

S38 THE ROLE OF THE RESPIRATORY MICROBIOME IN IDIOPATHIC PULMONARY FIBROSIS

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Introduction & Objectives Idiopathic pulmonary fibrosis (IPF) is a progressive and invariably fatal disease thought to result from repeated episodes of alveolar injury in individuals with dysfunctional alveolar epithelial repair mechanisms. Although multiple environmental exposures have been suggested, there has been no systematic search for potential bacterial sources of alveolar injury. This study aimed to use culture independent techniques to characterise and confirm the presence of a respiratory microbiome in IPF.

Methods Sixty five newly diagnosed patients with IPF and twenty seven healthy controls were prospectively recruited and underwent bronchoscopy with bronchoalveolar lavage (BAL). Bacterial DNA was extracted and a variable region of the 16S ribosomal RNA gene (16S rRNA) amplified, allowing quantification of bacterial load by 16S rRNA qPCR and pyrosequencing on the Roche 454 platform. Data curation, denoising, chimera removal and analysis were executed using QIIME (<http://qiime.org/>).

Results The IPF subjects had a mean age of 67 years and on average moderately severe disease (DLco $42 \pm 11\%$ predicted; FVC $77 \pm 20\%$ predicted). IPF subjects had a significantly higher bacterial load in BAL compared to controls ($P = 0.0063$). The IPF microbiota was less diverse ($P = 0.0019$) and less rich ($P = 0.015$) compared to controls. While the microbiota of both IPF and healthy subjects were dominated by the phylum Firmicutes (51% and 53% respectively), the IPF microbiota contained significantly more Proteobacterial ($P = 0.003$) sequences and fewer Actinobacteria ($P = 0.004$). Within the IPF cohort there was also inter-individual heterogeneity with one subject's bacterial community dominated by a *Burkholderia* sp. (49% of total 16S sequences) and another with an abundance of a *Moraxella* sp. (21%).

Conclusions We present the molecular characterisation of the airway microbiota in IPF, providing evidence that the lower airways in this disease are not sterile and identifying potentially pathogenic respiratory organisms previously not associated with the disease. The higher bacterial burden and differences in the composition of the respiratory bacterial communities in IPF may provide a low level antigenic stimulus for repetitive alveolar injury and thus be involved in the pathogenesis and/or progression of the disease.

S39 HUMAN MESENCHYMAL STEM CELLS MODULATE ALVEOLAR MACROPHAGE POLARISATION *IN VIVO* AND *IN VITRO*

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Rationale ALI is characterised by dysregulated and excessive pulmonary inflammation. Macrophages are key cellular mediators of the lung innate immune response. It is recognised that macrophages can be polarised towards an M1 (implicated in driving inflammation and the development of ALI) or an M2 (responsible for the resolution of inflammation) phenotype. We have previously reported that bone marrow-derived mesenchymal stem cell (MSC) treatment is protective in several models of ALI. To effectively translate MSCs into clinical practice, a better understanding of the mechanisms mediating their effect is needed. The current studies were

undertaken to test if human MSCs have a role in polarising alveolar macrophages towards an M2 phenotype *In vivo* and *in vitro*.

Methods *In vivo* studies were preformed using a mouse model of *E.coli* pneumonia. C57BL/6 mice were administered 10^6 CFU of *E. coli* intratracheally (IT), treatment with MSC was given IT 4 h later. BAL cytokine levels were measured by ELISA. For *in vitro* studies MSC and AM were co-cultured without cell contact, using a Transwell system. Expression of cell surface CD206 (mannose receptor, widely accepted to be a marker for M2 activation) and phagocytic activity were assessed by Flow Cytometry both *In vivo* and *in vitro*.

Results In the *In vivo* model of *E.coli* pneumonia, hMSC treatment demonstrated reduction in the severity of lung injury, improved bacterial clearance and reduced TNF- levels in the BAL 24 h after infection, compare to PBS-treated animals. hMSC administration was associated with significant up-regulation of CD206 on AM (Figure 1). In addition, *in vitro* co-culture with hMSC markedly decreased LPS-induced TNF- secretion by mouse AM, significantly up-regulated their expression of CD206 and enhanced their phagocytic activity towards bacteria. Interestingly, co-culture with MSC was associated with marked up-regulation of AM expression of CD11b and CD11c, both of which (among other functions) mediate bacterial phagocytosis.

Conclusions Bone marrow-derived hMSCs have the capacity to change the phenotype and functional properties of alveolar macrophages. hMSCs drive the polarisation of AMs towards a less inflammatory state but at the same time have the unique capacity to increase the capacity of AMs to phagocytose bacteria.

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Respiratory physiology

S40 A COMPARISON OF ASTHMATIC AND NON-ASTHMATIC SEVERE SMALL AIRWAY DISEASE

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Introduction We present a series of cases referred to our severe asthma clinic that symptomatically appear like asthma, yet physiology demonstrated one of the more uncommon types of severe Small Airway Disease (SAD), including: Allergic Bronchopulmonary Aspergillosis (ABPA), Obliterative Bronchiolitis (OB), follicular bronchiolitis and Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH). These diseases represent a diagnostic challenge. We have also reviewed cases of tracheomalacia and horseshoe lung, which in our report shared similar physiology to SAD.

Methods Our findings in these SAD patients are compared with randomly identified cases of genuine severe asthma.

Results ABPA, OB, follicular bronchiolitis and DIPNECH shared similar physiology in this project and are compared together as Non-Asthmatic SAD (NASAD). When compared to asthmatic SAD (ASAD), NASAD demonstrated a lower FEF_{25-75%} (NASAD range 4–26%, median 9%, 7 out of 8 < 18% v. ASAD range 8–32%, median 26.5%, 1 out of 6 < 18%) and a higher RV (NASAD range 74–203%, median 153%, 5 out of 8 > 120% v. ASAD range 66–121%, median 97.5%, 2 out of 6 > 120%). All patients, except one, demonstrated normal gas transfers strongly suggesting an absence of emphysema. HRCT alone did not provide any diagnostic benefit. Only asthma demonstrated significant and consistent bronchodilator reversibility. Tracheomalacia