potentially contributing to the observed differential protein release.

Conclusions Hypoxia augments NE release in a PI3K-dependent manner, further increased during COPD exacerbations, and hypoxic neutrophil supernatants injure endothelial cells *in vitro*. Unbiased characterisation of hypoxic neutrophil secretomes identified several upregulated proteins which may contribute to cellular/tissue damage. In addition to degranulation, NDMP release may underpin differential protein secretion under hypoxia. Hypoxia engenders a neutrophil phenotype with potential to cause local and distant tissue damage in COPD; novel targets in the hypoxic neutrophil secretome may identify new therapeutic opportunities.

S115 MECHANISMS TO REVERSE IMPAIRED MACROPHAGE EFFEROCYTOSIS IN COPD

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COPD patients have defective innate immunity, characterised in part by macrophage dysfunction. In established disease, both monocyte derived macrophages (MDM) and alveolar macrophages (AM), have impaired phagocytosis of bacteria and apoptotic cells (efferocytosis). Failure to adequately efferocytose dying cells leads to further release of inflammatory mediators and ongoing recruitment of immune cells, culminating in inflammation that is both damaging and ineffective. The transcription factor, Nrf2 is the master regulator of the antioxidant-response-element. Previous work using the non specific Nrf2 agonist, Sulforaphane, has partially restored defective bacterial phagocytosis in COPD AMs, however the specificity and mechanism of action in this context remains unknown. The role of Nrf2 activation in restoring macrophage efferocytosis in COPD has yet to be examined. We questioned if impaired macrophage phagocytosis and efferocytosis in COPD share a common mechanism and consequently if the defect in macrophage efferocytosis can be therapeutically

manipulated using highly selective Nrf2 agonists. AMs and MDMs were isolated from patients with established COPD (GOLD stage 1–3). Macrophage efferocytosis of apoptotic neutrophils from each donor was correlated with bacterial internalisation of *Streptococcus pneumoniae*. Efferocytosis assays were carried out in the presence or absence of Sulforaphane and Compound 7, a highly specific Nrf2 agonist (supplied by GSK). Both COPD MDMs and AMs have significantly impaired phagocytosis of bacteria and apoptotic cells compared to Healthy Controls (p values all <0.05). Moreover, there was a correlation between donor macrophage phagocytosis of apoptotic cells and of bacteria (r=0.71). *In vitro* studies using Sulforaphane enhanced efferocytosis of apoptotic cells in both COPD MDMs and AMs (p<0.01). This effect was replicated using Compound 7 in both MDMs (p<0.05) and AMs. This was more pronounced in current smokers. In summary, we observe a correlation between impaired macrophage phagocytosis and efferocytosis in COPD, suggesting a common mechanism. Furthermore, we describe partial rescue of defective COPD MDM and AM efferocytosis by Sulforaphane and via specific activation of the Nrf2 pathway using Compound 7. Together, these data highlight the importance of the Nrf2 pathway in reversing macrophage dysfunction in COPD patients and provide key mechanistic insights into the underlying defect.

S116 CELL-DISSOCIATED HAEMOPHILUS INFLUENZAE AND BACTERIA-ASSOCIATED INFLAMMATORY MEDIATORS IN THE AIRWAYS OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background Patients with COPD have a susceptibility to respiratory tract infections associated with increased pulmonary inflammation. Bacteria can reside within the host as cell-associated (attached to host cells via adhesins, pili or biofilm formation) or cell-dissociated bacteria. It is unclear how bacteria-to-cell interactions affect pulmonary inflammation and whether

Abstract S116 Figure 1 Levels of *H. influenzae* in the sputum plug (A) and in the sputum supernatant (B) during an exacerbation time course in 10 paired subjects with COPD.

these levels differ over an exacerbation time course. We sought to investigate the effects of *Haemophilus influenzae* cell-interaction upon airway inflammation and whether the levels of *H. influenzae* bacteria and cell-dissociated bacteria differ over an exacerbation time course.

Methods Cell differential counts were carried out on sputum samples as per standard protocol. Bacterial DNA was extracted and *H. influenzae* was quantified using qPCR from the sputum plug (contains cell-associated and dissociated bacteria) and the sputum cell-free supernatant (cell-dissociated bacteria only). Inflammatory mediators (IL-1 α , TNF- α , IL-8 and neutrophil elastase (NE)) were measured in the sputum supernatant using commercial assays.

Results 63 patients (77% male; average age of 69 (45–88); FEV₁ percentage predicted of 53%; mean percentage neutrophil count in sputum of 65%) at stable state were analysed. Levels of *H. influenzae* in the supernatant only correlated with the sputum total cell count ($r=0.38$; $p=0.03$). Levels of *H. influenzae* in the plug correlated with inflammatory mediators (sputum neutrophil percentage $r=0.42$, $p=0.01$; sputum macrophage percentage $r=-0.35$, $p=0.04$; IL-1 α $r=0.36$, $p=0.03$; IL-8 $r=0.49$, $p<0.01$; NE $r=0.40$, $p=0.02$). The exacerbation time course in 10 paired COPD subjects was examined. There was no significant difference in *H. influenzae* levels in the plug ($p=0.89$) (figure 1A). However, there was a significant increase in levels in the supernatant over the exacerbation time course ($p=0.05$) (figure 1B).

Conclusion *H. influenzae* levels in the sputum plug appear to have much more of an effect on airway inflammation than levels of cell-dissociated *H. influenzae* suggesting that cell-associated bacteria may be a driver of airway inflammation in COPD. Further investigation into this highly complicated relationship needs to be conducted.

S117 THE EFFECT OF CIGARETTE AND ELECTRONIC CIGARETTE VAPOUR ON BACTERIA IN CHRONIC LUNG INFECTION

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Introduction Cigarette smoke is a risk factor for the development of several chronic lung diseases, e.g., Chronic Obstructive Respiratory Disease (COPD) and bronchiectasis. This study aims to determine the effect of cigarette smoke and electronic cigarette vapour, on key bacteria involved in the progression and exacerbation of chronic lung disease.

Methods Cigarette smoke extract (CSE) and electronic cigarette vapour extract (ECVE) were prepared using a modified syringe driver apparatus and bubbling through appropriate growth media. Reference isolates [*Haemophilus influenzae*, ATCC49766 (HI), *Streptococcus pneumoniae* ATCC49619 (SP), *Staphylococcus aureus* ATCC29213 (SA) and *Pseudomonas aeruginosa* ATCC 27853 (PA)] were grown in broth +/- CSE or ECVE. Biofilm formation and survival in the *Galleria mellonella* infection model, following bacterial exposure to CSE/ECVE were determined. Levels of IL-8 and TNF α secretion from A549 cells, following infection by bacteria +/- CSE/ECVE and addition of cell-signalling inhibitors, were measured by ELISA.

Results A trend towards increased biofilm formation following exposure to either CSE or ECVE was observed, which reached statistical significance with SP and PA +CSE ($p=0.0047$ and 0.0043 , respectively) and SA+ECVE ($p<0.001$). There was decreased survival of *Galleria mellonella* following infection with bacteria+CSE/ECVE vs. bacteria only, suggestive of increased virulence; this was statistically significant in the case of HI ($p=0.016$) and PA ($p=0.0005$) +CSE. Statistically significant increases in IL-8 secretion in A549 cells were observed for HI and PA +CSE and SA +ECVE, and in TNF α , with SP and SA+ECVE. However, a clear trend towards increases in both cytokines following CSE/ECVE exposure was evident, with little difference observed between CSE/ECVE. Addition of pathway inhibitors following A549 cell infection with bacteria+CSE/ECVE resulted in a decrease in IL-8 or TNF α via the same pathways as with bacteria alone.

Conclusion Exposure of bacteria involved in the pathogenesis of chronic lung infection to both CSE and ECVE resulted in increased virulence, biofilm formation, and inflammation, and may contribute to establishment of chronic infection and persistence. Further work is required to determine the clinical effects of ECVE.

Of mice and men

S118 ELK1 GENE DELETION LEADS TO SPONTANEOUS EARLY FIBROTIC CHANGES IN THE AGEING LUNG

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Rationale Idiopathic pulmonary fibrosis is a chronic fibroproliferative disease with a median survival of approximately 3 years. α v β 6 integrins are upregulated in lung fibrosis and are associated with increased activation of the profibrotic cytokine TGF- β . The transcription factor Elk1 can repress gene expression of β 6. We therefore hypothesise that animals lacking functional Elk1 (*Elk1*^{-/-}) will develop age related pulmonary fibrosis.

Methods Elk1 knock-out (*Elk1*^{-/-}) and wild-type (*Elk1*^{+/+}) mice were allowed to age for 365 days. At 365 days old mice were sacrificed and their lungs harvested for evaluation of collagen gene expression, lung hydroxyproline concentration and histological assessment.

Results Lungs were extracted and lung wet weights were measured in *Elk1*^{-/-} mice and wild-type (*Elk1*^{+/+}) controls and no significant difference between the two genotypes was shown (175.7 mg, 161.8 mg respectively $n=6-8$). However, assessment of total lung hydroxyproline established that there was significantly more hydroxyproline in *Elk1*^{-/-} mice compared with *Elk1*^{+/+} controls (852.3, 758.4 μ g/lung set, respectively, $n=5-8$, $p=0.0346$). Assessment of Masson's trichrome stained *Elk1*^{-/-} lung tissue sections found a small number of fibrotic lesions were present. Furthermore, there was a trend towards increased alveolar wall median thickness in *Elk1*^{-/-} mice compared with *Elk1*^{+/+} animals (5.94 vs 5.56 μ m respectively). In a small number of 12 week old mice we identified a trend towards increased α -smooth muscle actin (α SMA) mRNA expression in the lungs of *Elk1*^{-/-} mice compared with