Disease pathogenesis in interstitial lung disease

**S67** A CRITICAL ROLE FOR ALTERNATIVELY ACTIVATED MONOCYTES AND MACROPHAGES IN THE PATHOGENESIS OF PULMONARY FIBROSIS

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**Introduction and Objectives** The pathogenesis of idiopathic pulmonary fibrosis remains a controversial subject. The prevailing hypothesis favours an aberrant wound healing response where epithelial injury stimulates myofibroblast differentiation with collagen deposition and resultant fibrosis. The role of the macrophage is controversial. We hypothesised that the profibrotic alternatively activated macrophage is critical to lung fibrosis progression. Using both the bleomycin and adenoviral transforming growth factor β (AdTGFβ) models we sought to determine the effects of depletion of circulating monocytes and alveolar macrophages on the degree of pulmonary fibrosis.

**Methods** Fibrosis was induced by intratracheal instillation of either 0.033 mg (0.05 U) of bleomycin or 3 × 10⁸ plaque-forming units (PFU) of AdTGFβ. Circulating monocytes and alveolar macrophages were depleted at various time points by administration of liposomal clodronate by intraperitoneal or intratracheal injection, respectively. In the bleomycin model fibrosis was assessed at both early and late stages (days 18 and 32). In the AdTGFβ model fibrosis was assessed at 14 days. Fibrosis was assessed by collagen quantification, histological fibrosis score and quantitative PCR (qPCR). Markers of macrophage phenotype were assessed by immunohistochemistry and qPCR.

**Results** In the bleomycin model depletion of circulating monocytes or alveolar macrophages at various time points had no effect on early fibrosis. Depletion at later points reduced the degree of pulmonary fibrosis (fig 1). Reduction in fibrosis was associated with a reduction in markers of alternative macrophage activation. In the AdTGFβ model, depletion of circulating monocytes reduced the degree of pulmonary fibrosis.

**Conclusions** We have shown for the first time the critical role that monocytes and macrophages play in the pathogenesis of pulmonary fibrosis. Our data suggest that it is the profibrotic alternatively activated macrophage that is the key player involved in this process. These results enhance our knowledge of a devastating and untreatable disease. By improving our understanding of a controversial disease process we hope to enable better targeted therapies to be identified. Specifically, with the advent of cell therapy and cell manipulation, we may be able to deplete or modulate the behaviour of these pro-fibrotic macrophages, and consequently halt, and even reverse, the degree of pulmonary fibrosis.

**S68** MURINE HAEMATOPOEITIC STEM CELLS BUT NOT MURINE MESENCHYMAL STEM CELLS AMELIORATE LUNG FIBROSIS USING GENE DELIVERY OF KERATINOCYTE GROWTH FACTOR

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**Introduction** Pulmonary fibrosis is the end stage of various conditions, lacks satisfactory treatment options and results in significant morbidity and mortality. Alveolar epithelial cell (AEC) injury plays a central role in the pathogenesis of pulmonary fibrosis; restoration of epithelial integrity is required for re-establishment of normal alveolar architecture. Keratinocyte growth factor (KGF) assists epithelial repair; KGF stimulates AEC proliferation, enhances DNA repair and reduces apoptosis. Intratracheal delivery of KGF attenuates fibrosis in animal models of pulmonary fibrosis. Circulating bone marrow-derived cells (BMDCs) are known to home to injured lung. We sought to determine whether BMDCs could be used as vehicles to deliver KGF to injured lung, to attenuate fibrosis.

**Methods** Murine haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) were transduced with a lentiviral vector conditionally expressing the KGF transgene under doxycycline control. Transduced HSCs and MSCs (or controls) were delivered to C57Bl/6 mice given oropharyngeal bleomycin to induce lung injury. The pathogenesis of idiopathic pulmonary fibrosis remains a controversial subject. The prevailing hypothesis favours an aberrant wound healing response where epithelial injury stimulates myofibroblast differentiation with collagen deposition and resultant fibrosis. The role of the macrophage is controversial. We hypothesised that the profibrotic alternatively activated macrophage is critical to lung fibrosis progression. Using both the bleomycin and adenoviral transforming growth factor β (AdTGFβ) models we sought to determine the effects of depletion of circulating monocytes and alveolar macrophages on the degree of pulmonary fibrosis.

**Results** In the bleomycin model depletion of circulating monocytes or alveolar macrophages at various time points had no effect on early fibrosis. Depletion at later points reduced the degree of pulmonary fibrosis (fig 1). Reduction in fibrosis was associated with a reduction in markers of alternative macrophage activation. In the AdTGFβ model, depletion of circulating monocytes reduced the degree of pulmonary fibrosis.

**Conclusions** We have shown for the first time the critical role that monocytes and macrophages play in the pathogenesis of pulmonary fibrosis. Our data suggest that it is the profibrotic alternatively activated macrophage that is the key player involved in this process. These results enhance our knowledge of a devastating and untreatable disease. By improving our understanding of a controversial disease process we hope to enable better targeted therapies to be identified. Specifically, with the advent of cell therapy and cell manipulation, we may be able to deplete or modulate the behaviour of these pro-fibrotic macrophages, and consequently halt, and even reverse, the degree of pulmonary fibrosis.
transplantation 8 weeks prior to bleomycin exposure; HSCs were delivered systemically following irradiation. MSCs were given systemically 8 h and 3 days after bleomycin exposure. Mice were sacrificed at day 14 following bleomycin exposure. Lung tissue was processed for histopathological examination (Ashcroft Score), immunohistochemistry, flow cytometry and quantitative reverse transcription-PCR (qRT-PCR).

Results Murine MSCs and HSCs were efficiently transduced with the lentiviral vector, and retained their differentiation capacity. MSCs engrafted at low levels in the lung and no significant difference was seen in KGF expression, AEC proliferation or lung architecture in mice given MSCs. However, MSC administration did result in a reduction in procollagen gene expression. Transplantation of transduced HSCs resulted in multilineage bone marrow engraftment. KGF–HSC-treated mice had significantly increased KGF expression in the lung. This was associated with significantly increased AEC proliferation, reduced profibrotic cytokines and improved fibrosis (fig 1). KGF–HSC-treated mice had significant improvements in weight loss and survival, suggesting the changes were physiologically relevant.

Conclusion We have shown that HSCs can be used as vectors to deliver KGF therapy to injured lung parenchyma. Genetically modified cell therapy represents a novel and exciting approach to the treatment of lung injury and fibrosis.

**S69 MARKERS OF SYSTEMIC AND ENDOTHELIAL INFLAMMATION AMONG PIGEON BREEDERS**

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**Introduction and Objectives** A characteristic histological feature of hypersensitivity pneumonitis (HP) is the lipid-laden foamy alveolar macrophage suggestive of altered lipid metabolism associated with interstitial inflammation. We have recently described increased serum cholesterol concentration among pigeon fanciers. Our hypothesis is that there is an altered inflammatory profile of biomarkers associated with endothelial cell activation in HP.

**Methods** In 100 pigeon fanciers we quantified the serum concentration of a variety of inflammatory mediators and immunoglobulin G (IgG) antibody titre to inhaled avian antigens by immunoaassay. These were compared with the clinical history of symptoms of HP and lung function.

**Results** All subjects had an IgG response to avian antigens (median [interquartile range] 29.0 μg/ml [12.5–54.0]), that was significantly inhibited by cigarette smoking (median μg/ml of never: excurrent 35.28±10, p<0.05). This antibody correlated with C-reactive protein (CRP) (r = 0.279, p = 0.015), with the cytokines interleukin-8 (IL-8), monokine induced by interferon γ (MIG), interferon-inducible protein 10 (IP10) and interferon α (IFNα) (each, p<0.05), and with E-selectin (r = 0.321, p = 0.004), intercellular adhesion molecule-1 (ICAM-1) (r = 0.242, p = 0.053) and soluble receptor for advanced glycation end-products (sRAGE) (r = −0.239, p = 0.053).

**Conclusions** These results suggest that it is sufficient to mount an antibody response to inhaled antigens in order to have associated systemic inflammation. This assertion may be taken further to include endothelial cell activation. Not all subjects were symptomatic or had abnormal lung function, therefore this suggests that subclinical inflammation is common and might help explain subacute development of HP.

**S70 LYSOPHOSPHATIDIC ACID INCREASES ITGB6 AND LPAR2 EXPRESSION VIA TGFβ-DEPENDENT AND TGFβ-INDEPENDENT MECHANISMS**

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**Introduction and Objectives** Idiopathic pulmonary fibrosis (IPF) is a debilitating disease with a poor prognosis and current treatment is ineffective. Transforming growth factor β (TGFβ) is a pleiotropic cytokine with a central role in the pathogenesis of IPF. Lysophosphatidic acid (LPA) induces αvβ6-mediated TGFβ activation via the LPA receptor 2 (LPAR2) and is increased in the lungs of patients with IPF. Both the αvβ6 integrin and LPAR2 are upregulated in the epithelium overlying fibrotic lung tissue. However, regulation of these molecules is poorly understood, and how they may be dysregulated in disease is unknown. This study tested the hypothesis that LPA induces the β6 integrin subunit (Itgb6) and Lpar2 gene expression, via an autocrine loop of TGFβ activation.

**Methods** Immortalised human bronchial epithelial cells (hHBECs) were stimulated with TGFβ1 or LPA. TGFβ1 (1D11) and αvβ6 integrin (6.3G9) blocking antibodies were used to inhibit αvβ6-mediated TGFβ activation. The transcription inhibitor actinomycin D was used for mRNA stability studies. Integrin β6 subunit (Itgb6) and LPAR2 (Lpar2) mRNA expression were assessed by real-time PCR.

**Results** Both TGFβ and LPA increased Itgb6 and Lpar2 gene expression in a time- and concentration-dependent manner. TGFβ and β6 blocking antibodies completely blocked LPA-induced Itgb6 gene expression, implicating αvβ6-mediated TGFβ activation. Conversely, TGFβ and β6 subunit blocking antibodies partially inhibited LPA-induced Lpar2 gene expression, indicating both TGFβ-dependent and -independent mechanisms. TGFβ did not enhance Itgb6 or Lpar2 mRNA stability, indicating that TGFβ increased gene transcription.

**Conclusions** LPA increases Itgb6 and Lpar2 expression via an autocrine loop of TGFβ activation. Lpar2 expression also involves TGFβ-independent mechanisms. Further dissection of this pathway may identify novel therapeutic targets in IPF.

**S71 MOLECULAR MECHANISMS OF LUNG EPITHELIAL CELL INJURY INDUCED BY INHIBITION OF GLUTATHIONE-S-TRANSFERASE**

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**Introduction** Glutathione-S-transferase (GST) polymorphisms are associated with a spectrum of acute and chronic lung pathologies, suggesting that GST expression and activity are important in lung inflammation. We have previously found that GST inhibition by ethacrynic acid (EA) caused cytotoxicity of mouse lung epithelial (MLE) cells and rendered them more susceptible to stress-related injury, such as exposure to hydrogen peroxide (H2O2). To explore potential mechanisms of such injury we investigated the influence of GST on cellular redox status, carbonyl stress and on global metabolic profiles.

**Methods** MLE cells were exposed to EA alone, or in combination with H2O2 for up to 5 h. Following cell extraction, adenine nucleotide content was measured by reversed-phase high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection, and ratios for ATP/ADP (reflecting energy status) and NAD+/ADPR (reflecting redox status) were calculated. Excess formation of carbonyl groups in proteins (‘carbonyl stress’)) was monitored...
Systemic aspects of COPD

S72 QUADRICEPS MUSCLE EXPRESSION OF MYOSTATIN IN PATIENTS WITH COPD

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Introduction and Objectives Quadriceps muscle weakness is well recognised in chronic obstructive pulmonary disease (COPD), and is associated with reduced exercise capacity, impaired health status and increased mortality. The cause of quadriceps weakness in COPD is multifactorial, but chronic daily inactivity is a likely major contributor. Myostatin is a negative regulator of muscle mass as demonstrated in naturally occurring human and animal genetic contributor. Myostatin is a negative regulator of muscle mass as expressed in naturally occurring human and animal genetic contributors. It is associated with muscle weakness in various conditions, including chronic obstructive pulmonary disease. Myostatin may play a role in the development of quadriceps muscle dysfunction in COPD.

Methods 18 patients with COPD were clinically phenotyped with 6MWD, 6 minute walk distance; SGRQ, St George’s Respiratory Questionnaire. Biopsy of the vastus lateralis muscle was performed using a Bergstrom needle. Real-time PCR for expression levels of transcripts for myostatin was performed in duplicate wells, and normalised to a housekeeping gene. The relationship between clinical variables and quadriceps myostatin expression was determined by Spearman rank correlation.

Results Baseline clinical characteristics are outlined in table 1. Quadriceps muscle myostatin mRNA expression was negatively correlated with QMVC/BMI (r = −0.50, p<0.04), T80 (r = −0.53, p<0.03), 6MWD (r = −0.62, p<0.01) and Lo (r = −0.54, p<0.04).

Conclusion We have demonstrated that EA is unlikely to compromise cellular energy state, but significantly reduces cellular redox status. Furthermore, our findings suggest that the presence of EA may enhance H2O2-induced cell injury via potentiation of protein carbonylation and by metabolic derangements. These observations highlight the importance of GST in the cellular response to oxidative stress and may help to understand the metabolic determinants of oxidative lung cell injury and adaptation.

Abstract S72 Table 1

| Age | 65 (7) |
| Gender (M/F) | 11/7 |
| FEV1, litres | 0.93 (0.40) |
| FEV1, % predicted | 34.5 (15.0) |
| BMI, kg/m² | 24.5 (4.7) |
| Fat-free mass, kg | 44.8 (10.9) |
| SGRQ | 56.8 (11.0) |
| 6MWD, m | 362 (145) |
| Locomotion time, min | 48.0 (44.8) |
| QMVC/BMI | 1.22 (0.29) |
| T80, s | 91.5 (46.6) |

F, female; FEV1, forced expiratory volume in 1 s; M, male; 6MWD, 6 minute walk distance; QMVC/BMI, quadriceps maximum voluntary contraction normalised to body mass index; SGRQ, St George’s Respiratory Questionnaire.

S73 SKELETAL MUSCLE GENE EXPRESSION IN PATIENTS WITH COPD WITH NORMAL AND LOW FFMI AND HEALTHY CONTROLS. A MICROARRAY GENE EXPRESSION ANALYSIS

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Skeletal dysfunction is one of the most important systemic effects of chronic obstructive pulmonary disease (COPD), leading to disability and poor prognosis; however, its underlying mechanisms are not fully understood. To gain insight into the molecular basis of this phenomenon we have undertaken a microarray gene expression analysis. 30 Agilent Human Whole Genome 4x44K Microarrays were hybridised following Agilent standard protocols with total RNA isolated from vastus lateralis of 20 patients with COPD (10 low (FFMI<17.5) and 10 normal (FFMI>17.5) fat-free mass index) and 10 matched healthy controls (C).

Expression measures were normalised using rma methodology from the Affy package of the Bioconductor project. Data analysis was performed using Rank Products method. Our ongoing work indicates that 542 well characterised genes were differentially expressed between COPD and C. Statistically significant gene ontology terms associate with relevant biological processes such as oxygen transport, muscle morphogenesis and contraction, inflammatory response and response to reactive oxygen species, among others.

In conclusion, our results identified a set of differentially expressed genes in both comparisons COPD vs C and FFMI<17.5 vs FFMI>17.5 that are relevant to muscle dysfunction/wasting. They also highlight the relevance of gene expression analysis in human tissue samples to identify molecular pathways involved in clinical abnormalities.

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