68. Experimental models and research in diffuse parenchymal lung diseases

P666
Late-breaking abstract: Serum and blood cell culture Th17 cytokines in pigeon fancies' hypersensitivity pneumonitis (HP)

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While HP affects a proportion of subjects, the factors that determine susceptibility are poorly understood. In HP experimental models IL-17 has pro-inflammatory function coordinating the inflammatory cytokines however little is known about this system in humans in HP among pigeon fanciers.

Aims: To quantify IL-1, IL-2, IL-4, IL-6, IL-10, IL-17, IL-22, TNFα, IFNγ, GM-CSF in serum and culture supernatant of immune blood cells of 45 pigeon fanciers and investigate their association with symptoms of HP.

Methods: Cytokines in antigen-stimulated lymphocyte and monocyte culture assay and serum antibody were measured by enzyme immunoassay.

Results: 11 subjects were categorised as HP probable, 6 were HP possible and 28 HP unlikely. The serum IgG antibody titre correlated with the number of symptoms, (r=0.288, p=0.048), and increased with the disease category of HP (r=0.397, p=0.007). There were no significant differences between clinical categories and any of the cytokines, but there were trends for a negative correlation between the antibody with IL-10 (r= -0.309, p=0.053) and with IL-4 (r= -0.297, p=0.066). The serum antibody levels correlated with antigen-specific proliferation stimulation index (r=0.537, p=0.007), and with IL-2, IL-4 and IL-5 (r= -0.05 for each). There was no disease specific serum or antigen-driven lymphocyte or monocyte culture production of IL-17 or IL-22.

Conclusions: In this study of serum and peripheral blood cell cytokines, the evidence suggested a potential role for Th2 cytokines associated with HP. There was no evidence of Th17 lymphocyte subset involvement.

P667
Reduction of lung injury and fibrosis by human embryonic stem cells in a mouse model of silica-induced lung fibrosis

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We assessed the ability of human embryonic stem cells (HUES-3) to differentiate into alveolar type II cells (ATIIcs), engraft into damaged lungs and ameliorate pulmonary fibrosis, thereby reducing mortality, in a mouse model of silica-induced pulmonary inflammation and fibrosis.

Nude mice (Charles River, Italy) were intranasally administered either silica oxide powder (99.9% Alfa Aesar) intranasally (5 mg/ml, 50 µl/mice/day) or saline for 15 days followed by intratracheal 2.5 x 106 HUES-3-ATIIcs (n=50, HUES-3-ATIIcs treated) or saline (n=50, Sham treated). Ten silica treated mice were instilled mature fibroblasts.

In vitro differentiated HUES-3-ATIIcs displayed an alveolar phenotype (multi-lamellar body, SP-B, SP-C and ZO-1 expression and CFTPr-mediated chloride ion transport). After transplantation into silica-damaged mice, HUES-3-ATIIcs were capable of engraftment as they showed persistence of human SP-C and of human DNA and effectively reduced inflammation and fibrosis. HUES-3-ATIIcs treated mice showed reduced expression of IL-6, TNF-α, MIP-2 and TGF-β and of collagen marker Col1-α1 (p<0.05) and reduced histological scores (Ashcroft score p<0.01). Also showing preserved oxyhemoglobin saturation and reduced mortality at 20 days (Sham treated, 100%; HUES-3-ATIIcs treated, no mortality with 95% weight recovery; p<0.05).

In conclusion, the data show that HUES-3-ATIIcs intratracheal instillation can reduce silica-induced lung injury and fibrosis in the mouse model of silica-induced lung fibrosis, indicating stem cell therapy as a viable approach for pulmonary fibrosis treatment.

P668
Rapid reversal of radiation-induced murine pneumonitis by treatment with the anti-CTGF monoclonal antibody FG-3019

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Rationale: Connective tissue growth factor (CTGF) is a matricellular protein that is a central mediator of tissue remodeling characteristic of fibrosis. CTGF expression is elevated in patients with IPF. CTGF is essential for sustained fibrosis, and can mediate multiple steps in the process of fibrosis. Therefore, inhibition of CTGF may be therapeutically beneficial to patients with pulmonary fibrosis.

Methods: Lung injury was initiated with a single, full thorax irradiation and administration of FG-3019 began 16 weeks after irradiation, when a significant increase in lung density was detectable by computed tomography (CT). Gene expression and histological analysis was performed at 18 weeks after irradiation.

Results: Lung density in placebo-treated mice increased between 12 weeks and 30 weeks after irradiation. Treatment with FG-3019 reversed the radiation-induced lung density increase, which progressively decreased during the 8 weeks of administration and remained constant for another 6 months. Gene expression analysis and histological examination showed considerable infiltration of mast cells and macrophages at 18 weeks after irradiation in placebo-treated animals, while two weeks of FG-3019 restored levels to that of unirradiated mice. Conclusions: FG-3019 reversed radiation-induced pneumonitis and lung remodeling very quickly after administration was initiated and produced a durable effect on lung density. Because these are key events in the process of fibrosis, the data suggest that inhibition of CTGF can disrupt fibrotic processes after they have begun and support continued clinical evaluation of FG-3019 for treatment of pulmonary fibrosis.

P669
Glycosphingolipids modulate TGF-β fibroblast signaling in human lung fibroblasts

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Background: In idiopathic pulmonary fibrosis (IPF), fibroblasts are recruited to the wounded area and exhibit a fibroproliferative phenotype. Tumor growth factor-beta-1 (TGF-beta) could play a role in mediating this process. TGF-beta receptors may cooperate with integrins and lipid rafts from cellular membrane micro-domains. We have investigated the role of PDEMP (D-threo-1-Phenyl-2-decanoylamino-3-morpholino-1-propanol-HCl.), a glycosphingo-lipid inhibitor, as a potential modulator of TGF-beta signaling in human lung fibroblasts.

Methods: MRC-5 cultures (human lung fibroblast cell line) were stimulated with TGF-beta1 (5ng/ml) in the presence or absence of PDEMP. Generation of fibronectin and collagen I was measured by real time PCR. We also studied the role of PDEMP in abrogating the conversion from epithelial cell to mesenchymal cells in TGF-beta stimulated rat epithelial cells (RLE) measuring alpha smooth muscle actin by rt-PCR.

Results: Our results indicate that PDEMP treated cells exhibit decreased collagen and fibronectin formation from a human lung fibroblast cell line. We also found that PDEMP reduced the expression of alpha smooth muscle in RLE cells as indicative of reduced myofibroblast transformation.

Conclusions: We have identified glycosphingolipids as important immunomodulators in TGF-beta signaling. Disruption of glycosphingolipids with PDEMP results in the decrease of extracellular matrix proteins and epithelial mesenchymal transformation, both important processes in IPF.
P670
The stimulator of soluble guanylate cyclase riociugat protects against bleomycin-induced pulmonary fibrosis in mice
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Background: Effective therapies for pulmonary fibrosis (PF) are currently lacking. Patients with PF develop pulmonary hypertension (PH), in part due to impaired production of endogenous nitric oxide (NO) that activates soluble guanylate cyclase (sGC). We hypothesized that the NO-independent stimulation of sGC might represent a new modality for treating PF and related conditions.

Methods: Male C57BL/6J mice (10-12 wks) were subjected to intratracheal bleomycin (0.5 U/kg) and gavage-feeding with the sGC stimulator riociugat (1, 3 or 10 mg/kg/day), the phosphodiesterase 5 (PDE5) inhibitor sildenafil (100 mg/kg/day), or a combination of riociugat (1 mg/kg/day) and sildenafil (100 mg/kg/day), or vehicle alone for two weeks.

Results: Bleomycin-induced PH (an increase in the right ventricle systolic pressure and a decrease in the pulmonary acceleration time/heart rate ratio) and the right ventricular hypertrophy were attenuated by riociugat and the combination of riociugat and sildenafil to a greater extent than by sildenafil alone. In the vehicle-treated mice, fibrosis and inflammation diffusely involved lung parenchyma. Riociugat and its combination with sildenafil but not sildenafil alone markedly ameliorated PH and inflammation that was mainly confined to subpleural areas and/or peripheral lung in a patchy distribution. Riociugat increased plasma cGMP levels and also reduced mortality.

Conclusions: Pharmacological stimulation of sGC with riociugat attenuates PH, right ventricular hypertrophy and mortality in the bleomycin-exposed mice. This therapeutic approach appears to be superior to treatment with sildenafil. Stimulation of sGC might represent a new modality for treating PF and related conditions.

P671
Can flaxseed oil reduce experimental lung fibrosis in rats?
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Background: The anti-inflammatory effects of polyunsaturated fatty acids (PUFA) is actually demonstrated. In this study we evaluated protective and therapeutic effects of Flaxseed Oil (FO) on Bleomycin (BLM) lung fibrosis in rats.

Methods: 30 males wistar rats were randomly divided into 3 equal groups: untreated group (G1) and 2 treated groups (FO, 1g/kg bw/day). G2 received FO during two months before inducing lung fibrosis than G3 received FO 2 days after inducing lung fibrosis during 10 days. Pulmonary fibrosis was induced by BLM (4 mg/kg, intratracheally single dose). Inflammatory index, fibrosis score (Ashcroft) and TGF-β density was evaluated in different areas of damaged lung by anatomo-histolological and immunohistological analysis.

Results: Independent-samples T-test revealed that comparatively to control group (G1), FO reduced significantly fibrosis score in G2 and TGF-β density in fibrocytes in G3 (p<0.05).

Conclusion: These data demonstrated a protective activity of FO against bleomycin-induced lung fibrosis model in rats. More investigations will be realized in human to prove relationship between diet and interstitial lung diseases.

P672
Experimental model resembling the histological pattern of usual interstitial pneumonia and reinforcing the epithelial injury as pathway
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The present experimental model in mice was developed to confront the histopathological features with that of the idiopathic pulmonary fibrosis (IPF)/usual interstitial pneumonia (UIP), as we suppose both are caused by the injury of type II pneumocytes (TIIp) and by the increase of collagen system. Twenty male Balb/c mice are injected ip with 400mg/kg of bleomycin (BLM) during two months before inducing lung fibrosis than G3 received FO 2 days after inducing lung fibrosis during 10 days. Pulmonary fibrosis was induced by BLM (4 mg/kg, intratracheally single dose). Inflammatory index, fibrosis score (Ashcroft) and TGF-β density was evaluated in different areas of damaged lung by anatomo-histolological and immunohistological analysis.

Results: Independent-samples T-test revealed that comparatively to control group (G1), FO reduced significantly fibrosis score in G2 and TGF-β density in fibrocytes in G3 (p<0.05).

Conclusion: These data demonstrated a protective activity of FO against bleomycin-induced lung fibrosis model in rats. More investigations will be realized in human to prove relationship between diet and interstitial lung diseases.

P673
Inhibition of PAI-1 in the cigarette smoke induced epithelial-mesenchymal transition
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Introduction: Plasminogen activator inhibitor-1 (PAI-1) has been known to play an essential role in pulmonary fibrosis by inhibiting plasminogen activator (PA). Recently, it is assumed that epithelial-mesenchymal transition (EMT) play a role in the pathogenesis of IFP. We tried to find out whether PAI-1 is involved in the bleomycin-induced pulmonary fibrosis in the rat and in smoking-induced rat alveolar EMT in vitro.

Methods: First, rats were received intratracheal bleomycin (4U/kg) and then administered tiplaxitinin (1mg/kg) at day 1, 3, 5, 7, 10 orally. Rats were sacrificed at day 14. Next, the type II alveolar epithelial cells were isolated from normal rats by percoll gradient methods. Type II epithelial cells were transfected with PAI-1-siRNA and were stimulated with cigarette smoking extract (CSE, 5%).

Results: In bleomycin-induced pulmonary fibrosis model, tiplaxitinin decreased the bleomycin-induced pulmonary fibrosis by Ashcroft score and also decreased the PAI-1 and TGF-β concentrations in the BAL fluids. In epithelial cell experiment, exposure to CSE increased the α-SMA and PAI-1 mRNA expression in real-time PCR. However, they were attenuated either after transfection with siRNA-targeted PAI-1 or treatment with tiplaxitinin (50 μM). TGF-β concentrations in the CSE exposed cell culture supernatants were also decreased either by PAI-1-siRNA transfection or treatment with tiplaxitinin. The up-regulation of Snail and pSMAD by smoking exposure in epithelial cells was decreased either by transfection with PAI-1-siRNA or treatment with tiplaxitinin.
P674
Bleomycin-induced remodeling – Inflammation and fibrosis are developing in parallel during initiation
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Background: Pulmonary fibrosis is a facet of Diffuse Parenchymal Lung Disease and has been considered a sequel to chronic inflammation. Yet the relation between connective tissue and immune system during the initiation phase has not been thoroughly investigated. Our aim was to investigate the initiation phase and the interplay between immune system and connective tissue.

Methods: C57BL/6 mice were given subcutaneous bleomycin injections (controls received saline) 3 times/week for 1, 2, 3, and 4 weeks. Following sacrifice, lungs were embedded in paraffin and 4 μm thick sections were used for analysis of lung parenchyma, Masson Trichrome (total collagen) and immunohistochemistry to detect neutrophils, macrophages and B-cells.

Results: Total collagen was significantly increased at 1, 2 and 4 weeks. A transient neutrophilia was found at 1w (2779±820 cells/mm3), but no difference compared to controls was found at 2, 3 and 4w. Macrophages were significantly increased at 2w (135±29 cells/mm3), whereas B-cells were significantly decreased at 3 and 4w, compared to controls.

Conclusions: Subcutaneous administration of bleomycin induced rapid pulmonary remodeling, exemplified by increased total collagen. The immune response was markedly different from the one following intratracheal administration, illustrated by a marked transient neutrophilia. Infiltration of macrophages at 2w in combination with decreased neutrophils suggests ongoing clearance. Decrease of B-cells may suggest migration to lymphoid organs, thus initiation of an adaptive immune response. In summary, we found fibrosis and inflammation occurring in parallel, both present early in the development of pulmonary fibrosis.

P675
Enhanced lung inflammatory response to intratracheal CpG-ODN in mice with bleomycin-induced lung fibrosis
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Introduction: Although the etiology of an acute exacerbation of idiopathic pulmonary fibrosis (IPF) is unknown, it may be represented by a clinically occult immune response. In summary, we found fibrosis and inflammation occurring in parallel, both present early in the development of pulmonary fibrosis.

Methods: Lung fibrosis was induced in female C57BL/6 mice by the intraperitoneal injection of bleomycin (4 mg/kg). Twenty-eight days after the injection of bleomycin, mice were killed. The neutrophil counts and cytokine levels were measured.

Results: The neutrophil counts and cytokine levels were significantly higher in the BLM/CpG group than in the saline/CpG group. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemical analysis revealed that the levels of macrophage inflammatory protein (MIP)-2 and interleukin (IL)-6 in the BALF were found to be significantly higher than those in the saline/CpG group. The levels of macrophage inflammatory protein (MIP)-2 and interleukin (IL)-6 in the BALF were also significantly higher in the BLM/CpG group than in the saline/CpG group. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemical analysis revealed that the levels of macrophage inflammatory protein (MIP)-2 and interleukin (IL)-6 in the BALF were found to be significantly higher than those in the saline/CpG group. The levels of macrophage inflammatory protein (MIP)-2 and interleukin (IL)-6 in the BALF were also significantly higher in the BLM/CpG group than in the saline/CpG group.

Conclusions: We found fibrosis and inflammation occurring in parallel, both present early in the development of pulmonary fibrosis.

P676
Anti-oxidative and anti-inflammatory effects of triglonga foroum gracum (fenugreek) seed extract in experimental pulmonary fibrosis
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Background: Oxidative stress participated in the pathogenesis of interstitial lung diseases. This prospective study is the first experimental work that evaluated the effect of Fenugreek (FG) and its phenolylic extract on oxidative stress and lung fibrosis in experimental model.

Methods: A single dose of Bleomycin was injected intratracheally in forty male wistar rats (4mg/kg). Two days after inducing lung injuries, animals were ran- domly divided in four groups of 10 rats: G1, G2, G3 and G4. G1 received FG Seed Polyphenol Extract at a dose of 200 mg kg by daily gavage.G2 received FG seeds powder mixed with food at levels of up to 20%. G3 received an equal volume of water (6.5 ml kg, daily gavage). G4 did not receive any treatment. Two weeks after inducing lung fibrosis, rats were sacrificed and we evaluated stress biomarkers (TAS:Total Antioxidant Status, Malon DiAldehyde MDA) in serum and inflammatory index, fibrosis score and TGF beta density in lung tissue.

Results: Comparatively to control groups G3 and G4, ANOVA statistical study, demonstrated that FG Seed Polyphenols Extracts ameliorated TAS and reduced inflammatory index and TGFβ density in G1 and seeds powder of FG reduced MDA a marker of oxidant stress and inflammatory score in G2 (p<0.05). However, Ashcroft semi-quantitative assessing of fibrosis score has not revealed significant differences between the four groups (p=0.05). Conclusion: This study demonstrated that FG has potent antioxidant and anti-inflammatory activities but has not an anti-fibrotic effect confirming that besides inflammation, other factors probably interfere in the pathogenesis of pulmonary fibrosis.

P677
Effect of experimental pulmonary fibrosis induced by bleomycin on the lung fatty acid composition in Wistar rats
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Background: Many recent studies are directed towards the use of natural substances for the treatment of pulmonary fibrosis and of these fatty acids. The main our study was first to verify the normal composition of fatty acids in the lung of control rats and collect the effect of experimental fibrosis induced by bleomycin in rats on the latter.

Methods: A total of twenty males wistar rats which received water and food ad libitum, were divided randomly into two groups: untreated group (G1, n=10) and treated group (G2, n=10).

Pulmonary fibrosis was induced in all treated rats (G2) by bleomycin (4 mg/kg, single dose) administrated intratracheally. Three day later, all rats were sacrificed, lungs were extracted and blood was collected for analysis of fatty acid composition in the lungs and red cells of rats studied, and this by using the technique of gas chromatography (GC).

Results: The statistical of independent-samples T test revealed that bleomycin filters significantly the composition means of various fatty acids in treated group (G2) compared to untreated group (G1) as shown in the figures below.

Conclusions: These results confirm that bleomycin affects the fatty acid composition of lung giving the opportunity to test the effects of substances rich in fatty acids on pulmonary fibrosis models in rats.
of unknown cause such as idiopathic pulmonary fibrosis. The aim of the current study was to characterize the mechanisms of pulmonary fibrosis and to determine whether IL-17A plays an important regulatory role.

**Methods:** Balb-Bleomycin (BLM), Balb-Paraquat, C57-BLM and C57-KO-IL17-β fibrosis animal models were used to evaluate the fibrosis and the IL-17 role. We used the picrosirius-polarization method, weigert's resorcinn-fuchsin histochemistry, immunohistochemistry, and morphometric analysis to evaluate the amounts of collagen and elastic fibers and the transforming growth factor-β (TGF-β) expression.

**Results:** We report here that BLM-mediated fibrosis is IL-17 independent, as IL17-deficient mice developed similar amounts of fibrosis when compared to control mice. In marked contrast, BLM-induced pulmonary fibrosis revealed increase of collagen fibers in all four groups (Fig 1). We found that only Balb-BLM and Balb-Paraquat presented increase of elastic fibers. A positive correlation was found between elastic and collagen fibers in Balb-BLM animals, as well as in Balb-BLM and Balb-Paraquat. In addition, we found a direct correlation between TGF-β and collagen in C57-KO-IL17-β fibrosis.

**Conclusions:** Bleomycin and paraquat-mediated fibrosis is IL-17 independent. However, more studies are necessary to validate the regulatory role of IL-17 on pulmonary fibrosis process.

**P679**

Modulation of fibroblast phenotype in idiopathic pulmonary fibrosis: Role of NrF2

Elise Artaud-Macari, Delphine Goven, Stéphanie Brayer, Joelle Marchal-Somme, Bruno Crestani, Anne Boutten, Marcel Bonay. U700, INSERM, Paris, France

**Rationale:** Oxidative stress has been implicated in Idiopathic Pulmonary Fibrosis (IPF) pathophysiology especially in myofibroblastic differentiation. NrF2 signaling pathway, the main regulator of endogenous antioxidant enzymes, could be involved in fibrogenesis. The aim of our study was to analyse human pulmonary fibroblast expression of NrF2, and, to assess the effects of NrF2 modulation on fibroblast phenotype in vitro.

**Methods:** We assessed antioxidant/antioxidant balance, NrF2 expression and phenotype of IPF and control fibroblasts in basal conditions, after stimulation by TGF-β, and NrF2 siRNA transfection.

**Results:** We showed a decrease of nuclear NrF2 expression in IPF fibroblasts concomitant with myofibroblast phenotype in basal conditions. TGF-β induced an inhibition of nuclear NrF2 expression and a myofibroblastic differentiation of control fibroblasts. NrF2 inhibition in control fibroblast led to an increased oxidative stress in association with a myofibroblastic differentiation. Conversely, NrF2 activation by Krap1 siRNA, resulted in antioxidant defenses restoration and myofibroblastic dedifferentiation in IPF fibroblasts.

**Discussion:** Our results suggest an association between decreased nuclear NrF2 expression and myofibroblastic phenotype of IPF fibroblasts. NrF2 modulation in human lung fibroblasts confirmed the increased susceptibility of NrF2 knockout mice to bleomycin induced pulmonary fibrosis.

**Conclusion:** Our study identified NrF2 as a novel therapeutic target in IPF fibroblasts and suggested a potential antifibrosis effects of NrF2 pharmacological activators.

**P680**

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**P681**

Serum SP-A and SP-D: Different cutoff values for German and Japanese patients to diagnose idiopathic interstitial pneumonia

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**Introduction:** Surfactant protein (SP)-A and SP-D are members of the C-type lectin superfamily. Serum levels of SP-A and SP-D are known to be elevated in patients with various interstitial lung diseases; however, the majority of these data have been obtained in Japanese.

**Objectives:** This study was conducted to compare serum levels of SP-A and SP-D between the German and Japanese population and to investigate whether SP-A and SP-D could be used as diagnostic biomarkers in German patients with idiopathic interstitial pneumonias (IPFs).

**Methods:** Serum samples were obtained from Germans (110 patients with IPFs and 57 healthy controls) and Japanese (68 patients with IPFs and 100 healthy controls).

**Results:** Serum SP-A and SP-D levels were measured and cutoff values to discriminate IPF patients from healthy subjects were assessed by receiver operating characteristic (ROC) analysis.

**Conclusions:** In healthy subjects, serum levels of SP-D were significantly higher in Germans than those in Japanese (63.9±30.4 ng/ml and 40.2±24.4 ng/ml, p<0.001) whereas serum levels of SP-A were not significantly different (32.1±16.9 ng/ml and 27.3±13.3 ng/ml, p=0.100). ROC analysis revealed that the optimal cutoff values to discriminate IPF patients from healthy subjects in Germans and Japanese were 133.5 ng/ml and 103.0 ng/ml for SP-D, and were 38.9 ng/ml and 32.4 ng/ml for SP-A, respectively.

**Conclusions:** Our data suggest the possibility of SP-A and SP-D to be used as diagnostic biomarkers for IPFs in Germans. Cutoff values to discriminate IPF patients from healthy subjects seem to be different between in Germans and in Japanese, however, further investigations will be required.

**P682**

LSC 2011 Abstract: Role of mast cells and chymase in idiopathic pulmonary fibrosis

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**Rationale:** Mast cell (MC) activation has been implicated in the pathogenesis of inflammatory lung diseases. Among the MC-derived mediators, MC chymase is involved in the processes such as activation of TGF-β involved in the processes such as activation of TGF-β and formation of collagen fibrils. The literature suggests that chymase may contribute to the pathogenesis of pulmonary interstitial remodeling; however, a systematic investigation is still missing.

**Methods:** Lung tissues obtained from donors and patients with idiopathic pulmonary fibrosis were formalin-fixed and paraffin-embbeded, followed by toluidine blue staining for MCs and by immunostaining for chymase. The total, perivascular and interstitial MC and chymase-positive MC (CMC) number in each section was evaluated by using a light microscopy and computerized morphometric system. Also, MCs were separated as granulated and degranulated (activated).

**Results:** We showed a decrease of nuclear NrF2 expression in IPF fibroblasts concomitant with myofibroblast phenotype in basal conditions. TGF-β induced an inhibition of nuclear NrF2 expression and a myofibroblastic differentiation of control fibroblasts. NrF2 inhibition in control fibroblast led to an increased oxidative stress in association with a myofibroblastic differentiation. Conversely, NrF2 activation by Krap1 siRNA, resulted in antioxidant defenses restoration and myofibroblastic dedifferentiation in IPF fibroblasts.

**Discussion:** Our results suggest an association between decreased nuclear NrF2 expression and myofibroblastic phenotype of IPF fibroblasts. NrF2 modulation in human lung fibroblasts confirmed the increased susceptibility of NrF2 knockout mice to bleomycin induced pulmonary fibrosis.

**Conclusion:** Our study identified NrF2 as a novel therapeutic target in IPF fibroblasts and suggested a potential antifibrosis effects of NrF2 pharmacological activators.
There was a preponderance of perivascular MCs and CMCs in IPF lungs (p<0.05 versus donor lungs). Furthermore, we found that there was about 8 fold decrease of IOG in IPF patients as compared with donors. Finally, there was a strong accumulation of both MCs and CMCs in interstitial regions of the tissues (∼65%) in comparison with other regions of the lungs.

Conclusion: The findings suggest that chymase released from activated MCs may be involved in the pathogenesis of IPF. Further investigations will unravel the underlying pathomechanism and substantiate chymase as a potential target for future therapeutic strategies.

P683
LSC 2011 Abstract: Histological markers of epithelial-mesenchymal transition in idiopathic pulmonary fibrosis provide evidence of an alternative repair process
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Introduction: Wound remodelling in the pathogenesis of idiopathic pulmonary fibrosis (IPF) has produced conflicting results from previous cell culture and animal model studies.

Aim: The aim of this study was to examine wound remodelling mechanisms in usual interstitial pneumonia (UIP), with emphasis on the role of epithelial mesenchymal transition (EMT).

Methods: Immunohistochemistry was used to assess cellular expressions of markers of EMT in paraffin embedded lung tissue samples from 21 patients with IPF, with comparisons made to histologically-defined normal lung sections from 19 control subjects.

Results: Hyperplastic type II pneumocytes in all UIP cases expressed the adhesion molecule E-cadherin with no expression of N-cadherin or TWIST. Expression of TWIST was restricted to the fibroblasts/myofibroblasts. TGF-β protein was consistently expressed by type II pneumocytes of UIP samples, but to varying degrees within the fibroblastic foci. Collagen I and smooth muscle actin were expressed in the fibroblastic foci.

Conclusions: Myofibroblasts may form a contractile repair response to a micro-injury via secretion of extracellular matrix proteins, providing scaffolding for type II pneumocytes which then divide at the edge of the insult and migrate over the fibroblastic foci surface. TGF-β signalling pathways may lead to the continued accumulation of type I collagen in the foci. Alternatively, abnormal collagen I is produced which is resistant to matrix metalloproteinases inhibiting wound repair. We conclude that tissue remodelling in IPF is a complex processes warranting further investigations to fully elucidate the role of EMT in the pathogenesis of IPF.