smokers with CB but without COPD (S, n=8) and patients without CB and without COPD (C, n=10).

In mini-BAL of COPD group, Foxp3 and IL-10 were significantly reduced while TLR4 was significantly increased in comparison to C. TLR4 was also increased in mini-BAL of S. In COPD peripheral blood, Foxp3 was reduced in comparison to C but not significant differences were observed for TLR4 and for IP-10. No significant differences were observed for IL-8 concentrations in the mini-BAL and for the blood of the recruited patients. The mini-BAL TLR4 expression correlated with the Clinical Infective Pulmonary Score.

In conclusion, in COPD patients with respiratory failure, lung and systemic regulatory immune responses (low TLR4 expression and increased immune responses (high TLR4 expression) may contribute to the increased inflammatory events leading to respiratory failure.

P1431 Lectins improve efferocytosis via changes to cytoskeletal remodeling: Relevance to COPD
Violet Mukaro1,2, Johan Byland2, Jessica Ahern3, Mark Holmes1,2, Paul Reynolds1,2, Sandra Hodge1,2. 1Thoracic Medicine, Royal Adelaide Hospital, Adelaide, SA, Australia; 2Sahlgrenska Academy, University of Gothenburg, Sweden

We have shown that the defective ability of alveolar macrophages (AM) to phagocytize apoptotic cells (efferocytosis) in COPD could be therapeutically improved using mannose binding lectin (MBL) although the exact mechanisms are unknown. A further lectin, galectin-3, is also known to regulate macrophage phenotype and function, via interaction with its receptor CD98 (an M2 mediator). We hypothesized that defective expression of MBL and galectin/CD98 would be associated with defective efferocytosis in COPD effects on cytoskeletal remodeling and macrophage phenotype.

Galectin-3 was measured by ELISA in BAL from controls, smokers and current/ex-smokers with COPD. CD98 was measured on AM using flow cytometry. We assessed the effects of MBL and galectin on efferocytosis, CD98, actin polymerisation, rac activation, and the involvement of PI3k (using β-actin probing and wortmannin inhibition) in vitro using human AM.

A significant decrease in galectin-3 was observed in BAL from smokers and COPD subjects vs controls. Galectin 3 and MBL increased efferocytosis via an increase in active GTP bound Rac1. This was confirmed with β-actin probing and the role of PI3k was confirmed using wortmannin inhibition. The increased efferocytosis was associated with increases in available glutathione and expression of CD98.

We provide evidence for a role of airway lectins in the failed efferocytosis in COPD, supporting their further investigation as potential macrophage-targeted therapies.

P1432 Enhanced cytotoxic function of NK and NKT-like cells associated with decreased CD94 (Kp43) in the airway in COPD
Greg Hodge1,2, Violet Mukaro1,2, Mark Holmes1,2, Paul Reynolds1,2, Sandra Hodge1,2. 1Thoracic Medicine, Royal Adelaide Hospital, Adelaide, Australia; 2Lung Research, Hanson Institute, Adelaide, Australia

NK and NKT-like cells represent a small but important proportion of effector lymphocytes that we have previously shown to be a major source of pro-inflammatory cytokines and granymes. We hypothesized that NK and NKT-like cells would be increased in the airway in COPD and that this would be accompanied by a reduction in expression of the inhibitory receptor CD94 (Kp43) and increased expression of air exposed mediators granzyme B and perforin.

We measured NK and NKT-like T-cells and their expression of CD94 in the blood of patients with COPD (n=61), smokers (16) and healthy controls (25) and BAL from a subset of subjects. We further assessed activation by expression of CD69 and cytokotoxic potential by production of granzymes A and B and using a cytotoxicity assay. In blood from COPD subjects, there were no significant changes in NK or NKT-like cell numbers or expression of granzyme A or cytokotoxic potential vs controls. There was, however, increased expression of granzyme B and decreased expression of CD94 by both cell types vs controls.

In the airway in COPD, NK and NKT-like numbers were increased, associated with increased NK cytotoxicity, increased expression of granzyme B and decreased expression of the inhibitory receptor CD94.

Treatment strategies that target NK and NKT-like cells, their cytotoxicity and production of inflammatory mediators in the airway may improve COPD morbidity.

P1433 The role of IL-17 and lymphoid follicles in the pathogenesis of COPD
Koshika Yadava1,2, Aurelien Trompette1,2, Anke Sichelschmidt1,2, Laurent Nicod1,2, Benjamin Marsland1,2. 1Laboratoire de Pneumologie, CHU, Lausanne, Switzerland; 2Faculty of Biology and Medicine, UNIL, Lausanne, Switzerland

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

2575s

Abstract printing supported by Chiesi Visit Chiesi at Stand B2.10
severities of COPD by incremental instillation of LPS and elastase in mice. We observed an increase in the levels of IL-17A following repeated challenges, which coincided with the progressive drop in lung function as well as the appearance of lymphoid follicles. IL-17A production was in part triggered by the engagement of Toll Like Receptor 3 implicating a role for an endogenous danger signal in COPD. Neutralization of IL-17A at specific times after the initiation of disease ameliorated the impaired function and affected B Cell hyperplasia activation states. Our data indicates that IL-17A is involved in COPD progression by induction of lymphoid follicles and regulation of both innate and adaptive immunity.

P1434 Expansion of alveolar-lymphoid interfaces in lungs of patients with COPD
Mieko Mori1, Cecilia Andersson1, Anders Bergqvist1, Medya Shikhagaie2, Jonas Erjefält1, 2, Department of Experimental Medical Science, Lund University, Lund, Sweden; 2Respiratory Medicine and Allergology, Lund University Hospital, Lund, Sweden

Rationale: Although adaptive immune responses are critical for combating distal airway infections in COPD, the structural basis for alveolar antigen uptake has remained poorly investigated. This study investigates the interface between alveolar lumen and the adaptive immune system at different severities of COPD.

Methods: Lung resections (n=31) from mild (GOLD I), moderate-severe (GOLD II-III), and very severe (GOLD IV) COPD were subjected to detailed histological assessment of components of the adaptive immune system in distal lung.

Results: COPD severity correlated with an increase in the number of lymphoid aggregates in small airways, pulmonary vessels, and the alveolar parenchyma. Irrespective of anatomical localization, the vast majority (88%) of the aggregates had direct contact with alveolar luminal spaces (37% of the aggregate perimeter). In advanced COPD, the epithelium at alveolar-lymphoid interfaces had transformed into a significantly higher columnar phenotype (p=0.02) that, apart from expressing luminalcytokeratins, contained increased numbers of langerin-positive dendritic cells (p=0.02). Also, the total alveolar macrophage associated dendritic cells were increased (p=0.02 and p=0.002, respectively).

Conclusions: The progression of COPD is linked with an expansion and remodeling of alveolar lymphoid interfaces. These alterations, which predict an increased capacity to respond to alveolar antigens, correlated with lung function parameters and may thus contribute to the aggravated inflammation in COPD lungs.

P1435 Diverse and altered distribution patterns of TLR5 and TLR7 in the distal lung of COPD patients
Medya Shikhagaie1, Mieko Mori1, Cecilia Andersson1, Anders Bergqvist1, Claes Görän Löfdahl2, Jonas Erjefält1, 2, Experimental Medical Science, Lund University, Lund, Sweden

Activation of the innate immune system in the distal lung is a hallmark of COPD. Toll-like receptors (TLRs) trigger innate immune responses to pathogens. Although COPD patients are susceptible to infections, the distribution patterns of TLRs at different stages of disease have remained poorly studied. The present study characterizes TLR5 and TLR7 in the distal lung of COPD patients.

Methods: GOLD I (n=6), GOLD II-III (n=13), GOLD IV patients (n=8), and controls (never-smokers and non-COPD smokers) (n=15) were enrolled in this study. Immunohistochemical staining was used to identify TLR5 and TLR7 positive cells.

Results: TLR5 immunoreactivity was identified in sub-epithelial glands, airway smooth muscle, CD68+ macrophages, CD138+ plasma cells, CD208+ type II pneumocytes and the small airway epithelium. In control subjects and mild COPD epithelial TLR5 had a foremost apical distribution. In contrast, in advanced COPD TLR5 immunoreactivity was identified in sub-epithelial glands, airway smooth muscle, CD68+ macrophages, B- and T-lymphocytes, and CD56+ NK cells.

Conclusion: Both epithelial TLR5 and TLR7 are upregulated in advanced COPD. Whether the altered expression reflects a natural adaption to the increased pathogen burden in advanced COPD or is part of a dysfunctional immune-regulation in COPD remains to be determined.

P1436 Metabolomic fingerprinting in the identification of biomarkers in COPD patients
Felipe Villan-Alvarez1, Antonia García-Fernández2, Alessia Ferrari2, Julio Gómez-Seco1, Marcel Rodríguez-Guzmán1, Jesús Ruiz-Cabello1, Coral Barbas-Arribas2, Germán Peces-Barba1, 1Pneumology, II. Fundación Jiménez Díaz, CIBERES, Madrid, Spain; 2CENIM, Facultad de Farmacia, Universidad CEU San Pablo, Madrid, Spain; 3IBEB, Universidad Complutense de Madrid, CIBERED, Madrid, Spain

Introduction: Metabolomics fingerprinting is able to achieve the identification of novel biomarkers the comprehensive characterization of the entire metabolome of a disease. The main objective is to use it to understand the pathological basis underlying COPD and its relationship to the severity and phenotypic characteristics.

Methods: Observational case-control study involving COPD patients and controls without COPD or cardiovascular history. COPD patients were grouped in chronic bronchitis and emphysema, and at different stages of GOLD. Different platforms are required to capture all metabolites in one sample of plasma and separation techniques such as gas chromatography coupled to mass spectrometry (GC-Q-MS).

Results: This study involved 48 participants; 32 COPD patients (22 men, 10 women) and 20 healthy controls (7 men, 9 women). Mean age: 58.3 and 56.6 respectively. Mean FEV1 value was 64.3%. We can not establish significant differences between COPD and controls in the total population. In women, we can differentiate COPD from the controls by metabolites such as glycolic acid, although its origin is uncertain and could be related to medication. This metabolite, in women, also helps to differentiate emphysema (and smokers) from controls, emphysema and chronic bronchitis from controls and smokers from non-smokers. With oxalic acid we can differentiate chronic bronchitis (and smokers) from controls in women, and emphysema from chronic bronchitis in men with COPD (and former smokers). Besides, in this last group, we can differentiate GOLD I from GOLD III–IV D with mystic acid.

Conclusion: Using metabolomic fingerprinting in plasma we could identify markers and differentiate phenotypes and early stages in COPD.

P1437 Comparable matrix alterations in the alveolar and small airway wall of COPD patients
M.L. Eurlings, N.L. Reynaert, E.F.M. Wouters, M.A. Dentener, Respiratory Medicine, Maastricht University, Maastricht, Netherlands

Rationale: Remodeling in COPD is considered twofold with thickening of the wall of airways >2mm on one hand and destruction of alveolar walls on the other. However, we hypothesize that matrix alterations in both alveolar and small airway (SA) walls of COPD patients differ from healthy people.

Methods: Lung tissue sections of 8 smoking controls and 10 moderate to severe COPD patients were stained for elastin by Weigert’s Resorcin-Fuchsin and collagen I, 2, and 3 by Perls’ red. In addition, hyaluronan, a glycosaminoglycan matrix component, was stained with a hyaluronan binding protein. All stainings were corrected for total surface area and data expressed as mean % of stained area ± SD.

Results: Elastin was significantly decreased in COPD in both alveolar (27.7% ± 5.3 vs. 17.9% ± 3.3, p<0.01) and SA walls (24.2% ± 4 vs. 15.2% ± 2.7, p<0.01). Both collagen in alveolar (11.6% ± 5.4 vs. 25.9% ± 10.4, p<0.01) and SA walls (11.8% ± 2.3 vs. 23.2% ± 6.7, p<0.01) and hyaluronan in alveolar (11.7% ± 3.2 vs. 19.8% ± 3.5, p<0.01) and SA walls (12.9% ± 5.4 vs. 25.6% ± 10.0, p<0.05) increased significantly. Alveolar and SA wall matrix components correlated significantly: elastin (r=0.664, p<0.01), collagen (r=0.741, p<0.01) and hyaluronan (r=0.626, p<0.05). Furthermore matrix compounds were significantly related with FEV1; alveolar elastin (r=0.742, p<0.01), SA elastin (r=0.824, p<0.01), alveolar collagen (r=0.755, p<0.01), SA collagen (r=0.675, p<0.01), alveolar hyaluronan (r=0.775, p<0.01) and SA hyaluronan (r=0.538, p<0.05).

Conclusion: These results indicate that remodeling in the alveolar and SA wall in COPD show marked similarities and both relate to FEV1.

P1438 Corticosteroid insensitivity in airway smooth muscle cells of severe asthma and COPD: Modulation by IFN-γ
Po-Ju Chang1,2, Josephine Baker1, Funkji K. Bhavsar1, Mark M. Perry1, Kian Fan Chung1, 2Experimental Studies Group, Airway Disease Section, National Heart and Lung Institute, Imperial College, London, United Kingdom; 2Department of Thoracic Medicine, Chang Gung Memorial Hospital, Linkou, Taoyuan County, Taiwan

Background: Patients with severe asthma or COPD respond poorly to corticosteroids (CS). IFN-γ impairs CS response in airway smooth muscle cells (ASMCs) of healthy subjects.

Aims and objectives: Compare cytokine-induced chemokines and CS response in ASMCs of severe asthma or COPD. Investigate the effect of IFN-γ on CS response.

Methods: ASMCs of healthy subjects (12), non-severe (NSA; 10) and severe asthma (SA; 10), smokers (8) and COPD (8), were obtained via endobronchial biopsy. At passage 4-5, cells were pretreated with dexamethasone (Dex; 10-10 M, 24 hr) and stimulated with TNF-α/IFN-γ (10 ng/mL each; 24 hr). CCL11 and CXCL8 release and mRNA were assessed by ELISA and qRT-PCR, p58 NF-κB promoter stimulation by CHIP, and p38 MAPK activity by Western Blot.

Results: Baseline and TNFα-induced CCL11 release/mRNA were not increased in NSA, and CXCL8 increased at baseline in smokers and COPD. Dex suppression of induced chemokines was impaired in SA, smokers and COPD. IFN-γ inhibited i) induced CCL11 and CXCL8, ii) p58 recruitment to chemokine promoters, and iii) Dex suppression of induced chemokines in the healthy. In SA, IFN-γ further reduced suppression of only CXCL8. Paradoxically, IFN-γ improved the suppressive effect of Dex on CXCL8 in smokers. COPD p38 MAPK activity was reduced in SA, and inhibition of p38 restored corticosteroid sensitivity. However, IFN-γ did not modulate induced p38 activity.
Conclusions: ASMCs of SA, smokers and COPD display CS insensitivity. IFN-γ impairs the suppressive effect of Dex on CXCL8 in the healthy and asthmatics but improves it in smokers and COPD, suggesting differential mechanisms underlying CS insensitivity in SA and smokers/COPD.

P1439
Inhaled corticosteroids (ICS) attenuates epithelial mesenchymal transition (EMT) in COPD: A key to understanding long term benefits?
Sukhwinder Singh Sohal1, Amir Soltani1, David Reid1,2, Chris Ward1,3, Steven Weston1, Hans Konrad Muller1, Richard Wood-Baker1, Eugene Haydn Walters1, 1NHMRC Centre for Research Excellence in Chronic Respiratory Disease, University of Tasmania, Hobart, TAS, Australia; 2Iron Metabolism Laboratory, Queensland Institute of Medical Research, Brisbane, QLD, Australia; 3Respiratory Medicine, Institute of Cellular Medicine, Newcastle upon Tyne, Newcastle, United Kingdom

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

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

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

Results:

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

<table>
<thead>
<tr>
<th>Markers</th>
<th>Before (FP)</th>
<th>After (FP)</th>
<th>Before (Placebo)</th>
<th>After (Placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Rbm fragmentation</td>
<td>24.0 (6.6-100)</td>
<td>2.6 (0-88.6)†</td>
<td>26.9 (2.5-48.5)</td>
<td></td>
</tr>
<tr>
<td>S100A4 positive cells in BE per mm of Rbm</td>
<td>25.8 (2.4-55.3)*</td>
<td>12.3 (0.6-24.9)*</td>
<td>19.8 (2.9-31.6)</td>
<td>17.4 (10.3-35.5)</td>
</tr>
<tr>
<td>S100A4 positive cells in Rbm per mm of Rbm</td>
<td>44.4 (15.3-92.6)*</td>
<td>20.8 (2.6-60.7)*</td>
<td>23.1 (14.8-52.9)</td>
<td>29.3 (3.6-48.1)</td>
</tr>
<tr>
<td>MMP-9 positive cells in Rbm clefts per mm of Rbm</td>
<td>0.6 (0-22.4)*</td>
<td>0 (0-10.6)*</td>
<td>1.1 (0-4.1)</td>
<td>1.3 (0-2.7)</td>
</tr>
<tr>
<td>EGFR % epithelium</td>
<td>34 (14.6-59.5)*</td>
<td>5.8 (2.6-43)*</td>
<td>14.4 (13.6-38.2)</td>
<td>10.3 (1.3-39.1)</td>
</tr>
</tbody>
</table>

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

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