

in pancreatic endocrine function. This may be important in CF, where glucose handling is deficient even in those without established CF-related diabetes (CFRD). To look at this further, we assessed the response to a glucose challenge throughout the day in CF patients and compared it with healthy controls.

**Method** We compared 20 CF patients (17 pancreatic insufficient) without known CFRD with 6 healthy age and BMI matched controls. Following an overnight fast subjects consumed a standardised mixed meal (the gold standard measure of endogenous insulin secretion<sup>3</sup>, providing an equivalent glucose load to a standard OGTT) at 0800, 1300 and 1800 hours on the same day. Blood glucose and insulin were measured over 120 minutes for each test meal and the area under the curve (AUC) calculated for the entire duration of each test.  $\beta$ -cell indices [ $\beta$ -cell function (%B), insulin sensitivity (%S), insulin resistance (IR)] were measured using the HOMA method<sup>4</sup>.

**Results** See Table (mean $\pm$ SEM). CF subjects had greater overall glucose levels throughout the day when compared to controls for all 3 tests ( $p<0.005$ ).  $\beta$ -cell function was highest in the afternoon in the CF group in keeping with a lower AUC<sub>glucose</sub> at this time and there was a decrease in %S and an increase ( $p=0.03$ ) in IR with progression of the day.

**Conclusions** This study demonstrates insulinopenia and reduced insulin sensitivity in the CF population resulting in glucose intolerance. Although not the primary defect in CF, there is an increase in insulin resistance as the day progresses. The clinical implications of this study are important not only for the diagnosis of CFRD but also its management in terms of the timing and profiling of exogenous insulin administration.

#### References

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**Abstract S124 Table 1** Blood Glucose and markers of pancreatic beta-cell function

	Controls			CF		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening
AUC <sub>glucose</sub> (mmol/L)	500 $\pm$ 27	560 $\pm$ 9	571 $\pm$ 22	820 $\pm$ 33	697 $\pm$ 20	831 $\pm$ 35
AUC <sub>insulin</sub> ( $\mu$ U/ml)	4128 $\pm$ 626	3662 $\pm$ 548	3582 $\pm$ 634	4005 $\pm$ 573	3263 $\pm$ 482	3449 $\pm$ 555
% B	127 $\pm$ 11	136 $\pm$ 23	152 $\pm$ 17	136 $\pm$ 19	166 $\pm$ 27	149 $\pm$ 19
% S	109 $\pm$ 22	112 $\pm$ 29	71 $\pm$ 9	91 $\pm$ 8	78 $\pm$ 9	77 $\pm$ 10
IR	1.0 $\pm$ 0.2	$\pm$ 0.2	1.6 $\pm$ 0.3	1.4 $\pm$ 0.2	1.7 $\pm$ 0.3	2.1 $\pm$ 0.5

## Novel mechanisms in lung fibrogenesis

### S125 PRO-FIBROTIC EFFECTS OF MULTI-WALLED CARBON NANOTUBE EXPOSURE ON PRIMARY HUMAN ALVEOLAR TYPE II EPITHELIAL CELLS AND FIBROBLASTS

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Multi-walled carbon nanotubes (MWCNTs) are hollow fibre-like nanomaterials which are being investigated for use in drug delivery and as biosensors. However, due to their structural similarity to asbestos fibres, inhaled MWCNTs may elicit similar adverse health effects such as fibrosis and mesothelioma. Animal studies have suggested that this may be possible, however there is currently limited human data.

We hypothesised that the pro-fibrotic potential of MWCNTs would be determined by their physicochemical properties i.e length and concentration of impurities. We exposed primary human alveolar type II epithelial (ATII) cells and pulmonary fibroblasts to 30nm diameter CNTs of increasing length (0.2–2 $\mu$ m, 3–5 $\mu$ m and 10–30 $\mu$ m) and increasing purity (49%, 69% and >97%) for up to 96 hours. Oxidative stress, TGF $\beta$  release, soluble collagen release, and cell proliferation were measured in fibroblasts and release of VEGF, MCP-1, TGF $\beta$  and surfactant proteins (SP) A and D were measured in ATII cells.

MWCNTs induced oxidative stress in fibroblasts within 4h and a significant dose-dependent cell proliferation after 96h ( $P<0.05$ ) that was not affected by MWCNT length or purity. Furthermore, there was a significant, dose-dependent, 4–7-fold increase in release of collagen, exceeding what could be accounted for by proliferation alone ( $P<0.05$ ). There was a trend towards shorter and less pure CNTs inducing greater collagen release ( $P<0.1$ ). Release of VEGF and MCP-1 from ATII cells was not induced by CNTs. However, TGF $\beta$ , SP-A and SP-D were released by ATII cells and were found bound to MWCNTs. More SP-A bound to the 0.6–2 $\mu$ m MWCNTs compared to the longer MWCNTs; the converse was true for SP-D. In addition the >97% pure MWCNTs bound more surfactant protein than the lower purity MWCNTs. Significantly more TGF $\beta$  was bound to the 10–30 $\mu$ m MWCNTs compared to shorter MWCNTs.

Our results demonstrate that MWCNTs can induce pro-fibrotic responses in primary human fibroblasts. Furthermore, our unique discovery of binding of TGF $\beta$  and surfactant proteins to MWCNTs suggests that this could exacerbate the fibrotic response if MWCNTs translocate across the epithelial barrier, due to the “Trojan horse” effect of MWCNTs delivering these mediators to the interstitium.

### S126 INTERLEUKIN-1 ALPHA (IL-1 $\alpha$ ) RELEASED FROM INJURED LUNG EPITHELIUM IS A CRITICAL ALARMIN DRIVING ACTIVATION OF A POTENT INFLAMMATORY PHENOTYPE IN LUNG FIBROBLASTS

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**Background** Activation of the innate immune system plays a key role in exacerbations of chronic lung disease. Myeloid cells are classically considered to drive innate immune responses yet the potential of fibroblasts to act as immune cells has been postulated. We hypothesized that alarmins released from lung epithelium during environmental insults such as oxidant injury and viral infection might induce innate immune responses in lung fibroblasts.

**Methods** Human bronchial epithelial cells (BECs) and human lung fibroblasts (HLFs) were cultured from brushings taken from lung transplant recipients and resected lung tissue respectively. Cytokine concentrations were measured by ELISA or multiplex platform (MSD). Gene expression was assessed by qRT-PCR. Wild-type and *Il1a*<sup>-/-</sup> mice were infected with Influenza (PR8). Data were analysed using t-Student or Mann-Whitney U Test. Correlations were assessed using Spearman rank correlation coefficient.

**Results** Conditioned media from PBECs subjected to oxidant injury contained elevated levels of alarmins. Treatment of HLFs with conditioned media significantly upregulated proinflammatory cytokine expression. Anti-IL-1 $\alpha$  or IL-1Ra significantly reduced induction of IL-8 (93% and 95%), IL-6 (90% and 91%), MCP-1 (92% and 93%) and GM-CSF (95% and 94%). Anti-IL-1 $\beta$  had no effect. *Co-stimulation with Poly I:C significantly accentuated the IL-1 $\alpha$  induced inflammatory phenotype in HLFs.* Bronchoalveolar lavage (BAL) form