Results Lung tissue treated with CSE showed a dose-dependent increase in IL-8 and MMP-9 secretion across a range 0.6250%–20% CSE. IL-8 response to 20% CSE was 71592.21 pg/mg/ml ±4680.7 SE compared to non-stimulated tissue 14 177 pg/mg/ml ±1088 SE (n=6, p<0.001), MMP-9 response to 20% CSE 206 pg/mg/ml ±30.55SE vs control 104 pg/mg/ml ±4.49 SE (n=6, p<0.001). However no demonstrable rise in TNF-α secretion from tissue treatedwith CSE was detectable. With LPS stimulation both TNF-α and IL-8 responses demonstrated adose-dependent increase within the range 0.01–100 ng/ml (n=5, p=0.0003).

Treatment effects Stimulated IL-8 and MMP-9 secretion was significantly reduced in tissue treated with 0.1% vitamin C. 25% reduction in IL8 (n=3, p=0.065) and 32% reduction in MMP9 (n=3, p=0.0133). Fluticasone treatment reduced LPS induced TNF-α and IL-8 in a dose dependent manner (n=4, p=0.03).

Conclusions A human lung tissue model of smoke and LPS induced inflammation demonstrates the importance of selecting appropriate readouts for a given stimulus or treatment and hence a potential utility in selecting trial endpoints. Furthermore it demonstrates that vitamin C and corticosteroids can reduce oxidative stress and inflammation in a complex tissue system- their combined effects warrant investigation in COPD.

P117 3D CRYO-ELECTRON MICROSCOPIC ANALYSIS OF THE DISEASE MECHANISM OF α1-ANTITRYPSIN DEFICIENCY

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α_1-antitrypsin deficiency is characterised by predispositions to liver disease and severe, early-onset emphysema. It is caused by point mutations that destabilise the molecular structure of α_1-antitrypsin, leading it to self-associate into chains known as polymers. Polymerisation abolishes the antiprotease activity of α_1-antitrypsin and causes circulating deficiency of the protein. These loss-of-function effects result in dysregulated elastase activity within the lung parenchyma. In addition, polymerisation has pro-inflammatory gain-of-function effects that must be mediated through the structural characteristics of the polymer itself. It is therefore important to understand the structure of the α_1-antitrypsin polymer to identify targets for drug design. We have used cryo-electron microscopy, single particle reconstruction techniques to study α_1-antitrypsin polymers. Analysis of the 3D arrangement of α_1-antitrypsin molecules within the directly observed polymer chain allows us to evaluate current competing models of polymer assembly.

P118 THE ROLE OF THE RETINOIC ACID PATHWAY IN HUMAN LUNG REGENERATION

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Recent studies into retinoic acid (RA)-induced alveolar regeneration in rodent models of emphysema and bronchopulmonary dyplasia strongly suggest a latent regenerative potential of the adult mammalian lung. RA signalling is known to have an important role during alveolar formation in the developing lung in rodents and man. However, critical differences between animal and human physiology and development qualify the relevance of animal models of regeneration. Here we explore the role of RA signalling in human lung regeneration. Using RT-PCR with primers specific to RA signalling genes, we confirm expression of the RA synthesising enzymes Raldh1, 2 and 3, RA degrading enzymes Cyp26 A1, B1 and C1, retinoic acid receptors RAR-α, β, γ and retinoid binding proteins CRBPI and II, and CRABP I and II in normal, human peripheral lung tissue. To determine which cell types of peripheral lung are involved in RA signalling we have isolated primary alveolar type 2 epithelial cells and primary vascular endothelial cells and confirm expression of RA signalling genes. We have developed an high performance liquid chromatography (HPLC) method to identify and quantify endogenous human lung retinoids and demonstrate the ability to separate known standard retinoids by characteristic elution times. Finally, using precision cut lung slices we are developing an experimental system to determine the effects of retinoids in an architecturally complex human tissue model. Future work will include characterisation and optimisation of a primary human type 2 alveolar epithelial cell wound healing model and the effects of various selective retinoic acid receptor agonists and antagonists.

P119 CHANGES IN AIRWAY AND SYSTEMIC IL-18 LEVELS AT EXACERBATION IN COPD PATIENTS

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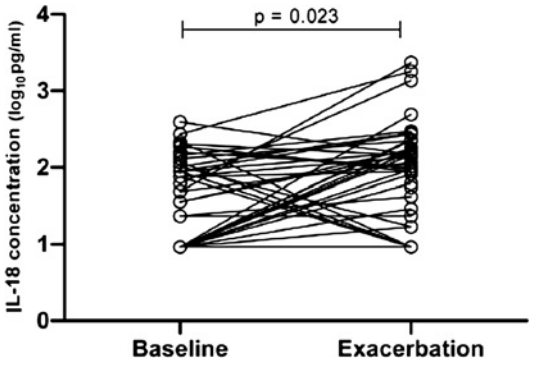
Introduction IL-18 is a proinflammatory cytokine implicated in COPD pathophysiology, causing pulmonary inflammation and emphysema in murine models. IL-18 receptor expression is increased on alveolar macrophages in COPD.[1] IL-18 levels are elevated in serum and sputum of stable COPD patients. We hypothesised that airway and systemic IL-18 concentrations increase further at exacerbation.

Methods Sputa and sera prospectively collected from the London COPD cohort were analysed using ELISA (eBioscience®, Vienna). Patients had an FEV_1=80% predicted and FEV_1/FVC ratio=0.7. Baseline was defined as at least 6 weeks after, and 2 weeks before, an exacerbation. An exacerbation was defined as an increase for two consecutive days in respiratory symptoms, with at least one major symptom (dyspnoea, sputum purulence or volume) plus another major or minor (wheeze, cold, sore throat or cough) symptom. Exacerbation frequency was calculated from daily diary cards collected over the previous 12 months. When unavailable, patient's recall of exacerbations over the preceding year was used. Frequent exacerbators had ≥2 exacerbations in the preceding year, infrequent exacerbators <2.

Results 94 COPD patients had serum analysed, of whom 48 also had sputum analysed. 60% were male, mean age was 71.6 years (SD 8.5), mean FEV_1 predicted 50.6% (18.1). Sputum IL-18 levels increased significantly from baseline to exacerbation (median 1.46 $\log_{10}$ pg/ml (IQR 0.96–2.01) vs 1.95 (0.96–2.23), n=48, p=0.023, Abstract P119 figure 1). However, serum IL-18 concentrations were not significantly greater at exacerbation compared to paired baseline levels (median 2.17 $\log_{10}$ pg/ml (1.97–2.37) vs 2.08 (1.91–2.36), n=31, p=0.299).There was no correlation between baseline serum IL-18 concentrations and exacerbation frequency (n=94, ρ=0.096, p=0.357) or FEV_1% predicted (n=93, ρ=0.081, p=0.443). No significant difference was found in baseline serum IL-18 concentrations between frequent and infrequent exacerbators (median 148 pg/ml (95–235; n=46) vs 120 (78–219; n=48), p=0.431). There was no correlation between baseline serum IL-18 concentrations and paired sputum levels (n=42, ρ=−0.149, p=0.348).

Conclusions Sputum but not serum IL-18 increases at COPD exacerbation. Treatment options for exacerbations are limited and there is a need for novel anti-inflammatories. The results of this study suggest IL-18 as a potential target for exacerbation therapy.



Abstract P119 Figure 1 Paired sputum IL-18 levels at baseline and exacerbation.

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P120 COMPARISON OF CELLULAR INFLAMMATION AND TLR EXPRESSION PROFILES BETWEEN HEALTHY AND COPD SUBJECTS

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Introduction and Objectives Chronic obstructive pulmonary disease (COPD) is a complex inflammatory disease of the lungs Initiated by inhalation of toxic particles or gases. Periodic exacerbations triggered by respiratory pathogens are a major cause of morbidity/mortality in these patients. Microbial pathogens are recognised by pattern recognition receptors such as the toll-like receptors (TLRs), initiating innate immune defences. We hypothesised that abnormal TLR expression, and not resident inflammatory cell load in the lung parenchyma, contributes to exacerbation in COPD.

Methods Human lung tissue, distant from tumour margins, was taken from ex-smoker patients undergoing lobectomy for lung cancer. Patients were classified according to GOLD guidelines as healthy control subjects (HC) or those with COPD. Resected tissue was digested and cells analysed by flow cytometry for phenotypic markers of epithelial cells and inflammatory cell subtypes (macrophages, CD8 + and CD8− T lymphocytes) and the TLR2 and four expressions on these subtypes. Quantitative data of cell numbers and TLR staining intensity were compared using Mann–Whitney U tests.

Results Seven COPD patients and nine age-matched HC were analysed. No significant differences in the numbers of inflammatory or epithelial cells in the parenchymal tissue of these groups were observed, although a trend was observed to a reduction in macrophage numbers in the COPD group (median HC=4.2, median COPD=3.2 p=0.17). Similarly, no significant difference was found in the level ofTLR2 or TLR4 expression on any of the cell types examined. However, a trend was observed towards a decrease in TLR2 expression on epithelial cells in the COPD patients (median sMFI 3399 (HC) vs 2462 (COPD), p=0.094).

Conclusions This preliminary analysis has demonstrated that, as hypothesised, there was no significant difference in inflammatory cell load in parenchymal tissue between the two groups. The trend towards a reduced expression of TLR2 in the epithelial cells may reflect an abnormal down regulation of this receptor due to constant exposure to bacterial pathogens. The lack of surveillance of microbial pathogens by TLRs is a potential mechanism by which patients with COPD are more susceptible to infection by new bacterial strains and thus could contribute to exacerbation frequency.

P121 DIFFERENTIAL RESPONSES OF M1 AND M2 MONOCYTE-DERIVED MACROPHAGE PHENOTYPES IN COPD

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Introduction Inflammation in chronic obstructive pulmonary disease (COPD) is associated with increased numbers of highly activated macrophages with a reduced phagocytic capacity. Macrophages may exist as M1 "classically activated" or M2 "alternatively activated" with different phagocytic and inflammatory mediator profiles, suggesting in COPD a more persistent, M1 macrophage predominates. It is unknown whether circulating monocytes in COPD patients predetermine whether M1 macrophages will be preferentially activated, thus driving an inflammatory phenotype.

Objectives This study investigated differences between monocyte-derived macrophages (MDM) from non-smokers, smokers and COPD patients driven towards M1and M2 phenotypes.

Methods Monocytes were isolated from whole blood and cultured with GM-CSF (2 ng/ml) or M-CSF (100 ng/ml) for 12d to generate M1 and M2 MDM respectively. Cells were stimulated with LPS (0.01–100 ng/ml) for 24 h and TNFα, CXCL8 and IL-10 measured by ELISA. Phagocytosis was measured fluorimetrically following exposure to fluorescent beads, *H influenzae* or *S pneumoniae* for 4 h.

Results There were no differences in baseline release of any of the cytokines measured between subject groups. Cells released cytokines in response to LPS in a concentration-dependent manner. M1MDM derived from non-smokers and COPD patients released greater concentrations of LPS-stimulated (10 ng/ml) TNFα compared to M2 MDM. (Non-smokers: 7.4±2.3 vs 1.5±0.2 ng/ml, n=4; p<0.01; COPD: 7.0±1.8 vs 2.1±0.9 ng/ml, n=4) and significantly less IL-10 (Non-smokers: 0.4±0.2 vs 3.0±0.6 ng/ml, n=4; p<0.05; COPD: 0.3±0.04 vs 1.5±0.5 ng/ml, n=3) than M2 MDM. These differences were not apparent in cells from smokers. Both M1 and M2 MDM released LPS-stimulated CXCL8 similarly with no difference between subject groups. Phagocytosis of polystyrene beads was similar by both MDM phenotypes in all subject groups. However, there was a trend for M2 MDM to phagocytose more bacteria compared with M1 MDM which reached significance in healthy subjects (p<0.05).

Conclusions M1 and M2 MDM from non-smokers and COPD subjects showed distinct differences with respect to LPS-stimulated cytokine release and phagocytosis, however these differences were not apparent in cells from smokers without COPD. This suggests that smokers without COPD have altered circulating monocytes that do not differentiate into the pro-inflammatory M1 macrophage and may be protective against the development of COPD.

P122 NO ABSTRACT ASSIGNED TO THIS NUMBER

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Challenges in smoking cessation

P123 A RETROSPECTIVE COHORT STUDY OF THE LONG TERM EFFECTIVENESS OF SMOKING CESSATION COUNSELLING

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Introduction and Objectives A regional smoking cessation counselling service provides one-to-one counselling with follow-up by telephone and appointments for up to 1 year. Previously, no long-term