407. Immunology and cell biology of asthma and COPD

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Antigen presenting cell populations in the respiratory tract take up size-dependently 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 bronchoalveolar lavage 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: 63.3±6.1%, 200nm: 63.4±5.3%, 1000nm: 62.1±10.8%, p=0.0516) and was significantly increased during EAAD for 50nm particles only (64.1±1.6%, p=0.0014). In contrast, in both LPDC subsets, particle uptake was generally lower and size dependent (CD11b+ LPDC: 50nm: 4.3±0.7%, 100nm: 5.0±0.9%, 200nm: 8.2±2.4%, 1000nm: 1.5% ±0.3%, p=0.0005 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 (6.2±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.

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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 PDGF-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, eotaxin and GM-CSF were determined in the cell culture medium. PDGF-BB dose dependently increased both IL-6 and eotaxin 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, eotaxin 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 in a disease and stimulus specific pattern.

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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 instillated with lipopolysaccharide (LPS) (10 μg, i.t) or zymosan (100μg i.t.). A significant increase in neutrophil numbers in the lung were observed 24h later compared to saline controls (saline: 0.3±0.01 *vs* LPS: 9.4±1.9; zymosan: $6.2\pm0.8\times10^5$/ml, n=9. $p\leq0.05$) or MKK3$^{-/-}$ KO mice (LPS: 0.36±0.04; zymosan: $0.57\pm0.3\times10^5$/ml, n=10). WT mice also produced significant levels of IL-6, IL-12, TNF-α and INF-α as compared to saline mice. MKK3$^{-/-}$ mice did not release these cytokine in response to LPS or zymosan. WT mice were sensitized twice to ovalbumin (ova, 10mg/mouse i.p in alum). 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 eosinophils number in the lungs of ova-sensitized mice as compared with sham-immunized mice (sham: 0±0 *vs* ova WT: 0.45±0.11, n=11; ova MKK3$^{-/-}$: 2.83±0.74, n=13×10^5/ml). Ova-MKK3$^{-/-}$ mice showed significant increase of IL-5 compared to WT ova and sham-immunized mice (sham: 0.38±0.98 vs ova WT: 3.5±1.9 and 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±0; ova WT: 6062.3±560 pg/ml, n=6; sham MKK3$^{-/-}$: 4414.5±637, ova MKK3$^{-/-}$: 5328.8±415.8 pg/ml, n=13). In conclusion, MKK3 differentially regulates allergic and non-allergic responses in the lung.

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Airway epithelial toll-like receptor expression in asthma and its relationship to disease severity

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Asthma is classically considered a Th2 mediated disease. However severe, treatment-resistant disease is more heterogeneous and often associated with airway neutrophil recruitment. This could be due to altered airway bacterial colonisation. Bacteria express molecular patterns that are recognised as non-self by pattern recognition receptors expressed on innate cell surfaces such as Toll-like receptors (TLR's). This study has investigated the expression of mRNA for TLR-2, -4 and -5 in airway epithelial cells in asthmatics and healthy volunteers.

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. RT-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 bacterial responses within the airways. These features are indicative of ongoing innate immune airway responses in asthma. The relevance of this to clinical disease expression requires understanding.

P3843

Putative roles of leptin on airways reactivity

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There is growing evidence that sustain the associations between the airway hiperre-

activity and obesity. Leptin is an adipokine with a not 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 hight 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 10μM 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 rats but restored terbutaline effects on OP bronchi. Inhibition of all NO synthases (NOS) with 10μM 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 modify 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

Immunocytochemical 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 via method of liquid cytology. Cytologic centriphugates 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 comorbide digestive system diseases

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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 comorbide 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%), duodenum anabrosis-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 BA±DSD

	Controls	BA	BA+dsd
CD 3+, %	68,2±2,9	58,0±0,74*	54,4±1,6*
CD 4+, %	41,2±2,8	30,0±0,54*	24,8±0,8*#
CD 8+, %	23,9±2,4	18,9±0,62*	17,0±0,5*#
CD 16+, %	12,4±1,6	7,7±0,32*	7,8±0,5*
CD 22+, %	11,5±1,3	8,4±0,66*	7,1±0,6*
CD 25+, %	3,1±0,1	6,4±0,33*	5,5±0,4*
IgG, g/l	14,9±0,9	12,1±1,01*	10,5±0,5*
IgA, g/l	3±0,2	2,2±0,21*	1,3±0,1*#
IgM, g/l	2±0,2	1,5±0,14	1,2±0,1*
IgE, U/mL	56±24	324±35*	329±47*
CIC, un.	41,3±5	43,8±3,72	45,9±5,1

*p<0,05 with controls, #with BA.

Conclusions: Comorbide 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 T-regs are important in balancing 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 Tregs 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 asthmatic patients compared to 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 the severity of asthma.

P3847

Superoxide dismutase as a longitudinal biomarker of lung function in asthma

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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=47) [age:40+3, mean%FEV1:77+5, FEV1/FVC: 0.69+0.1, gender: M/F=17/30, severe asthma n=12] were evaluated longitudinally over 4 years. SOD levels at the start of the study were significantly lower in Asthmatics as compared to Control subjects ($p<0.001$). Asthmatic adults had greater than the normale ~25 ml/year decline in vital capacity; severe asthmatic adults tended to lose more than nonsevere asthmatics [decline FVC ml/year: nonsevere, 51+16; Severe, 120+30; p=0.04). Asthmatics were classified as high or low SOD depending on whether their serum SOD was above or below the median (15 U/ml). Asthmatics classified as high SOD had the most accelerated FVC loss [decline FVC ml/year: Low SOD, 42+13, High SOD, 108+27;p=0.05). The decline in FVC/year was related to SOD activity at enrollment (R= -0.624, p=0.006). 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 B2-agonists. Role of IgE mediated inflammation in asthma is esthablished. 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 subepitelial basement membrane in severe asthmatic is associated to increased bronchial mucosal eosinophils, tipical 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. RBM thickness was measured by morphometric anaysis by using light microscope image analysis. The analisys 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-

tion in RBM thickness and different cut-off. The difference between Responder and Non-Responders proved statistically significant. Present data showed that 9/11 patients reduced the original RBM after treatment with anti-IgE, thus emphasizing the role of omalizumab in affecting asthma remodeling.

P3849

Reverbα 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 10% of the genome.
Aims: We investigated the function and mechanism of Reverbα, part of this "molecular clock", concerning COPD inflammation.
Results: LPS stimulated macrophages showed a diurnal response for IL-6. A novel Reverbα ligand (GSK414112) corrected this but had no effect if Reverbα 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 PPARγ 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 Reverbα binds. This mechanism was confirmed experimentally with a LXR antagonist. Luciferase reporter constructs, focused on the IL-6 proximal promoter, identified that the ligand's cell specific effects were due to the C/EBP transcription factor binding site. GSK414112 doubled the IC50 of dexamethasone on IL-8.
Conclusion: Reverbα 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.

P3850

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 was still unclear. We sought to determine the the role of autophagy in airway inflammation induced by CS using systems-level analysis by amino acids in cell culture (SILAC)-based quantitative proteomics.
Methods: SILAC-labeled human lung mucoepidermoid 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 validate 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 microtubule-associated proteins 1A/1B light chain 3C(LC3A/B), 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 LC3A/B, Cystatin-C and HMGB1 expression in rat lung (respectively, $p<0.05$).
Conclusions: Autophagy may play a role in airway inflammation induced by CS.

P3851

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 α chain of IL-4 receptor (IL4Rα) which has been proposed as a marker for these cells. IL4Rα was quantified by flow cytometry in peripheral blood from 19 smokers with COPD and no cancer (FEV1=56±6%pred), 6 smokers with COPD and lung cancer (FEV1=67±9%pred) and 11 controls with normal lung function and no cancer (5 smoking, 6 non-smoking; FEV1=106±6%pred).
IL4Rα expression was increased in monocytes from smokers with COPD and concomitant lung cancer (23;17-37%) but also in COPD subjects without cancer (median;range: 12;5-25%) compared to smoking and non-smoking controls (10;3-15% and 9;7-11%. all $p<0.05$). A similar IL4Rα upregulation was also observed in granulocytes. Of note, in all patients considered together, IL4Rα expression was related to the degree of airway obstruction ($p=0.003, r=-0.50$).
In conclusion, our study shows that IL4Rα expression is upregulated in monocytes and granulocytes 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 and potentially identify inflammatory alterations that might increase the risk of developing lung cancer.

P3852

Comparison of fluticasone propionate and budesonide on phagocytosis of common respiratory pathogens in COPD patients

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Background: Inhaled corticosteroids are recommended for COPD patients with $FEV_1 \leq 50\%$ predicted, experiencing ≥ 2 exacerbations in 12 months. However, post-hoc analysis of the TORCH study revealed an increased risk of pneumonia of 50% in patients taking inhaled fluticasone propionate (FP) (ERJ 2009;34:641). This contrasts with a meta-analysis of patients taking budesonide (BUD) where there was no increased risk of pneumonia (Lancet 2009;374:712). The reason for this discrepancy is unclear, however, we hypothesise that the particulate nature of FP, due to high lipophilicity and low water solubility, leads to its uptake by phagocytes resulting in impaired function.
Methods: Neutrophils and monocytes were isolated from the blood of COPD patients (n=10). Monocyte-derived macrophages (MDM) were generated from monocytes by 12d culture in media containing GM-CSF. All cells were incubated with steroid for 1h prior to phagocytosis assays with fluorescently-labelled polystyrene beads, *Haemophilus influenzae* (HI) or *Streptococcus pneumoniae* (SP).
Results: BUD (1μM) increased MDM phagocytosis of beads by 17% ($p<0.05$), whereas FP had no effect. Neither steroid altered MDM phagocytosis of either HI or SP. Neither steroid altered neutrophil phagocytosis of beads or SP. In contrast, BUD (10^{-10}-10^{-6}M) significantly ($p<0.05$) increased neutrophil phagocytosis of HI with a maximal effect of 67%. FP (1μM) showed a smaller effect, increasing phagocytosis of HI by 38% ($p<0.05$).
Conclusions: FP did not reduce phagocytic function of either MDM or neutrophils. BUD improved neutrophil phagocytosis of HI, which may explain differences in pneumonia incidence between FP and BUD in COPD patients.

P3853

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 visualization of mucosal (MC_T, positive for the protease tryptase) and connective tissue (MC_{TC}, positive for the proteases chymase and tryptase) mast cells as well as for IL-17A and MC_T resp. MC_{TC}. Image analysis was performed using spectral microscopy. Values are expressed as cells/BM 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 MC_T and MC_{TC}, 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.

Abstract P3853 – Table 1

Cell type	Airway	Layer	Cells/mm BM
Mast cells (total)	LA	inner	13* (11; 20)
		outer	12 (9; 15)
	SA	inner	3# (2; 6)
		outer	10 (6; 16)
Mucosal mast cells (MC_T)	LA	inner	8*# (6; 12)
		outer	4* (0; 7)
	SA	inner	2# (1; 5)
		outer	7 (4; 9)
Connective tissue mast cells (MC_{TC})	LA	inner	6* (1; 9)
		outer	5 (1; 10)
	SA	inner	1# (0; 1)
		outer	3 (2; 4)
Mast cells (total)/IL-17A double positive	LA	inner	3* (3; 12)
		outer	6* (5; 12)
	SA	inner	3# (0; 3)
		outer	2 (1; 4)
MC_{TC}/IL-17A double positive	LA	inner	0# (0; 2)
		outer	5* (3; 9)
	SA	inner	0# (0; 1)
		outer	2 (0; 4)

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.

P3854

Carbocysteine and N-acetyl cysteine inhibit cigarette smoke mediated acetylation of the PMN chemoattractant peptide PGP

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Cigarette smoke contains reactive compounds that result in epithelial damage and stimulate neutrophil chemotaxis, airway invasion, and collagen breakdown. Proline-Glycine-Proline (PGP) is a degradation product of collagen proteolysis. PGP activates neutrophil receptors stimulating further chemotaxis and inflammation.

PGP and an N-terminal acetylated PGP (NAcPGP) are elevated in clinical samples of COPD patients and correlate with disease activity. NAcPGP confers increased potency for neutrophil stimulation and bioavailability as leukotriene A4 Hydrolase, responsible for PGP destruction, is incapable of acting on NAcPGP.

N-acetyl cysteine (NAC) and carbocysteine (CC) are scavengers of reactive compounds and used as mucolytics in chronic lung diseases. We hypothesize that NAC and/or CC will prevent PGP acetylation by cigarette smoke. These compounds may provide better understanding of targeted therapeutics in cigarette smoking-related lung disease.

Cigarette smoke extract (CSE) or condensate (CSC) was prepared and incubated with native PGP +/- NAC or CC. Samples were incubated and analyzed for NAcPGP. NAcPGP concentration was measured with mass spectrometry. This data was repeated in a vapor experiment, where PGP mixed with either NAC or CC was placed in a separate well from cigarette smoke in a 96-well plate and incubated.

Our data demonstrate NAcPGP generation by cigarette smoke extract, condensate, and vapor that is inhibited by NAC or CC.

Our data support a possible mechanism where reducing compounds such as NAC and CC may diminish lung inflammation in COPD and other chronic lung diseases by inhibiting PGP acetylation thereby facilitating PGP degradation.

P3855

Interactions between the effects of a 4-week NO_2 and carbon nanoparticle exposure with allergen sensitization on bronchial responsiveness in Brown Norway rat

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Goals: The aim of this study was to assess the effect of combinations of allergen sensitization, NO_2 and carbon nanoparticle (CNP) exposure on airway reactivity (AR).

Methods: Brown-Norway rats were divided into the following groups: Control, CNP (FW2: Ø13nm, 0,5mg/kg) instilled intratracheally x3 at 7-day intervals, ovalbumine-sensitised (OVA), and CNP+OVA. Equal groups of animals were exposed either to air or to NO_2, 10 ppm, 5h/d, 5d/wk for 4 weeks. 24h after exposure, airway resistance (Raw) was measured using forced oscillation technique (FOT) at baseline and after intravenous infusion of methacholine (MCH) (5-10 and 15 μg/kg/min (γ)). Bronchoalveolar lavage fluid (BALF) cell counts were obtained.

Results: See Table 1.

Conclusions: NO_2 exposure and allergen sensitization caused a synergistic increase in AR. However, CNP exposure during the sensitization process decreased this effect. The observed interactions may be due to different inflammatory mechanisms induced by CNP and NO_2.

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 burning biomass, such as is used in household cooking. Biomass induced COPD has been reported to be associated with a more fibrotic phenotype than cigarette smoke induced COPD. We aimed to investigate if biomass smoke induced extracellular matrix (ECM) protein production from primary human lung cells in vitro.

Methods: Primary human lung fibroblasts were stimulated for up to 72 hours with increasing concentrations of biomass smoke extract (BME) or cigarette smoke extract (CSE) as a comparison. Cells were lysed and deposition of ECM proteins were assessed using ELISA. Activation of intracellular signaling molecules was analysed using western blotting. Release of interleukin (IL)-8 into the supernatant was analysed using capture ELISA. Cell viability was assessed using manual cell counts and a commercially available MTT assay.

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.

P3857

Hypoxia Inducible Factor-1α binding to HDAC2 and MIF promoter regions is associated with changes in gene expression levels in chronic ozone exposed mice

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Ozone is an oxidizing environmental pollutant that significantly contributes to respiratory health risk. Exposure to increased ambient levels of ozone has been associated to worsening of symptoms of patients with lung diseases like asthma and COPD. In this study we investigate the chronic effects of ozone exposure on Hypoxia Inducible Factor (HIF)-1α binding to Histone Deacetylase (HDAC) 2 and Macrophage Migration Inhibitory Factor (MIF) promoter regions *in vivo*. C57BL/6J mice were exposed to ozone (2.5 ppm) for 3 hours a day, 2 times a week for a period of 6 weeks. After the last exposure mice were sacrificed for 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) levels in ozone exposed mice indicating the presence of an inflammatory environment in the lung. Lung HIF-1α protein levels were increased in cytoplasm and nuclear fractions of ozone exposed mice compared to normal air exposed mice. Using chromatin immunoprecipitation assays were demonstrate that HIF-1α binds to the promoter regions of both HDAC2 and MIF genes *in vivo*. This was associated with a reduction in HDAC2 mRNA, protein and activity and an increase in MIF protein and mRNA levels. Ozone-induced HIF-1α protein is able to bind to HDAC2 and MIF promoter regions *in vivo* identifying these as two novel HIF-1α target genes.

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Abstract P3855 – Table 1

	Air – 4 weeks			NO2 (10 ppm) – 4 weeks		
	Raw (cmH2O·s/l)		BALF Eosinophils (%)	Raw (cmH2O·s/l)		BALF Eosinophils (%)
	Baseline	MCH15γ		Baseline	MCH15γ	
Control	55.6±8.5	166.4±83.9	5.5±6.4	65.7±6.4	255.6±63.9*	6.6±3.6
CNP	59.4±8.5	125.2±28.2	0.6±0.5	67.3±8.4	229±58.8*§	1.5±1.3
OVA	61.3±10.5	146.5±56.3	43.5±7.2#	73.6±10.4	362.6±164.1*#	45.3±13.1#
CNP + OVA	62.7±11.2	128±30.1	36.2±9.5#	78.8±8.8	287.1±141.9*§	51.6±13.2*#

Data are m±SD (n=6 per group); *p<0.05 vs. Air exposure, within a group and a condition; #p<0.05 vs. control, within a condition; §: p<0.05 vs. OVA, within a condition, by ANOVA.

P3858

Characterisation of T cell populations in proximal and distal airways

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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/mm^2 compared to 1.4/mm^2) 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.