P3839 Antigen presenting cell populations in the respiratory tract take up size-dependent particles during steady state and allergic airways inflammation
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Effects of biomedical particles on lung antigen presenting cells (APC) such as lung parenchyma dendritic cells (LPDC) and bronchus-associated lymph fluid (BALF) alveolar macrophages (AM) remain poorly understood to date. The size-dependent fate of particles and trafficking by APC populations is crucial to understand downstream immunological effects. To study particle uptake in lung APC during steady state and in an experimental allergic airways disease (EAAD) model, ovalbumin (OVA)-sensitised BALB/c mice received intra-nasally different sized fluorescent polystyrene particles. Two hours after instillation, BALF/lung parenchyma were collected and analysed by FACS and confocal microscopy. Lung APC populations (AM, CD11b+ LPDC, CD11b- LPDC) were tracked during particle exposure in naive and EAAD mice. In AM, particle uptake occurred independently of size (frequency of particle-positive AM for 50nm: 47±5.1%, 100nm: 63±6.1%, 200nm: 63±4.5%, 1000nm: 62±10.8%, p=0.0516) and was significantly increased during EAAD for 50nm particles only (64±1.6%, p=0.0014). In contrast, in both LPDC subsets, particle uptake was generally lower and size dependent (CD11b+ LPDC: 50nm: 4.1±0.7%, 100nm: 5.0±0.9%, 200nm: 8.2±2.4%, 1000nm: 1.5%±0.3%, p=0.005 and CD11b- LPDC: 50nm: 21.6±6.5%, 100nm: 4.2±1.1%, 200nm: 10.4±1.7%, 1000nm: 1.9%±0.3%, p=0.0001). During EAAD CD11b+LPDC were predominant and reduced their capacity for uptake of 50nm particles during EAAD (62±0.6%, p=0.044). We conclude that in APC populations, particle size, and the presence of inflammation critically determine uptake of particles in the lung.

P3840 Disease and stimulus specific pro-inflammatory cytokine secretion by human fibroblast of asthma patients
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It is well documented that the asthmatic lung contains increased levels of pro-inflammatory cytokines. We provide evidence that compared to healthy primary human fibroblasts isolated from medium size airways of asthma patients secrete significantly more pro-inflammatory cytokines in a stimulus and disease specific pattern. Bronchial fibroblasts from 5 asthma patients and 5 healthy controls were isolated and stimulated with PGDF-BB, TNF-alpha or IL-1beta (0.1, 1.0, 10 ng/ml), all of which are well known asthma-relevant factors. After 24 hours, secreted IL-6, IL-8, etoxin and GM-CSF were determined in the cell culture medium. PGDF-BB dose dependently increased both IL-6 and etoxin secretion by 3 and 6 fold, respectively, but with no difference between asthma and control. In contrast, IL-8 and GM-CSF levels were significantly increased in asthma TNF-alpha increased IL-6, etoxin and GM-CSF significantly stronger in asthma fibroblasts relative to control. IL-8 secretion dose-dependently increased after TNF-alpha treatment, but no difference between asthma and control was observed. IL-1beta dose-dependently increased the secretion of all 4 cytokines, however, with a disease specific increase of GM-CSF at 10 ng/ml. Our data suggest that bronchial fibroblasts contribute to chronic inflammation in asthma and stimulus specific pattern.

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P3841 The role of MKK3 in allergic and non-allergic inflammatory responses in the lung
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MKK3 is a member of the p38 MAPK signaling pathway and is an important factor in non allergic inflammatory and Th1 responses. Less is known about the role of MKK3 in allergic inflammation. We investigated the role of MKK3 in murine models of non-allergic and allergic lung inflammation. Wild Type (WT) mice were intranasally instilled with lipopolysaccharide (LPS) (10 μg, i.t.) or zymosan (100μg i.t.). A significant increase in neutrophil numbers in the lungs were observed 24h later compared to saline controls (saline: 0.3±0.01 × 10⁶ μ, LPS: 9.4±1.9 × 10⁶ μ; zymosan: 6.2±0.8 × 10⁶ μ). WT mice also produced significantly larger levels of IL-6, IL-12, TNF-α and INF-α as compared to saline mice. MKK3⁻/⁻ mice did not release these cytokines in response to LPS or zymosan. WT mice were sensitized twice to ovalbumin (ova, 10ng/mouse i.p in albuin). From day 14 all mice were exposed to 3% ova once daily for 3 days. Lung lavages were performed 24 h after the last exposure. Exposure to ova significantly increased eosinophil numbers in the lungs of ova-sensitized mice as compared with sham-immunized mice (sham: 0.4±0.2 × 10⁵ μ vs ova WT: 0.45±0.1, n=11; ova: MKK3⁻/⁻: 2.8±0.7 × 10⁵ μ). Ova-MKK3⁻/⁻ mice showed significantly increase of IL-5 compared to both ova WT and ova sham-immunized mice (sham: 0.8±0.8 × 10⁵ μ vs ova WT: 3.8±1.9 μ vs ova MKK3⁻/⁻: 20.8±10.7 pg/ml). MKK3⁻/⁻ mice showed significantly higher levels of IgE compared to WT mice, irrespective of ova treatment (sham: WT: 0.01 μ, ova WT: 6.062±3.56 μg/ml, n=6; sham MKK3⁻/⁻: 441±50 μg/ml, n=13). In conclusion, MKK3 differentially regulates allergic and non-allergic responses in the lung. 

Airway epithelial toll-like receptor expression in asthma and its relationship to disease severity
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Airway epithelial toll-like receptor (TLR) expression is an important feature in the development of asthma. TLR expression is increased in asthmatic airway epithelium and is associated with disease severity. TLR expression in asthma is known to be increased in airway epithelial cells, airway submucosal glands, and bronchial epithelial cells. However, the role of TLR in the development of asthma and exacerbations is not well understood. In this study, we investigated the expression of TLR in the airways of healthy volunteers and patients with mild and severe asthma. Epithelial brushings were obtained from the large airways at bronchoscopy from 18 healthy non-asthmatic volunteers and 34 asthmatic volunteers. The asthmatic group comprised 7 non-steroid treated and 27 steroid treated asthmatics. FT-qPCR analysis was performed on extracted RNA for TLR-2,-4 and -5 and IL-8. Gene expression for TLR-2 (p=0.008) and TLR-4 (p=0.012) was significantly increased within the asthmatic sample compared to healthy subjects whilst TLR5 expression did not differ significantly. IL-8 mRNA was also increased in the asthmatic population (p=0.007) compared to that of healthy subjects. These significant differences from the healthy population were also individually present in both the mild and severe asthmatics groups, with no significant difference being evident between mild and severe asthma. These findings reveal up-regulation of epithelial gene expression for TLR family members and IL-8 relevant to respiratory responses within the airways. These findings are indicative of ongoing innate immune airway responses in asthma. The relevance of this to clinical disease expression requires understanding.

Putative roles of lepin on airways reactivity
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There is growing evidence that the associations between the airway hyper-
activity and obesity. Leptin is an adipokine with a well-established functional role on airways function. The specific airway resistance (sRaw) and isolated bronchial rings reactivity were assessed on obese resistant (OR) and obese prone (OP) rats, fed with high fat diet. Results were compared with data from Sprague Dawley (SD) rats fed with standard diet. In vitro experiments were performed on main left bronchus rings. Both contraction induced by 10 mM acetylcholine (ACh) and terbutaline-induced relaxation were assessed. On OP bronchi, the terbutaline dose-response curve was shifted to the right and the maximal relaxant effect was decreased up to 20%. Leptin pretreatment did not significantly modify terbutaline relaxant effects on OR but restored terbutaline effects on OP bronchi. Inhibition of all NO synthases (NOS) with 10 mM N(G)-nitro-L-arginine methyl ester blocked the leptin effects. Aminoguanidine (inhibitor of inducible NOS) partially prevented the leptin effects. The ACh-induced sRaw variation was not significantly modified by intratracheal administration of leptin on OP as compared with OR. On the other hand, the exogenous leptin significantly increased the sRaw on OR as compared with SD. These results suggest that leptin could have protective effects on airway hyperresponsiveness by stimulation of NO synthesis due to the activation mainly of constitutive NOS (cNOS), and not only the obesity but also the diet could modify the airways function.

P3844
Immunochemical study of interleukin-1β in alveolar macrophages of patients with well-controlled and non-controlled asthma
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We have conducted dynamic immunocytochemical investigation of the level of Interleukin-1β (IL-1β) in alveolar macrophages of a group of patients (n=16) with well-controlled and non-controlled asthma. Induced sputum specimens from each of the patients were collected twice. Cytologic preparations of sputum were obtained using the method of liquid cytology. Cytologic centrifugates were coloured on slides via immunocytochemical method with the use of monoclonal anti-IL-1β antibodies. The results of immunocytochemistry were evaluated using Histology Score method (HScore).

The average value of the investigated index “content of IL-1β in alveolar macrophages” in patients with non-controlled asthma was 251.5±8.3 HScore, while in patients with well controlled asthma it came to 66.4±18.0 HScore. The level of IL-1β decreased more than five times in 14 of 16 patients as a result of their treatment which included education.

Thus, the results of our study indicate that IL-1β is produced by alveolar macrophages in significant amount during non-controlled asthma and that its production decreases abruptly when asthma becomes well-controlled.

P3845
Immune status in patients with bronchial asthma exacerbation and comorbid digestive system diseases
Shamil Zagidullin, Elena Galimova, Guev Nurtinova, Rustem Zulkareev

Bronchial asthma (BA) is frequently linked with digestive system pathology. Aim: To evaluate features of immune status in bronchial asthma (BA) patients with and without comorbid digestive system diseases (DSD). Materials and methods: Prospective, descriptive study of 90 hospitalized patients with bronchial asthma exacerbation and 31 healthy controls. Immune status was assessed via quantitation of lymphocytes subpopulations, immunoglobulins, A,E,G,M, circulating immune complexes. Digestive system diseases were diagnosed via gastroscopy and ultrasonic scanning. Results: 61 (67.8%) patients with BA exacerbation suffered from digestive system diseases: gastritis, duodenitis-27 patients (30.0%), duodenal aneurism-4 (4.5%), gastric-duodenum reflux-20 (22.2%), biliary dyskinesia-10 (11.1%). Immune response parameters were decreased in both groups of BA patients vs. controls. Patients with BA and DSD showed significant decrease of CD4, CD8 and IgA vs. controls and patients with BA without comorbidities.

Immune status in patients with BAxDSD

<table>
<thead>
<tr>
<th>BA</th>
<th>BAxDSD</th>
</tr>
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<tbody>
<tr>
<td>CD4+</td>
<td>68.2±2.0</td>
</tr>
<tr>
<td>CD8+</td>
<td>41.2±2.8</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>19.8±6.2*</td>
</tr>
<tr>
<td>CD16a</td>
<td>12.4±1.6</td>
</tr>
<tr>
<td>CD22a</td>
<td>11.5±1.3</td>
</tr>
<tr>
<td>CD25+</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>IgG</td>
<td>14.9±0.9</td>
</tr>
<tr>
<td>IgA</td>
<td>3.0±0.2</td>
</tr>
<tr>
<td>IgM</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>IgE</td>
<td>32.4±1.3</td>
</tr>
<tr>
<td>CIC, unc.</td>
<td>41.4±5</td>
</tr>
</tbody>
</table>

*p<0.05 with controls, with BA.

Conclusions: Comorbid digestive system diseases in patients with BA are associated with significant decrease of activity of cell and humoral parts of immune system.

P3846
Increased levels of CD4+CD25high and CD4+FoxP3+ T-regulatory cells (Tregs) in patients with different severity of bronchial asthma (BA)
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Background: BA is characterized by persistent inflammation in the airways in response to allergens or other triggers. The intensity of the inflammation determines the severity of asthma. Levels of FoxP3+ regulatory T cells (Tregs) are important in maintaining immune responses and maintaining peripheral tolerance. Our study sought to determine whether numbers of CD4+CD25high and CD4+FoxP3+ Tregs are related to airway inflammation and disease severity.

Aim and objectives: To investigate the expression of surface molecules on Treg cells in patients with different severity of bronchial asthma compared to healthy persons.

Methods: Peripheral blood mononuclear cells of control (n=17) and asthmatic patients (mild BA, n=11; severe BA, n=17) were labeled for CD4, CD25 and intracellular FoxP3 and analysed using flow cytometry.

Results: Numbers of peripheral blood CD4+CD25high was significantly decreased in severe asthmatic patients compared with healthy control. Patients with severe asthma had increased levels of CD4+CD25high and CD4+FoxP3+ compared to mild BA.

Conclusions: Our findings suggest that decreased levels of Tregs in peripheral blood might contribute airway inflammation. Increase CD4+CD25high and CD4+FoxP3+ is important in balancing immune responses for prevention of the severity of asthma.

P3847
Superoxide dismutase as a longitudinal biomarker of lung function in asthma
Stary Combair, Aman Khan, Serpi Esmaeili

Asthma is a chronic inflammatory disease related to oxidative stress. Previously, we reported that superoxide dismutase activity (SOD) is lower in asthma as compared to healthy controls and related to airflow obstruction and reactivity. In this study, we hypothesized that serum SOD would be informative for progression of asthma over time. To test this, asthmatic adults (n=77) were followed for 5 years. At baseline, patients with severe asthma had increased levels of CD4+CD25high and CD4+FoxP3+ compared to mild BA. These results suggest that asthmatics with high SOD experience a progressive loss of SOD activity over time and are at risk for accelerated loss of lung functions.

P3848
Anti-IgE and airway remodelling: Omalizumab affects reticular basement membrane thickness in severe persistent atopic asthma
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Asthma is a complex genetic disorder that is characterized by airway inflammation and reversible airflow obstruction. Severe asthmatics are inadequately controlled despite the use of high-dose inhaled corticosteroids (ICS) and long-acting β2-agonists. Role of IgE mediated inflammation in asthma is established. Allergic inflammatory process underlies the pathogenesis of severe persistent asthma. The most widely used and currently only approved monoclonal antibody against IgE for use in asthma is Omalizumab. The efficacy and safety of omalizumab have been evaluated in several studies which showed a significant drop in asthma exacerbations, and emergency visits. The thickening of subepithelial basement membrane in severe asthma is associated to increased bronchial mucosal eosinophils, typical allergic cellular effectors. The aim of the study is to investigate the effect of anti-IgE on the basement membrane thickness. Biopsies were obtained from 11 patients with Severe Persistent Allergic Asthma. Before e post (12 months) treatment with omalizumab. RMB thickness was measured by morphometric analysis by using light microscope image analysis. The analyses proved a significantly statistical difference, p<0.005, in a narrow population. Nonetheless we explored more in detail the entire population discriminating Responder and Non Responder on the basis of the obtained reduc-
Reverub is a novel regulator of COPD macrophage inflammation and glucocorticoid resistance

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Introduction: Human inflammation shows circadian oscillation of inflammatory mediators e.g. IL-6. The potency of many anti-inflammatory drugs e.g. glucocorticoids also oscillate in a similar manner. The mechanisms causing these phenomena are poorly understood. A recent discovery describes that cells have a “molecular clock”, regulating 50% of the genome.

Aims: We investigated the function and mechanism of Reverub, part of this “molecular clock”, concerning COPD inflammation.

Results: LPS stimulated macrophages showed a diurnal response for IL-6. A novel Reverub ligand (GSK414112) corrected this but had no effect if Reverub expression was suppressed. The ligand had no effect in two epithelial cell lines (A549, Hela), demonstrating cell specific actions. An array and luminex analysis on cellular supernatants from human healthy and COPD macrophages defined the targets and mechanism of GSK414112 suppression. A number of key COPD targets were suppressed. GSK414112 up regulated PPARα and LXRα, two nuclear receptors, and their respective cholesterol target genes e.g. ABCA1. Distal regulatory element analysis identified the importance of the LXR DR4 motif, which Reverub binds. This mechanism was confirmed experimentally with a LXR antagonist. Luciferase reporter constructs, focused on the IL-6 proximal promoter, identified three key transcription factor binding sites. GSK414112 doubled the IC50 of dexamethasone on IL-8.

Conclusion: Reverub affects both inflammation and glucocorticoid resistance. Synthetic ligands can modify its function potentially allowing for the first time temporal control of the inflammatory response and glucocorticoid potency.

Quantitative proteomics study reveals cross-talk between autophagy and inflammation induced by cigarette smoke in airway

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Introduction: Cigarette smoke (CS) is an important risk factor for the development of airway inflammation. Autophagy can be induced by CS, while the role of autophagy in airway inflammation induced by CS is still unclear. We sought to determine the key role of autophagy in autophagy induction induced by CS using system-level analysis by amino acids in cell culture (SILAC)-based quantitative proteomics.

Methods: SILAC-labeled human lung mucocoeipithelial cells (NCI-H292) was exposed to cigarette smoke extract for 24h, and then was used to identify the differentially expressed proteins. In order to validate the results in vivo, rats were exposed to CS for 4 weeks. Besides the analysis of bronchoalveolar lavage fluid (BALF) and histological changes, immunohistochemistry and western blot validated the selected proteins. Autophagy was observed by electron microscope (EM).

Results: Three proteins associated with autophagy were significantly up-regulated after CS exposure in NCI-H292 cell (p<0.05), including macrophage-associated proteins 1A/1B light chain 3GLC3/AB1, high mobility group protein (HMGB1), and Cystatin-C. EM revealed that autophagic vacuoles were dramatically increased in rat lung tissues exposed to CS (p<0.05). Moreover, airway inflammation induced by CS was demonstrated by histological changes, increased cell counts and pro-inflammatory cytokines in BALF (respectively, p<0.05).

Immunohistochemistry and western blot demonstrated that CS markedly increased L3CA/B, Cystatin-C and HMGB1 expression in rat lung (respectively, p<0.05).

Conclusions: Autophagy may play a role in inflammation induced by CS.

Myeloid derived suppressor cells in the crosstalk between COPD and lung cancer

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COPD is associated to increased lung cancer risk, even independently from cigarette smoke. The chronic inflammation present in COPD patients may represent an important link with lung cancer, but the relationship has yet to be elucidated. Of note, tumors to promote their own survival, may influence the immune response by inducing a subset of myeloid cells with immunosuppressor properties (MDSC). We evaluated the induction of MDSC in patients with COPD, with or without lung cancer, by measuring the m1 of IL-4 receptor (IL-4Rα) which has been proposed as a marker for these cells. IL-4Rα was quantified by flow cytometry in peripheral blood from 19 smokers with COPD and no cancer (FEV1=66±66/ pred), 6 smokers with COPD and lung cancer (FEV1=67±69/ pred) and 11 controls with normal lung function and no cancer (5 smoking, 6 non-smoking; FEV1=106±66/ pred). IL-4Rα expression was increased in monocytes from smokers with COPD and concomitant lung cancer (23;17-37%) but also in COPD patients without cancer (median range: 12.5-25%). Comparison to smoking and non-smoking controls (10.3-15% and 97-11%; all p<0.05). A similar IL-4Rα upregulation was also observed in granulocytes. Of note, in all patients considered together, IL-4Rα expression was related to the degree of airway obstruction (p=0.03, r=0.50). In conclusion, our study shows that IL-4Rα expression is upregulated in monocytes from smokers with COPD and lung cancer, but interestingly even in COPD patients without cancer. Evaluation of the alleged suppressive activity of these cells could improve our understanding of the immune response in COPD potentially identify inflammatory alterations that might increase the risk of developing lung cancer.

IL-17A expression by mast cells in smokers

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Background: Mast cells are increasingly recognized as important contributors to smoking-induced inflammation. IL-17A is a cytokine produced by inflammatory cells which promotes neutrophilic inflammation. We evaluated whether IL-17A is expressed by mast cells in the airways of smokers.

Methods: Immunohistochemical staining was performed in large (LA) and small (SA) airways from 12 (ex-)smokers undergoing lung cancer resection. In airways free of tumour a double staining protocol was used for the simultaneous visualisation of mucosal (MCp, positive for the protease trypstatin) and connective tissue (MCc, positive for the proteases chymase and tryptase) mast cell as well as for IL-17A and MCcresp. MCc. Image analysis was performed using spectral microscopy. Values are expressed as cells/µm length for inner and outer layers.

Results: See Table 1 (p. 698).

Conclusion: A marked proportion of mast cells in LA and SA of smokers express IL-17A. High expression of IL-17A was observed in both MCp and MCc, and mast cells situated in the outer layer. These findings suggest that mast cells contribute to inflammation in LA and SA via IL-17A expression.

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Abstract P3854 – Table 1

**P3854**

**Carboxylyseine and N-acetyl cysteine inhibit cigarette smoke mediated acetylation of the PMN chemotractant peptide PGP**

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Objectives: The objectives were to investigate the chronic effects of ozone exposure on bronchoalveolar lavage (BAL) fluid and lung tissue collections. BAL total cell counts were elevated (predominately macrophages and neutrophils) and was associated with increased levels of cytokines (KC and TNFα) and elevated protein levels.

Methods: Ozone exposure and allergen sensitization caused a synergistic increase in MCH15 γ of ozone exposed mice compared to normal air exposed mice. Using chromatin immunoprecipitation assays of ozone exposed mice, we identified 2 and Macrophage Migration Inhibitory Factor (MIF) promoter regions associated with increased levels in chronic ozone exposed mice.

Results: Using chromatin immunoprecipitation (ChIP) and DNA microarrays, we identified 2 and Macrophage Migration Inhibitory Factor (MIF) promoter regions associated with increased levels in chronic ozone exposed mice.

Conclusions: Our results support the hypothesis that ozone exposure directly induces fibrosis by upregulating production of ECM proteins. The mechanisms by which both biomass and cigarette smoke exposure cause lung damage may be similar.

N=12. Medians (quartiles). *p<0.05 vs corresponding SA layer within same cell type. #p<0.05 vs corresponding outer layer within same airway type.

### P3855

### Interactions between the effects of a 4-week NO2 and carbon nanoparticle exposure with allergen sensitization on bronchial responsiveness in Brown Norway rat

Skander Layachi1, Françoise Rogerieux2, Christelle Gamez2, Kelly Blazy2, Norwals1, Francis Bobil2, Anthony Lecomte2, Ghislaine Lacroix2, Sam Bayat1,3.

**Methods:** Brown-Norway rats were divided into the following groups: Control, NO2, NO2 + CNP, OVA, NO2 + OVA, and CNP. Approximately 50% of deaths from chronic obstructive pulmonary disease (COPD) in developing countries have resulted from repeated exposure to biomass smoke and the capacity to directly induce fibrosis by upregulating production of ECM proteins. The mechanisms by which both biomass and cigarette smoke exposure cause lung damage may be similar.

**Results:** Deposition of the ECM proteins perlecan and fibronectin was upregulated by both 5% CSE and 1% BME. 1%, 5%, 10% and 20% BME and 5% CSE significantly upregulated the phosphorylation of ERK1 and ERK2 following 30 minutes exposure and these remained elevated to 24 hours exposure. Release of IL-8 in the supernatant was increased by 5% CSE and 1% BME. Exposure to concentrations of BME >10% caused significant decreases in cell viability.

**Conclusion:** BME has similar effects to CSE in vitro and has the capacity to directly induce fibrosis by upregulating production of ECM proteins. The mechanisms by which both biomass and cigarette smoke exposure cause lung damage may be similar.

### P3856

**Biomass smoke extract increases fibronectin and perlecan release from human lung fibroblasts**

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**Introduction:** Approximately 50% of deaths from chronic obstructive pulmonary disease (COPD) in developing countries have resulted from repeated exposure to biomass smoke and the capacity to directly induce fibrosis by upregulating production of ECM proteins. The mechanisms by which both biomass and cigarette smoke exposure cause lung damage may be similar.

N=12. Medians (quartiles). *p<0.05 vs corresponding SA layer within same cell type. #p<0.05 vs corresponding outer layer within same airway type.
P388S
Characterisation of T cell populations in proximal and distal airways
Rebecca Spruce, Gregory Rankin, Christopher Pickard, Jane Warner. School of Medicine, University of Southampton, Southampton, Hampshire, United Kingdom

Introduction: CD8+ T cells are known to be involved in the pathogenesis of COPD but less is known about CD4+ T cells. We have characterised T cell populations from the distal and proximal airways of patients with and without airways obstruction.

Method: Macroscopically normal matched proximal and distal airway tissue was obtained from 23 patients (mean age 66±2.7 years, FEV1/FVC=0.66±0.02) undergoing lung resection. Samples were dissected into explants, fixed and processed for GMA immunohistochemistry. Sections were stained for CD3, CD4 and CD8. To examine a larger population of T cells, explants from the same patients were incubated for 24 hours to encourage T cell migration into supernatant. Recovered cells were stained for T cell markers (CD3-FITC, CD8-APC) and analyzed via FACS.

Results: Immunohistochemical analysis revealed relatively few T cells in either proximal or distal airways (median CD3+ cells distal airways =1.6/mm² compared to 1.4/mm²) in the proximal airways. We found more CD4+ than CD8+ cells in both compartments but numbers of cells counted were low. In order to characterise a larger number of T cells FACS was performed on cells that migrated out of the explants. This confirmed that there were more CD4+ cells than CD8+ cells in the distal airways though this was not seen in the proximal airways. The distribution was not significantly affected by the presence of airways obstruction.

Conclusions: Similar numbers of CD3+ cells were found in the proximal and distal airways. CD4+ cells were predominant in the distal airways while the distribution of CD4 and CD8+ cells was equivalent in the proximal airways. The presence of mild/moderate COPD did not affect T cell number or distribution.