92. The ageing pulmonary interstitium

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Ambrisentan attenuates lung and heart injury in a rat model of bronchopulmonary dysplasia

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The selective endothelin receptor type A antagonist ambrisentan may be a novel therapeutic agent in neonatal chronic lung disease by blocking the adverse effects of the potent vasoconstrictor endothelin-1, including pulmonary arterial hypertension (PAH)-induced right ventricular hypertrophy (RVH). The cardiopulmonary effects of ambrisentan were studied in neonatal rats with hyperoxia-induced lung injury. Ambrisentan treatment was investigated in 2 models of experimental BPD: a prophylactic model, in which pups were continuously exposed to hyperoxia and treated daily with either saline or ambrisentan (20 mg/kg body weight/day; injected subcutaneously), and an injury-recovery model, in which pups were exposed to hyperoxia for 9 days, followed by 9 days of recovery in room-air and treatment with ambrisentan was started on day 6 of oxygen exposure and continued during the recovery period. In the prophylactic model treatment with ambrisentan improved survival (p <0.01) by reducing lung fibrin deposition (3-fold, p <0.001), alveolar septum thickness (1.7-fold, p < 0.001) and medial wall thickness of small arterioles as a marker for PAH (1.7-fold, p <0.001), and preventing associated RVH (p <0.001). Treatment with ambrisentan did not have beneficial effects on alveolar enlargement, vascularization, the pulmonary influx of macrophages and neutrophils, and the mRNA expression of procoagulant and inflammatory markers. In the injury-recovery model treatment with ambrisentan attenuated PAH and RVH (p <0.001), demonstrating that established PAH-induced RVH is still reversible in the neonatal period. Beneficial effects on reduced pulmonary vascularization and alveolarization were absent.

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Serum HSP47 is a novel blood marker for rapidly progressive interstitial pneumonia

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Heat shock protein (HSP) 47, a collagen-specific molecular chaperone, is involved in the processing and/or secretion of procollagen. The aim of this study was comparative analysis of the diagnostic values of serum HSP47, Krebs von den Lungen-6 (KL-6), surfactant protein (SP)-A, SP-D and lactate dehydrogenase (LDH) levels for rapidly progressive interstitial pneumonia.

Subjects comprised 27 patients with rapidly progressive interstitial pneumonia, 12 with cryptogenic organizing pneumonia (COP), 19 with idiopathic usual interstitial pneumonia (UIP), 16 with idiopathic nonspecific interstitial pneumonia (NSIP), 11 with collagen vascular disease-associated UIP, 11 with collagen vascular disease-associated NSIP, and 18 healthy adult volunteers.

Serum levels of HSP47 in patients with rapidly progressive interstitial pneumonia were significantly higher than those in patients with COP, idiopathic UIP, idiopathic NSIP, collagen vascular disease-associated UIP, collagen vascular disease-associated NSIP and healthy volunteers. Receiver operating characteristic curves revealed that HSP47 was superior to the other markers. The cut-off level for HSP47 that resulted in the highest diagnostic accuracy was 896.9 pg/ml. The sensitivity, specificity, and diagnostic accuracy were 92.6%, 100%, and 98.2%, respectively.

These results suggest that of the markers studied, HSP47 is the best serum marker for rapidly progressive interstitial pneumonia.

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Protease-activated receptor-2 triggers epithelial to mesenchymal transition: Potential relevance in pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) constitutes the most devastating form of fibrotic lung disorders. The destructive fibroblast foci characteristic of IPF originate, at least partly, via epithelial to mesenchymal transition (EMT). The extracellular signals and cellular receptors triggering EMT in IPF remain incompletely understood however. Recently, we showed that protease-activated receptor-2 (PAR-2), a transmembrane G-protein-coupled receptor expressed ubiquitously in the lung, is an essential player in fibrotic lung disorders by directly targeting fibroblasts. Here, we explore the role of PAR-2 on epithelial cells by focussing on PAR-2-induced EMT in pulmonary fibrosis. Immunostaining of lung biopsies of IPF patients showed prominent PAR-2 expression by fibroblasts and epithelial cells overlying fibroblast foci. Double stainings indicated that PAR-2 co-localized on cells expressing both epithelial (cytokeratins) and mesenchymal (vimentin) markers, indeed suggesting a role of PAR-2 in EMT in IPF. Subsequent *in vitro* experiments showed that PAR-2 stimulation induced a fibroblast-like morphology in type II lung epithelial cells, the expression of the myofibroblast markers vimentin and α-SMA, and the secretion of collagen. Interestingly, PAR-2 stimulation triggered β-catenin accumulation and translocation to the nucleus. In conclusion, PAR-2 triggers EMT of epithelial cells and PAR-2 dependent activation of the β-catenin/WNT signaling pathway is probably the main driver of PAR-2-induced EMT. Overall our data thus suggest that inhibition of the PAR-WNT axis may be a clinically relevant treatment option in IPF but also in other disorders in which EMT is essential.

P783

Disruption of Nrf2 enhances susceptibility to pulmonary fibrosis induced by bleomycin in mice

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Introduction: Recently studies suggest that N-acetylcysteine improve the idiopathic pulmonary fibrosis. Oxidant/antioxidant balances may play an important role in many of the processes of inflammation and fibrosis. Nrf2 is involved in the transcriptional regulation of many antioxidant genes. We therefore investigated the role of Nrf2 against the development of pulmonary fibrosis in mice.
Materials and methods: Both $Nrf2^{-/-}$ and $Nrf2^{+/+}$ C57BL/6J mice were used. Bleomycin was administered intravenously to the mice at a dosage of 0, 70, 80, and 90 mg/kg body weight on day 0, and the fibroblastic foci were assessed histologically by Ashcroft score determined in the lung tissues on day 28. Furthermore, bleomycin was administered intravenously to the mice at a dosage of 80 mg/kg body weight on day 0, and the bronchoalveolar lavage (BAL) fluid examined for cell populations on days 0, 3, 7, 10, 14, 21, and 28.
Results: The fibroblastic foci were induced by bleomycin at a dosage of 90mg/kg body weight in the lung tissues on day 28 in $Nrf2^{+/+}$ mice. In contrast, the fibroblastic foci were induced by bleomycin at a dosage of 70mg/kg body weight in $Nrf2^{-/-}$ mice. The total number of cells and macrophages in the BAL fluid were significantly increased from day7 after bleomycin administered in both $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice. The increased cells number were significantly greater in $Nrf2^{+/+}$ mice than in $Nrf2^{-/-}$ mice.
Conclusions: These findings suggest that Nrf2 might be an important genetic factor in the determination of susceptibility to bleomycin induced pulmonary fibrosis by regulating the macrophages defense mechanisms.

P784

αB-crystallin is involved in the process of pulmonary fibrosis

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Introduction: Idiopathic Pulmonary Fibrosis (IPF) is a devastating disease with currently no treatment. In the presence of TGF-β, epithelial cells differentiate into myofibroblats, key pro-fibrotic cells in a proccess called epithelial-to-mesemchymal transition (EMT).
αB-crystallin belongs to the small heat shock protein family and is constitutively expressed in many tissues including lungs. αB-crystallin is inducible by stress and has a major role in cell cytoskeleton architecture homeostasis by interacting with intermediate filament elements. The role of αB-crystallin in fibrogenesis is unknown.
Methods: In vitro we induced EMT on A549 cells after rTGF-β treatment. In vivo, Sprague Dawley rats received intra-tracheal administration of AdTGF-β or AdDL control. SV129 mice wild type (WT) or knock out (KO) for αB-crystallin received intra-tracheal bleomycin (0.07U/mouse).
Results: In vitro during TGF-β1-induced EMT: 1. αB-crystallin is early overexpressed, 12 hours before α-SMA overexpression; 2. αB-crystallin colocalize with α-SMA; 3. αB-crystallin interacts with HSP27; 4. The modulation of αB-crystallin plays a role on TGF/SMAD pathway.
In vivo: 1. αB-crystallin is overexpressed in fibrotic areas induced by TGF-β overexpression in rats or bleomycin administration in mice; 2. By day 21, collagen accumulation in the lung was significantly higher in WT mice (3 fold increase, $p<0.05$) compared to KO mice; 3. The level of TGF-β1 was lower in KO mice after bleomycin injection.
Conclusion: These results provide evidence that αB-crystallin is involved in pulmonary fibrosis maybe through a role in EMT.
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Systemic sclerosis patients' sera recognize collagen V in experimental scleroderma lung fibrosis

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Background: Type V collagen (COLV) has been recognized as an auto antigen involved in lung transplantation rejection and may be involved in systemic sclerosis pathogenesis (SSc), since immunization of healthy rabbits with this collagen induced an experimental model with similar characteristic of SSc patients.
Objective: To investigate if SSc patient's sera recognize COLV present in pulmonary tissue from this model and collagen isolated from supernatant culture of pulmonary fibroblasts from these patients.
Methods: Immunofluorescence using collagen type I, III and IV adsorved sera from 8 SSc treatment naive patients and from 8 controls was performed to evaluate COLV reactivity in lung tissue from experimental model. These sera were also tested by immunoblotting to evaluate COLV reactivity in collagen isolated from SSc patient's lung fibroblasts supernatants.
Results: Positive immunofluorescence reaction was observed when the sera of SSc patients recognized epitopes of COLV in the experimetal model vessels walls and pulmonary interstitium contrasting with the negative reaction observed in the control. Morphometric analysis confirmed these findings, demonstrating a higher COLV reactivity to sera from SSc patients when compared to the control ($p=0.0028$). Immunoblotting showed that SSc patients sera strongly reacted to COLV standard and to the high molecular weight fragments of COLV isolated from SSc lung fibroblasts culture supernatants, different from control sera reactivity ($p=0,02$).
Conclusions: SSc patient's sera recognize COLV epitopes in experimental model lung tissue and SSc patient's lung fibroblasts culture suggesting that this protein is an antigen involved in SSc pathogenesis.

P786

Work of inflation is the best correlate to lung fibrosis induced by bleomycin in mice

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Introduction: Bleomycin (BLM) induces a transient lung fibrosis that has been used to investigate mechanisms related to idiopathic pulmonary fibrosis. Our aim was to determine the method for assessing lung function that provided the strongest correlation to the lung fibrosis.
Methods: BLM (2U/kg) or saline was intratracheally microsprayed to male, C57BL/6 mice under isofluorane. Lung function was assessed using the flexivent system and after euthanasia, lungs were inflated in situ under a constant pressure of 25 cm H2O starting 1–35d after BLM administration (n=8). Right and left lung sections, stained with Masson's trichrome, were graded for fibrosis (0-4, i.e. no fibrosis to severe) and the scores were combined (0-8).
Results: Lung fibrosis scores were progressive 1-14d after BLM challenge. From 14-35d, the fibrosis scores did not significantly change. Lung function changes were more subtle and no significant changes in resistance or elastance were observed at 21d post-BLM. The only significant change occurred in work of inflation (WoI).

Day 21 post-BLM	Saline	Bleomycin	% Difference
Resistance (cmH2O)/mL)	0.72±0.03	0.86±0.07	20%
Elastance (cm H2O/mL)	34.4±1.2	41.0±4.2	19%
WoI (J/l)[#]	9.4±0.2	10.5±0.5	12%*
Fibrosis Score	0	3.3±0.6	N/A

[#]Data acquired at 3× tidal volume; *$P<0.05$ (n=7 control & n=8 BLM).

Compared to other indicators of lung function, WoI showed the most significant correlation to fibrotic score with the highest Pearson correlation coefficient = 0.74 ($P < 0.05$).
Conclusion: Scores for lung fibrosis are the robust indicator of BLM-induced changes to the lung. Although changes in lung function were less obvious, WoI provided a greatest level of precision to detect significant changes.

P787

Levels of cytokines and chemokines in BAL fluid in patients with idiopathic interstitial pneumonitis and collagen vascular disease associated interstitial pneumonitis

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Inflammatory cytokines and chemokines have been reported to play important roles in the pathogenesis of interstitial lung diseases. However, their individual roles in idiopathic interstitial pneumonitis (IIP) and in the other types of interstitial pneumonitis (IP), including collagen vascular disease associated interstitial pneumonitis (CVD-IP), remain unknown. BAL fluid levels of IL-1β, -2, -4, -5, -6, -7, -8, -10, -12, -13, -17, G-CSF, IFN-γ, MCP-1, MIP-1, and TNF-α were measured using a bead suspension array in 8 patients (5 men, 3 women; mean age, 60.0±9.9 years) with idiopathic nonspecific interstitial pneumonitis (NSIP), 6 patients (3 men, 3 women; mean age, 69.0±4.8 years) with idiopathic usual interstitial pneumonitis (UIP), 3 patients (2 men, 1 woman; mean age, 66.3±5.5 years) with rheumatoid arthritis (RA), and 3 patients (1 man, 2 women; mean age, 52.3±14.5 years) with dermatomyositis (DM) in CVD-IP, as well as in 13 patients (2 men, 11 women; mean age, 51.8±17.2 years) with sarcoidosis, as a disease control. Levels of IL-7 were highest for DM (19.0±6.8 pg/ml), compared with other IPs (9.6±7.8 pg/ml for UIP, 8.6±3.7 pg/ml for NSIP, 7.0±6.9 pg/ml for RA) and sarcoidosis (4.2±2.5 pg/ml). On the other hand, levels of TNF-α were highest for RA (27.8±37.0 pg/ml), compared with other IPs (2.3±1.1 pg/ml in UIP, 1.3±0.8 pg/ml in NSIP, 10.7±9.3 pg/ml in DM) and sarcoidosis (5.9±6.1 pg/ml). Interestingly, levels of IL-17 were detectable only in RA (5.2±5.0 pg/ml). Differences seen in the level of each cytokine and chemokine between patients with IIPs and CVD-IP might reflect the pathogenesis of the IP.

P788

Enhanced acute pulmonary inflammation and reduced fibrotic response in quartz-exposed p47phox-deficient mice

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Previous studies have shown that quartz (crystalline silica) provides a useful tool to study experimental fibrosis in rodents. In the present study, we have investigated the involvement of phagocyte-derived reactive oxygen species (ROS) in quartz-induced inflammatory and fibrotic responses.
NADPH oxidase p47phox subunit deficient mice and their wild type counterparts were exposed to 100 mg/kg b.w. quartz via a single pharyngeal aspiration. After 24 hours markers of inflammation and oxidative stress were investigated in bronchoalveolar lavage fluid (BALF) and lung tissue. Quartz elicited a strong acute inflammatory response, characterised by a remarkably similar pulmonary influx of neutrophils in both strains. Interestingly, however, luminex multiplex analysis of BALF revealed stronger increases of interleukin (IL)-1β, IL-6, keratinocyte-derived chemokine (KC), monocyte chemoattractant protein-1 (MCP-1) and granulocyte colony stimulating factor (G-CSF) in the knockout mice compared to wild type animals. Differences in IL-4, IL-10, IL-13 and tumour necrosis factor-alpha (TNF-α) were not detectable. In contrast, pulmonary mRNA levels of the oxidative stress markers γ-glutamyl cysteine synthetase (γ-GCS) and heme oxygenase-1 (HO-1) were significantly enhanced only in the wild type mice in response to quartz-treatment. Three months after quartz treatment, significantly less fibrosis occurred in the lungs of knockout mice, as indicated by hydroxyproline content and Masson's trichrome staining.
These data show that impairment of NADPH oxidase increases acute inflammatory responses, whereas it reduces oxidative stress and fibrosis in the lungs of quartz-exposed mice.

P789

Muscarinic receptor stimulation differentially regulates extracellular matrix gene expression in lung fibroblasts

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Airway fibrosis is a characteristic feature of both asthma and COPD, in which fibroblasts are importantly involved. Increased activity of the cholinergic system may contribute to airway fibrosis, as muscarinic receptor stimulation has been shown to enhance collagen deposition by fibroblasts. The effects of muscarinic receptor stimulation on the expression profile of other extracellular matrix (ECM) proteins, however, remains to be established. To assess the effects of muscarinic receptor stimulation on ECM expression, MRC5 lung fibroblasts were stimulated for 24 h with increasing concentrations (10 nM – 100 μM) of the muscarinic receptor agonist methacholine in the absence and presence of the fibrogenic growth factor TGF-β1 (2 ng/ml), after which ECM gene expression was determined by quantitative PCR. The results demonstrate that methacholine concentration-dependently enhanced gene expression of the laminin α1 chain, whereas expression of the decorin gene was decreased. No effects of methacholine were observed on the gene expression of collagen I, collagen III, fibronectin, biglycan, versican or laminin α2, β1 and γ1 chains. In the presence of TGF-β1, methacholine (10 μM) enhanced the gene expression of fibronectin and collagen I. No additional effects of methacholine were observed on the expression of the other ECM genes investigated. Collectively, these results indicate that muscarinic receptor stimulation selectively changes the expression of specific ECM genes, which may contribute to the airway remodelling as observed in asthma and COPD.

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Collagen V and decorin is involved in systemic sclerosis pulmonary fibrosis

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Background: Systemic sclerosis (SSc) is characterized by vasculopathy, inflammation, autoimmunity and fibrosis. Collagen V (COLV) is involved in SSc since immunization of health rabbits with this protein induces an experimental model reproducing pathogenic manifestation of disease. We have demonstrated an increased amount of unusual COLV in SSc lung patients indicating a role for this protein in fibrosis. Fibrosis can be induced by cytokines and cell-matrix interactions that involves signalization mechanism. COLV and decorin participate of this mechanism interfering with fibrilogenesis.
Objectives: To evaluate COLV and decorin in tissue and to characterize COLV lung fibroblasts culture biochemically.
Methods: We evaluated COLV and decorin expression and tridimensional reconstruction by immunofluorescence in SSc patients without pulmonary hypertension that underwent surgical lung biopsy (n=6) and health controls from trauma (n=6). Biochemical characterization of COLV from lung fibroblasts culture used quantitative imunoblotting.
Results: COLV fibers was distorted and thickened in SSc lung tissue compared to thin fibers of controls. Decorin was distributed around COLV fibrils in the bronchovascular interstitium and vascular walls. Histomorphometric analysis of SSc demonstrated increased expression of COLV ($p<0,01$) and decorin ($p=0,01$) when compared to control. Imunoblotting detected an increased high molecular weight COLV fraction in SSc ($p=0.02$).
Conclusion: Over expression and unusual organization of COLV fibers with biochemical changes associated to increased decorin indicates that matrix signalization pathway is involved in COLV fibrillogenesis process in SSc pulmonary fibrosis.

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The effect of erythropoietin (EPO) on cyclooxygenase-2 (COX-2) and cytochrome-c (CYT-c) in the bleomycin (BLM)-induced pulmonary fibrosis (PF) in rats

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Purpose: The enzymes COX-2 and CYT-c are known to be a part of the fibrotic pathway.EPO is a multiple functional cytokine with anti-inflammatory and anti-apoptotic properties. Aim of this study was to investigate the role of EPO on the expression of both enzymes in BLM- induced PF in rats.
Methods: Fifty Wistar rats (300gr) were divided into five groups of 10 animals each:1)control animals,2)intratracheal (i.t) and intraperitoneal (i.p) injection of saline (0.5ml/kg),3)BLM hydrochloride (7.5mg/kg) i.t injection,4)BLM hydrochloride (7.5mg/kg) i.t injection followed by EPO i.p injection (2000 iu/kg),5)saline (0.5ml/kg) i.t injection followed by EPO i.p injection (2000 iu/kg). All rats were sacrified after 14 days.Immunohistochemical evaluation was performed for the expression of COX-2 and Cyt-c.A scale of 4 grades was used for the evaluation of the results: 0-25% (A),25-50% (B),50-75% (C),75-100% (D).
Results: In groups 1,2 and 5, both COX-2 and CYT-c were expressed in the grade A (80%) and in the grade B (20%). In group 3,COX-2 was expressed in the high grades B (20%),C (60%) and D (20%),and CYT-c only in the two higher grades C (70%) and D (30%). In group 4,both enzymes were expressed only in the low grades A (80% and 70% respectively) and B (20% and 30% respectively). The expression of COX-2 and CYT-c took place in the high grades for BLM group and in the lower grades for BLM+EPO group ($p<0.001$ and $p<0.05$ respectively).
Conclusions: BLM injection followed by EPO resulted in significant lower expression of COX-2 and CYT-c compared with BLM group. The protective mechanisms of EPO on PF must be further clarified.

P792

Microparticles-associated tissue factor activity is increased in bronchoalveolar lavage of patients with pulmonary fibrosis and correlates with functional impairment

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Background: The activation of the coagulation cascade plays a role in the pathogenesis of fibrotic lung diseases. Furthermore, anticoagulants are effective in experimental lung fibrosis and possibly in patients with idiopathic pulmonary fibrosis. Microparticles (MP) are cell derived procoagulant and proinflammatory vesicles that can express tissue factor (TF); MP represent a storage pool of bioactive effectors and are emerging as a new family of physiologically relevant mediators.
Aim: To evaluate the presence of TF-bearing MP in the bronchoalveolar lavage fluid (BALF) of patients with pulmonary fibrosis (PF) in comparison with control patients, and to correlate their concentration with the degree of functional impairment.
Methods: Seven patients with PF and 10 control patients with suspected lung cancer or infectious diseases underwent bronchoscopy. The presence of MP was evaluated through a prothrombinase assay that measures phosphatidylserine (PS) concentration; TF activity was assessed by a one-stage clotting assay.
Results: The BALF of patients with PF had a higher concentration of microparticles (87.23 [67.21-108.8] vs 49.19 [29.50-77.22] nM PS, p=.05) and a greater TF activity (27144 [17253-29998] vs 8596 [4019-21962] arbitrary U, p=.05) (data expressed as median [interquartile range]). We found a significant negative correlation between MP-associated TF activity and forced vital capacity% predicted (r^2=.95, p<.001) and DLCO% predicted (r^2=.56, p=.05).
Conclusions: Our preliminary data are consistent with an involvement of TF-bearing procoagulant MP in the pathogenesis of PF and in disease progression.

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Study of tetrahydrobiopterin in idiopathic pulmonary fibrosis and COPD

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Introduction: Tetrahydrobiopterin (BH4) is an essential cofactor for the activity

of nitric oxide synthase enzyme (eNOS). Deficiency of BH4 induced by oxidative stress could produce eNOS uncoupling and contribute to pulmonary damage.
Objective: To study the role of endogenous BH4 in patients with stable idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD).
Materials and methods: Twenty eight patients (15 IPF, 13 COPD; 61 (13) years) and 9 healthy controls were studied. Lung function tests (spirometry, pletismography and lung diffusion capacity), HRCT (High Resolution Computed Tomography) lung scan and 6-min. walk test were performed in all patients. Blood neutrophils, plasma BH4 (reverse-phase high-performance liquid chromatographic (RP-HPLC) with fluorescence detection), hemoglobin, fibrinogen, and CRP were also measured. Differences in BH4 levels between groups and its relationship with clinical, functional or biological parameters of disease severity were analized.
Results: BH4 was significantly reduced in IPF 1.32 (0.16)), and COPD (1.44 (0.27)) patients versus controls (2.42 (0.29)). There were no differences in BH4 levels between either IPF and COPD or bronchitis and emphysema phenotypes. BH4 levels were not related with parameters of lung function, radiological extension, inflammatory markers or smoking severity. In patients with COPD, BH4 levels were related with the number of previous exacerbations.
Conclusions: Plasma BH4 levels are reduced in IPF and COPD, which may be of potential value as a future biomarker of oxidative stress related diseases.
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P794
Increase of nitrosative stress in patients with eosinophilic pneumonia
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Background: Exhaled nitric oxide (NO) production is increased in asthma and reflects the degree of airway inflammation. The alveolar NO concentration (Calv) in interstitial pneumonia is reported to be increased compared to that in asthma. However, it remains unknown whether NO production is increased and nitrosative stress occurs in eosinophilic pneumonia (EP).
Objective: We hypothesized that nitrosative stress markers including Calv, inducible type of NO synthase (iNOS), and 3-nitrotyrosine (3-NT), are upregulated in EP.
Methods: Exhaled NO was measured in healthy subjects and in patients with interstitial pneumonia including idiopathic pulmonary fibrosis (IPF), cryptogenic organizing pneumonia, hypersensitivity pneumonitis, sarcoidosis and EP. iNOS expression and 3-NT formation were assessed by immunocytochemistory in BALf cells. The exhaled NO, lung function, and systemic inflammatory markers of the EP patients were investigated after corticosteroid treatment.
Results: The Calv levels in the EP group were significantly higher than those in the healthy subjects and the other interstitial pneumonia groups as well as the fractional exhaled NO (FE_{NO}) levels. More iNOS and 3-NT positive cells were observed in the EP group compared to the healthy subject and IPF patient. The Calv levels had significant correlations with both iNOS ($p<0.05$) and 3-NT positive cells ($p<0.01$). Corticosteroid treatment significantly reduced both the FE_{NO} ($p<0.05$) and the Calv levels ($p<0.01$). The magnitude of reduction in the Calv levels had a significant correlation with the peripheral blood eosinophil counts ($p<0.05$).
Conclusion: These results suggested that nitrosative stress was augmented in EP and may be involved in the pathogenesis.

P795
Molecular mechanism of lung aging in senescence-accelerated mouse (SAM)
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The SAM strains are a collection of inbred mouse strains developed as models of accelerated aging, and include nine short-lived, the senescence-prone strains (SAMP) and three longer lived control strains designated the senescence-resistant strains (SAMR). The SAMP was suggested as a new murine model of aging lung. However, molecular mechanism of accelerated lung aging remains to be elucidated. By using quantitative real time RT-PCR and western blot, here we show that expression of FOXO, a forkhead transcription factor that acts downstream of the PTEN/PI3K/Akt pathway and a key regulator of stress resistance, metabolism and ageing, was significantly decreased in aged SAMP mice compared to normal aging control SAMR mice. The decreased expression of FOXO gene was correlated with elevation of reactive oxidative species (ROS) and thiobarbituric acid reactive substances (TBARS), reduced mRNA expression levels of superoxide dismutase (SOD2) and catalase, as well as greater mean linear intercept (MLI) in SAMP mice lungs. Based on these findings we concluded that reduced FOXO activity may contribute to accelerated lung aging in this animal model. Given that FOXO proteins play a critical role in maintaining the quiescence and self-renewal capacity in hematopoietic stem cells and neural stem cells, manipulation of FOXO in lung progenitor cells may represent a novel approach to lung regenerative medicine and chronic lung disease such as COPD.

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Ageing and smoking contribute to plasma surfactant proteins and protease imbalance with correlations to airway obstruction
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Background: A significant number of young people start smoking at an age of 13-15, which means that serious smoking-evoked changes may have been occurred by their twenties. Surfactant proteins (SP) and matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) have been linked to cigarette smoke induced lung remodelling and COPD. However, the level of these proteins has not been examined during ageing or in young individuals with short smoking histories.
Methods: Plasma levels of SP-A, SP-D, MMP-9, and TIMP-1 were measured by EIA/ELISA from young (18-23 years) non-smoking controls (YNS) (n=36), smokers (YS) (n=51), middle aged/elderly (37-77 years) non-smoking controls (ONS) (n=40), smokers (OS) (n= 64) (FEV1/FVC >0.7 in all subjects) and patients with COPD (n=44, 35-79 years).
Results: Plasma levels of SP-A increased with age and in the older group in relation to smoking and COPD. Plasma SP-D and MMP-9 levels did not change with age but were elevated in OS and COPD as compared to ONS. The TIMP-1 level declined with age but increased in chronic smokers when compared to ONS. The clearest correlations could be detected between plasma SP-A vs age, pack years and FEV1/FVC. The receiver operating characteristic (ROC) curve analysis revealed SP-A to be the best marker for discriminating between patients with COPD and the controls (area under ROC curve of 0.842; 95% confidence interval, 0.785-0.899; $p<0.001$).
Conclusions: Age has a significant contribution to potential markers related to smoking and COPD; SP-A seems to be the best factor in differentiating COPD from the controls.

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Endothelial dysfunction in preterm infants with respiratory disorders
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Actuality. Respiratory disorders accompanied by endothelial disorders, but the clinical significance of changes in rates which characterize endothelial function in neonatology is neglected, because the purpose of our study was to establish clinical significance of endothelial dysfunction in preterm infants with respiratory disorders by the value of serum vascular endothelial growth factor (VEGF).
Materials and methods: The main group 67 children, who at birth had diagnosed respiratory distress syndrome. The average birth weight 1473,1±97,4 g, gestational age - 29,9±0,6 weeks. In the controling group were included 20 premature infants with birth weight 1529,5±82,8 g and gestational age 30,6±0,6 weeks without respiratory disorders.
Results and discussion: The values of serum VEGF at 5-7 day of life in children of all investigated groups did not differ significantly (by 124,9±31,9 and 135,8±32,2 pg/ml, $p>0,05$). In dynamics, 28 day life, we set the negative trend in the core group of children (114,1±24,5 to 328,8±92,3 pg/ml in the comparison group, $p<0,05$). Regression analysis established the relationship between values of serum VEGF at 5-7 day of life and indicators of body weight ($r=0,62$, $p<0,05$), duration of gestation at birth ($r=0,64$, $p<0,05$) and duration of mechanical ventilation ($r=0,89$, $p<0,05$)
Conclusions: The low value of VEGF for 5-7 day of life in preterm infants with respiratory failure and lack of growth in the dynamics indicate low ability to restore damaged capillaries and the risk of chronic disease.

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Neutral sphingomyelinase 2 (nSMase2) has a protective role in emphysema
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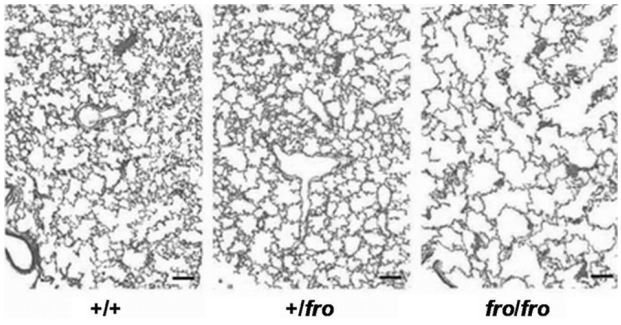
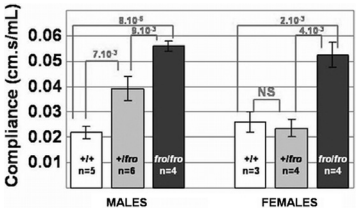
Introduction & background: nSMase2 is an enzyme converting sphingomyelin into ceramide. Others have proposed that nSMase2 activation and ceramide production are linked to emphysema development. The goal of this study was to test the effect of nSMase2 deficiency on lung phenotype in mice.
Animals: The mouse mutation *fro* carries a deletion within the nSMase2-encoding gene, rendering this enzyme inactive. In this study, we analyzed lung histology and lung function in adult *fro* mice.
Methods: Mean linear intercept (Lm) and alveolar destructive index (DI) were used to assess morphological changes in lungs. Lung compliance was measured to assess changes in lung function.
Results: We found that lungs of nSMase2-deficient mice exhibited emphysematous changes.
Both Lm and DI were significantly increased in *fro/fro* mice.



Consistent with emphysema, lung compliance was increased in *fro/fro* mice.



We found that heterozygous females were less affected than heterozygous males. **Conclusion:** In contradiction with the current hypothesis, we have shown that nSMase2-deficient mice develop emphysema. We conclude that at least some levels of ceramide are necessary to ensure proper lung development.

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Late intervention with a myeloperoxidase inhibitor prevents emphysema and small airway remodeling in the guinea pig

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Considerable evidence links both inflammation and oxidative stress to the pathogenesis of COPD. Myeloperoxidase (MPO), a neutrophil product, plays a major role in bacterial killing via production of the powerful oxidant, HOCl. However, oxidants generated by MPO can damage tissue and MPO exerts a variety of other effects that drive inflammation. We examined the effects of an MPO inhibitor, AZ11938920 on chronic (6 month) cigarette smoke-induced lesions in the guinea pig. One group of animals received compound from smoking day 1 (prophylactic arm), whereas another group was only treated after 3 months of smoke exposure (therapeutic arm). Analysis of lavage fluid showed that both treatments abolished smoke-induced increases in lavage inflammatory cells. Both treatments prevented smoke-induced increases in airspace size (emphysema) and small airway remodeling. Physiologically, both treatments largely reversed smoke-induced shifts of the pressure-volume and flow-volume curves and returned resistance to control values. Both treatments prevented muscularization of the small intrapulmonary arteries, but only partially ameliorated smoke-induced pulmonary hypertension. Immunohistochemical staining for the oxidation product, dityrosine, was increased in smoke-exposed animals and this effect was largely reversed by both treatments. We conclude that a myeloperoxidase inhibitor is able to prevent the development of emphysema and small airway remodeling and to partially protect against pulmonary hypertension, even when treatment starts after 3 months of smoke exposure. This protection appears to be related to prevention of oxidant damage and suppression of inflammation.