409. Cell biology of lung disease

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Late-breaking abstract: Repeated analysis of alpha-1-antitrypsin concentrations in sputum, lavage and serum of smokers with and without COPD

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Introduction: Alpha-1-antitrypsin (A1AT)-deficiency is a hereditary disease that can lead to the development of emphysema. Serum levels of A1AT are used for diagnosis and monitoring during substitution therapy.
Aim: We have previously shown that A1AT levels differ between smokers with and without COPD (Roepcke et al. ERS2010). Here we assessed the repeatability of A1AT levels in serum, induced sputum (IS) and bronchoalveolar lavage (BAL) over a period of 6 weeks and compared the concentrations between compartments.
Methods: 24 COPD patients (GOLD II) and 23 age and gender matched healthy controls were included into the study. All were current smokers ($\geq$ ten pack-years). Blood, BAL, and IS were collected on two occasions. A1AT was analyzed by ELISA, CRP by Luminex.
Results: The median (IQR) A1AT concentrations was 1.69 (0.53) g/L in serum, 505 (596) μg/L in BAL and 568 (475) μg/L in IS. The reproducibility between visits within each matrix was good (serum: $r=0.55$, $p<0.001$; BAL: $r=0.72$, $p<0.001$; IS: $r=0.72$, $p<0.001$). While there was a weak relationship between mean BAL and IS A1AT concentrations ($r=0.36$, $p=0.03$), no relationship was observed between serum and lung concentrations. BAL and IS A1AT levels did not correlate with neutrophils in the respective compartment or serum CRP, and only a weak correlation between serum A1AT, blood neutrophils ($r=0.31$, $p=0.04$) and CRP ($r=0.32$, $p=0.04$) was observed.
Conclusion: A1AT appears to be stable within compartments in healthy smokers and smokers with moderate COPD. The lack of relationship between lung and serum A1AT should be considered when interpreting serum A1AT for diagnostic and monitoring purposes.

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Late-breaking abstract: Repeated bronchoconstriction without additional inflammation is sufficient to induce airway remodelling in asthma

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Asthma is characterised clinically by intermittent bronchoconstriction and pathologically by structural airway changes termed airway remodelling. Remodelling is associated with adverse long term outcomes and has been attributed to eosinophilic inflammation. In vitro studies suggest that mechanical forces occurring during bronchoconstriction may induce remodelling independent of inflammation. This hypothesis was examined in human volunteers with asthma.
Methods: 48 asthmatics were randomised to 1 of 4 inhalation challenges involving 3 challenges at 48hr intervals: Challenge substances were; allergen (house dust mite), methacholine, saline or salbutamol followed by methacholine. Bronchoalveolar lavage (BAL) and bronchial biopsies were obtained before and 4 days after the challenges.
Results: Allergen and methacholine challenges induced similar immediate bronchoconstriction. Eosinophilic inflammation increased only in the allergen group (BAL eosinophils ($p=0.01$), BAL eosinophil cationic protein ($p=0.002$) and tissue eosinophils ($p=0.05$)). Markers of remodelling increased in both the allergen and methacholine groups, with no increase in saline or salbutamol/methacholine groups. Sub-basement membrane collagen thickness ($p<0.001$), epithelial mucus staining ($p=0.003$) and cell division in the epithelium ($p=0.001$) and the submucosa ($p<0.001$) all increased as did epithelial TGF-β immunoreactivity ($p<0.01$). There were no differences between the allergen and methacholine groups.
Conclusions: Experimentally induced bronchoconstriction without additional airway inflammation is sufficient to induce airway remodelling in asthma.

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Late-breaking abstract: Role of IL-13-producing BLT1-positive CD8 T cells in asthmatic airway obstruction

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Background: Asthma is characterized by reversible airflow obstruction, persistent inflammation and airway hyperresponsiveness. Recent animal studies identified a subset of CD8 T cells expressing BLT1, the high affinity receptor for leukotriene B4. These cells accumulate in the lungs and alter airway function via interleukin-13 (IL-13) production (Nat Med 2004, 10:865-9).

Aims: To determine if a similar subset of CD8 T cells are present in asthmatic human airways and if their presence is associated with evidence for asthmatic airway obstruction.
Methods: BAL cells from asthmatics (n=39) and healthy controls (n= 28) were stimulated in culture and immuno-stained for CD8, BLT1 and IL-13. The data were correlated to lung function, serum IgE and airway basement membrane (BM) thickness.
Results: Compared to controls, asthmatics showed higher proportion of CD8-positive lymphocytes in BAL fluid ($p < 0.001$). A significant proportion of these CD8 T cells expressed BLT1 in both groups. Most importantly, the proportion of BLT1-positive CD8 T cells expressing IL-13 was significantly higher in asthmatic airways compared to controls ($p < 0.001$). Furthermore, the proportion of IL-13-producing BLT1-positive CD8 T cells negatively correlated with FEV1 (% predicted) values and FEF[25-75] values ($p < 0.001$). Interestingly, a positive correlation was detected between the proportion of these cells and serum IgE levels as well as with BM thickness ($p < 0.01$).
Conclusions: IL-13-producing BLT1-positive CD8 T cells are present in the airways of asthmatics, and their accumulation correlates with airway obstruction, serum IgE levels and BM thickness, suggesting a pathogenic role for these cells in human asthma.

P3879
In vivo imaging of NF-kB pathway in acute lung inflammation mouse model can predict a pharmacological response
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NF-kB plays a central role in immunity, inflammation, development, cell survival and has been indicated under a number of pathological conditions of lung disease, including asthma, chronic bronchitis, and chronic obstructive pulmonary disease. In this study, we assessed the in vivo activation of NF-kB signaling in lung tissue using a bioluminescence imaging system (IVIS) to monitor activation of an NF-kB promoter in response to lipopolysaccharide stimulation. A plasmid contained responsive elements of NF-kB and luciferase as a reporter gene has been delivered intravenously in nude mice at the concentration of 40 ug per mouse using in vivo-jetPEI™ from Polyplus as a transfectant agent. One week after DNA delivery the transient transgenic mice had been imaged in order to check the baseline activation of the NF-kB pathway. The day after, the mice have been treated with LPS 15 ug per mouse intratracheally and the lungs imaged using bioluminescence (BLI) at 2, 4, 7 and 24 hs. The ability of the IKK2 inhibitor MLN120B orally administered at the dose of 300 mg/kg to counteract NF-kB activation has been evaluated. The maximum peak of NF-kB activation was reached at 4 hs with 7-10 folds of induction in comparison to the saline group and at 24 hs the signal dropped down at basal level. In the group treated with MLN120B was observed a 50% inhibition of LPS-induced NF-kB stimulation, an effect that was in good agreement with the inhibition of p65 nuclear translocation evaluated ex vivo in lung homogenates.
In this experiment we showed that is feasible to monitor NF-kB activation in vivo in lung tissue in a non-invasive way by BLI and the pharmacological response as well.

P3880
Comparison of distal lung innervation in human and guinea pig precision-cut lung slices (PCLS)
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Introduction: PCLS from human tissue are well suited to study peripheral airway responses. Since autonomic nerves are involved in airway control and in several diseases including allergic airway hyperreactivity, we characterized distal lung innervation in guinea pigs (GP) with respect to bronchoconstriction (BC) and compared the results to those with human PCLS.
Methods: PCLS were prepared from GP or human lung tissue. Neural activation was triggered by electric field stimulation (EFS) or capsaicin addition. Cholinergic innervation was verified by atropine. Capsaicin was used to show excitatory non-adrenergic non-cholinergic (eNANC) nerves. Application of Ruthenium red or SKF96365 confirmed transient receptor potential (TRP) channel contributions upon eNANC activation.
Results: GP and human PCLS were both sensitive to EFS and airways contracted to 39±26% of the initial airway area (%-IAA) and 42±30%-IAA, respectively. Frequency response curves were also similar. For both species, EFS-induced BC was decreased after the addition of atropine. Capsaicin contracted GP and human airways to 18±15%-IAA and to 62±6%-IAA, respectively. Capsaicin-triggered BC was inhibited in GP by Ruthenium red and SKF96365. Both inhibitors also reduced EFS-induced BC in PCLS from GP.
Conclusion: Both species contain atropine sensitive cholinergic and capsaicin sensitive eNANC nerves. In addition, GP PCLS were sensitive to TRP channel inhibitors allowing the study of their contribution in the pathogenesis of lung diseases. In conclusion, GP PCLS represent a useful model to study pharmacological aspects of lung innervation and resemble the human distal lung innervation.

P3881
The clinical significance of markers of endothelial dysfunction (ED) inprogression of idiopathic interstitial pneumonias (IIP)
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Angiogenesis and ED in IIP are considered as key mechanisms of remodeling in lung tissue and pulmonary hypertension (PH). The
Aim of present study was to define some mediators of ED and angiogenesis (endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), Plasminogen Activator Inhibitor-1 (PAI-1) in patients with different clinical forms of IIP.
Patients and methods: 39 patients (22 – usual interstitial pneumonia, 17 – non-specific interstitial pneumonia) with morphologically proved IIP were investigated. Plasma concentrations of ET-1, VEGF and PAI-1 (ELISA) and morphometrics of lung biopsy for estimation of intensity of neoangiogenesis were performed.
Results: Markes of ED positively correlated with HRCT patterns of lung fibrosis (VEGF r=0,31, p=0,004; ET-1 r=0,37 p=0,003, PAI-1 r=0,38, p=0,006 respectively) the level of VEGF in the blood correlated with parameters of neoangiogenesis in the lung tissue (r=0,37, p=0,001). Patients with PH in comparison with patients without PH demonstrated higher concentrations of ET-1 and PAI-1 (r=0,34, p=0,005 and r=0,37, p=0,002 respectively). Invert correlation was established between PAI-1 and DL_{CO} (r = -0,71, p<0,05), PAI-1 and FVC (r = -0,72, p<0,05).
Conclusion: These data demonstrate the important role of the studied markers of ED and neoangiogenesis in the mechanisms of IIP progression and may be used as predictors of survival.

P3882
Increased polysialylation in lung tissue of patients with idiopathic pulmonary fibrosis
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Idiopathic pulmonary fibrosis (IPF) is a chronic fibrosing interstitial lung disease of unknown etiology. The disease is characterized by alveolar destruction, uncontrolled fibroblast proliferation and excess matrix production, resulting in progressive dyspnea, a decline in lung function and loss of gas exchange properties. So far, only pirfenidone has been shown to exert some efficacy in IPF and lung transplantation represents the only option to prevent death.
Polysialic acid (polySia) is a developmentally regulated negative charged glycan which is predominantly found in neural tissue and tumours, where polySia is involved in the modulation of cell adhesion and migration processes. Therefore, we asked the extent of polysialylation in IPF (n=22) and donor (n=20) lung tissues obtained during lung transplantation.
We observed an up-regulation of the polysialyltransferases ST8SiaII and ST8SiaIV, the key enzymes of polySia biosynthesis, using quantitative real time PCR in IPF patients. In agreement with an increased mRNA expression level of both transferases we detected increased polySia levels in tissue samples of IPF patients in comparison to donor tissue by Western blotting. Using a glycoproteomics approach we were able to identify NCAM as the polySia carrier which could be confirmed by Western blot analysis. Surprisingly, polySia-NCAM was located intracellularly in vesicles of ciliated bronchiolar epithelial cells as well as clara cells. However, the role of polySia-NCAM in the bronchoalveolar system and especially during the development and the pathophysiology of IPF needs to be further investigated.

P3883
Human neutrophil peptides as biomarkers for monitoring respiratory functional impairment in sarcoidosis
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Background: Human Neutrophil Peptides (HNP) are cationic peptides with a

broad spectrum of inflammatory activities which have been found increased in plasma and BALF of individuals with sarcoidosis.
Aim: We designed a study to analyze HNP's role as markers of pulmonary involvement and functional impairment during sarcoidosis.
Methods: We enrolled 42 consecutive individuals with sarcoidosis, and 12 normal volunteers. Participants underwent pulmonary function tests, fiber-optic bronchoscopy and radiological evaluation. HNP concentration in BALF were measured by an ELISA test.
Results: Patients with sarcoidosis had higher BALF HNP concentrations as compared to volunteers (3.9±0.3 ng/ml vs 0.4±0.07 ng/ml, p< 0.0001). Individuals with parenchymal involvement had higher BAL HNP levels than subjects without parenchymal involvement (4.5±0.3 ng/ml vs 2.2±0.3 ng/ml, p=0.02). A negative correlation was observed between HNP levels and pulmonary functional impairment (HNP-%FEV1 rho= -0.33, p= 0.03; HNP%TLC rho=-0.33,p=0.03). ROC curve analysis revealed HNP as markers to discriminate patients with sarcoidosis from normal volunteers (Area Under the Curve =0.99, with 95%CI: 0.98-1.00; positive likelihood ratio equal to infinity, negative likelihood ratio= 0.02), and patients with pulmonary parenchymal involvement from patients with only bilateral hilar adenopathy (AUC of 0.83,with 95% CI: 0.69-0.96 positive likelihood ratio=2.7, negative likelihood ratio=0.09)
Conclusions: Our results suggest that HNP may have a role as biomarkers for sarcoidosis diagnosis and as indicators of parenchymal involvement, functional impairment and disease severity during pulmonary sarcoidosis clinical course.

P3884
A role for cathepsin S in the pathogenesis of cystic fibrosis lung disease?
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The pathogenesis of lung disease in cystic fibrosis (CF) has not been fully elucidated, however, neutrophil-dominated inflammation is thought to play a major role. Nonetheless, a number of proteases produced by other cells in the lung may play a pivotal role in CF lung damage. Human lysosomal cysteine proteases are a family of proteases that have been relatively unexplored in the area of CF lung disease. We have shown that cathepsin S activity is increased in CF bronchoalveolar lavage fluid. In addition to lung tissue degradation, cathepsins have been found to contribute significantly to the destruction of host defence proteins such as SLPI, β-defensins and lactoferrin. These findings indicate a role for cathepsin S in the diminution of the lung antiprotease and antimicrobial screen possibly leading to lung destruction and favouring conditions for bacterial infection. We have identified epithelial cells as a source of cathepsin S in the CF lung with the demonstration that CF bronchial and tracheal epithelial cell lines express and secrete significantly more active cathepsin S than normal cells in the absence of proinflammatory stimulation. These findings were confirmed in primary human bronchial epithelial cells from CF patients. On the basis of our results to date, we postulate that upregulated cathepsin S plays an important role in CF lung disease and we are currently investigating reasons for this upregulation of cathepsin S in CF epithelial cells. This data will shed valuable light on the role of cathepsin S in CF, an area that has been overshadowed to date, and may open up new avenues for exploration in the search for an effective therapeutic target in CF lung disease.

P3885
Immune and inflammatory responses in induced sputum (IS) in Anderson Fabry disease (AFD), COPD and healthy volunteers
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AFD is an X-linked lysosomal storage disorder caused by mutations of the GLA gene. Reduced enzyme results in accumulation of storage material, leading to multi-organ pathology including airflow obstruction. Given the association between intra-cellular lipid and disease, we hypothesised subjects with pulmonary AFD would have detectable changes in airway immune/inflammatory cell profiles.
Methods: AFD and COPD subjects matched for GOLD stage plus healthy age/sex matched volunteers underwent lung function testing and sputum induction.
IS cells were stained with combinations of CD3, CD4, CD8, CD16 & CD56 antibodies and examined using flow cytometry for T cell populations including NKT cells, thought to be relevant to AFD immuno-pathology.
Results: 46% of AFD patients had evidence of airflow obstruction (AO+).The% of total T cells in IS in AFD AO+ was greater than AFD AO- (p<0.01), and similar to COPD patients. AFD AO- had lower T cell% than seen in COPD (p<0.01). Other T cell populations including NKT cells were similar between groups.
Conclusion: Sputum T cell profiles in AFD and airways obstruction are similar to those of COPD whilst AFD AO- are more like healthy volunteers. This suggests a role for T cells in AFD lung disease.

T cell populations from IS

Group (median)	COPD	AFD AO+	AFD AO–	Normals
n	12	21	24	7
CD3+ T cell%	74.5* (53.5–92.5)	68.4* (18.8–89.9)	21.3(3.7–64.7)	40.1 (21.3–85.3)
CD4+ T cell%	40.3 (18.7–67.7)	36.6 (6.19–65.1)	59.5 (5.81–78.7)	50.0 (16.9–66.3)
CD8+ T cell%	11.26 (1.38–55.3)	22.0 (2.3–45.1)	14.3 (5.16–35.9)	14.4 (9.08–52.4)
NKT cell%	0.94 (0.1–2.98)	1.02 (0.72–2.98)	1.83 (0.77–5.32)	1.16 (0.13–2.23)

*p<0.01 compared to AFD AO–.

P3886
Surfactant protein D is critical for local immunomodulation in the distal lung
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Surfactant Protein D (SP-D) is a multifunctional product of lung epithelia that modulates pulmonary host defense. SP-D deficient mice (SP-DKO) develop a progressive baseline phenotype of pulmonary inflammation, enhanced oxidative-nitrative stress, and lung remodeling punctuated by a morphologically heterogeneous population of mononuclear alveolar cells and poor clearance of pathogens. To further define the role of SP-D as a local modulator of this immune cell population, bronchoalveolar lavage (BAL) cells recovered from SP-DKO and C57/BL6 controls (WT) were characterized using FACS, qRT-PCR, and functional assays. By FACS, over 95% of BAL cells from WT mice consisted of a morphologically homogeneous population (FSC/SSC) with an expression profile of F4/80+, CD11c+, Dectin-1+, CD45+, CD3-, LY6G- consistent with alveolar macrophages. In contrast, SP-DKO BAL cells exhibited a dispersed FSC/SSC profile which separated into 2 major populations: Approximately 50% were F4/80+, CD11c+, Dectin-1+, CD45+ while a second group was F4/80-,CD11c-,CD45-,PECAM-, and c-Kit-. Less than 10% of the second population expressed CD3, Ly6G or CD11b. By qRT-PCR, when normalized to F4/80, BAL cells from SPDKO expressed greater amounts of iNOS and Cox2. Functionally F4/80+ SP-DKO cells internalized significantly less zymosan particulate than F4/80+ WT cells. SPDKO BAL cells in culture were hyporesponsive to stimulation by LPS and zymosan. In vivo, SP-DKO mice produced less TNF-α in response to intratracheal zymosan or LPS. These data are supportive of a critical role for SP-D in modulation of the local immune response in the lung through effects on antigen-presenting cell differentiation, composition, and function.

P3887
Increased mast cell numbers in alveolar parenchyma in infants with respiratory viral infections
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Background: Mast cells (MCs) play a sentinel role in innate immunity. However, little is known regarding the role of MCs in viral infections in human in vivo conditions. This study characterizes MCs in the lungs from infants who have died in acute respiratory viral infections.
Methods: Lung tissue from infants who died in respiratory syncytial virus (RSV, n=5), adenovirus (n=11) and influenza (n=6) infections was processed for immunohistochemical identification of MC_T and MC_{TC} and related mediators. Ten infants who died of non-respiratory causes were used as controls. We also examined MC alterations in a mouse model of exposure to the common aeroallergen house dust mite (HDM) during the course of an influenza infection.
Results: An increase in both MC_T and MC_{TC} numbers was observed in the alveolar parenchyma in infants infected with RSV (p=0.02), adenovirus (p=0.001) and influenza (p=0.02) compared to the controls. No differences were found in small airways or pulmonary vessels. High MC expression of pattern recognition receptors and pro-inflammatory cytokines were present in the infected lungs. In the mouse model, alveolar MC numbers were increased 3 weeks after infection (p=0.006), HDM (p=0.01) and when combining HDM and influenza (p=0.002) compared to saline treated animals. Increased MC numbers were still significant 6 week after infection.
Conclusions: These data demonstrate that a viral infection in peripheral airways evokes a rapid expansion of MC populations both in mice and humans. These findings support a role for MCs in the immune response to respiratory viral infections. Our animal data also indicates an important link between allergic sensitization and viral infection.

P3888

Establishment of reference values for differential cell counts in nasal lavage of healthy young adults

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Background: Upper airway inflammation could be reflected by nasal lavage cytology test, which is characterized by advantages of non-invasive, simple, objective and costless. However, reference values nasal lavage cytology was not established.
Objectives: To establish reference values and positive standard for nasal lavage cytology through screening normal healthy subjects and patients with allergic rhinitis according to strict inclusion criteria.
Methods: 143 normal healthy volunteers (control) and 166 subjects with allergic rhinitis (AR) were enrolled after detailed history inquiry, physical examination and allergen skin prick test. Nasal lavage cytology tests were performed, and the standard for judgment was defined as the average count of different inflammatory cells per 20 fields under 200× microscopic vision.
Results: There was no statistical significance in gender constitutional proportion, age, height and weight among each group. 95% CI of neutrophils, eosinophils was (0∼12.61)/×200 and (0∼1.70)/×200, respectively. The median (interquartile range) of neutrophils were 0 (0.65)/×200 in AR group, which showed no statistical difference ($P>0.05$) with that of normal group [0(0)/×200]. A significant difference was found in the median (interquartile range) of eosinophils [6.90(22.40)/×200] in AR group as compared with that of normal control group [0(0.10)/×200, $P<0.001$].
Conclusions: Establishment of reference values of nasal lavage cytology test is helpful to discriminate normal individuals and patients with allergic rhinitis, but also a non-invasive tool for objective reflection on upper airway inflammation, which is of great value for scientific and clinical purposes.

P3889

The dietary antioxidant quercetin boosts pulmonary antioxidant defenses

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We have demonstrated that single oral supplementation of the dietary antioxidant quercetin reduces oxidative stress in sarcoidosis patients. Apart from its direct oxidant scavenging properties, quercetin has also been suggested to boost endogenous antioxidant defenses indirectly by activation of redox sensitive signaling pathways. Therefore, we investigated the effect of orally applied quercetin on pulmonary redox balance.
C57BL/6J mice were sacrificed 3 or 16 hours after receiving an oral quercetin bolus (4 mg/animal). Total antioxidant capacity, quercetin concentration and the expression of nuclear factor erythroid derived (Nrf)2-regulated antioxidant genes were evaluated. The possible influence of Nrf2was also explored *in vitro* by treating quercetin-preloaded BEAS-2B human bronchial epithelial cells with the pro-fibrotic trigger bleomycin.
Total quercetin concentrations in plasma and lung tissue displayed a rapid but transient increase, which was associated with enhanced total antioxidant capacity. After 16 hours, amplified pulmonary mRNA levels of various antioxidant genes including catalase, superoxide dismutase, heme oxygenase 1 and γ-glutamyl cysteine synthetase were observed. Additionally, oral quercetin administration increased Nrf2 gene expression. In the BEAS-2B cells, quercetin also activated Nrf2 and, interestingly, this induction was augmented by bleomycin. Moreover, quercetin pre-treatment inhibited bleomycin-induced ROS production.
In conclusion, our results indicate that oral quercetin may exert beneficial effects by boosting pulmonary antioxidant defenses and suggest a possible involvement of Nrf2 herein. The therapeutic value of our findings is currently explored in a murine fibrosis model.

P3890

Clinical evaluation of angiogenesis and coagulation in pulmonary sarcoidosis

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The aim of our study was to evaluate the influence on prognosis of patients with pulmonary sarcoidosis of some mediators angioneogenesis and activation of coagulation (vascular endothelial growth factor (VEGF) and plasminogen activator inhibitor-1 (PAI-1).
Patients and methods: 46 patients with morphologically proved pulmonary sarcoidosis were examined. Standart clinical examination, pulmonary function testing, 6-min walk test (6 MWT), echocardiography, high-resolution CT (HRCT) were evaluated. Plasma concentrations and expression in lung biopsies of VEGF and PAI-1 were evaluated by immunoassay (ELISA) and immunohistochemistry. Morphometrics of lung vessels were performed.
Results: 12 (26%) patients presented pulmonary hypertension (PH) (sPAP 46,7 (35,3- 53,8) mmHg, r=0.45). Plasma concentrations of PAI-1 and VEGF correlated positively with HRCT patterns of fibrosis in lung (r=0,38, p=0,006 and r=0,37, p=0,002 respectively) and were more higher in patients with PH in comparing to patients without PH (PAI-1 20,6 (15,1-27,2)ng/ml vs 14,1 (9,1-18,2)Hr/Ml, p=0,001 respectively and VEGF 427,4 (316,1-554,2)ng/ml vs 280,1 (180,1-360,2) Hr/Ml, p=0,0021 respectively). VEGF expression in lung tissue correlated positively with morphology of pulmonary vasculitis (r=0,34, p=0,001), HRCT honeycomb patterns (r=0,45, p=0,002).
Conclusion: Degree of alterations of the coagulation system and angioneogenesis may be discussed as survival prognostic markers for pulmonary sarcoidosis.

P3891

Detection of serum anti-endothelial cell antibodies (AECA) in COPD rats

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Background: Chronic obstructive pulmonary disease (COPD) may be a systemic disease of autoimmune abnormality. Alveoli disorganization in COPD includes micro-vascular destruction. Anti-endothelial cell antibodies (AECA) are a type of circulating antibodies which bind to endothelial antigens and induce endothelial cell damage. It is unclear whether the AECA play a role in COPD mechanisms.
Objective: To detect the serum level of anti-endothelial cell antibody in adult rats of COPD in order to investigate the significance of AECA in COPD mechanism.
Methods: Replicated COPD rat models with passive-smoking and lipopolysaccharide trachea injections. The expression of AECA was examined by ELIZA.
Results: In smoking rats with simple airway inflammation, the alveolar septum thickened but was not destroyed, AECA149.48±7.47 ng/L. COPD rats had airway inflammation and emphysema, AECA138.46±7.85 ng/L. Compared with the normal control group (62.89±10.68 ng/L), AECA levels in the two test groups were much higher.
Conclusion: Autoimmunity constituents which induce endothelial cell lesions may participate in the pathogenesis of COPD; detection of serum AECA levels in COPD may have some clinical significance.

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CD146 in the pathogenesis of COPD

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Background: Chronic obstructive lung diseases (COPD) are the major cause of death and disability worldwide. The major risk factor for COPD is tobacco smoke. Cell adhesion molecule CD146 is expressed in all types of human endothelial cells (EC) and exists in a membrane-anchored and a soluble form (sCD146). The plasma concentration of sCD146 is modulated in inflammatory diseases associated with endothelial alterations.
Aims and objectives: To investigate the role of endothelial CD146 in the pathogenesis of cigarette smoke (CS)-induced emphysema.
Methods: Sprague Dawley rats were exposed to second hand CS for two months. The lung tissue and bronchoalveolar lavage (BAL) cells were examined for CD146 gene and protein expression and emphysema development as measured by MLI. sCD146 levels were determined in circulation and BAL fluid in rats and in patients with COPD. CD146 expression and function was also examined in rat pulmonary EC exposed to CS in vitro.
Results: Sprague Dawley rats exposed to cigarette smoke for 2 months developed significant emphysematous changes (as measured by mean linear intercept) and had increased levels of circulating and bronchoalveolar lavage fluid sCD146. Treatment of rat pulmonary EC with cigarette smoke extract in vitro also resulted in a decreased membrane-bound CD146 expression and increased sCD146 levels in the medium. Moreover, circulating levels of sCD146 were significantly increased in serum of patients with COPD and correlated with the disease severity.
Conclusion: Our data indicate that CD146 is involved in CS-induced vascular dysfunction and that sCD146 can be a candidate marker for COPD/emphysema.
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Effects of steroids on inflammatory cell number and function in the proximal and distal airways

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Introduction: Steroids are often prescribed for patients with mild/moderate COPD but it is not clear how these community prescribed drugs affect inflammatory cell numbers/function in the proximal and distal airways.
Methods: Matched proximal and distal airways tissue was obtained from 37 patients. Fourteen patients had no evidence of airways obstruction (FEV1/FVC=0.76±0.01) and 23 had evidence of mild/moderate COPD (FEV1/FVC=0.58±0.02). Eleven of these patients were prescribed steroids at the time of

surgery. There were no differences in the characteristics of the patients prescribed steroids. Tissue was fixed in GMA and inflammatory cells enumerated by immunohistochemistry. Tissue explants were also stimulated with 100μg/ml anti-IgE and the release of TNFα assessed by ELISA.

Results: Patients prescribed steroids had fewer mast cells in their distal airways (median=23.6 AA1+ve cells/mm^2) than either patients with no evidence of airways obstruction (median = 40.3 AA1+ve cells/mm^2) or those with COPD but not prescribed steroids (median = 59.9 AA1+ve cells/mm^2). There were fewer mast cells in the proximal airways of all three groups and no differences between the groups. The number of other inflammatory cells in either proximal or distal airways such as macrophages, neutrophils and CD3+ve cells were unaffected by either disease status or steroids. Anti-IgE induced TNFα release from tissue explants from proximal and distal airways was not affected by either the presence of airways obstruction or steroids.

Conclusions: Mast cell numbers are reduced in the distal airways of patients with mild/moderate COPD prescribed steroids. The distribution of other inflammatory cells are not affected.