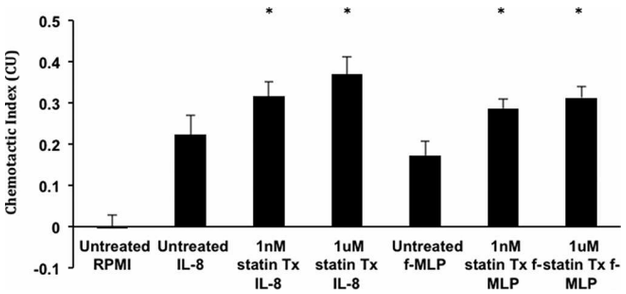

Abstract S114 Figure1 Simvastatin improves neutrophil migration in COPD. Legend. Isolated neutrophils from COPD patients (n = 13) migrated towards IL8 (100nM) or fMLP (100nM) following incubation with carrier control or Simvastatin (1nM or 1uM). Measurements were taken from 10 randomly selected cells from each individual. The average results for each subject were calculated, and an overall average was used for comparisons across groups using analysis of variance. Bars represent the mean migratory parameter with standard deviation shown as the error line. * = significant difference in migratory parameter from carrier control data across groups ($p < 0.05$).

have shown neutrophil migratory accuracy to be reduced in COPD. This is thought to contribute to the destruction of lung parenchyma and the poor responses seen in infective exacerbations. We aimed to characterise neutrophil migration in COPD and assess whether physiologically relevant concentrations of simvastatin altered neutrophil migration.

Methods Neutrophils were isolated from COPD patients and healthy smoking age-matched controls (age > 60yrs, n = 13 per group) and incubated with 1nM - 1μM Simvastatin or with a carrier control before migratory dynamics were assessed towards IL8 and fMLP using time-lapse photography. Data is expressed as means with standard deviation in parentheses.

Results COPD neutrophils displayed reduced chemotaxis (directional speed of migration) and reduced chemotactic accuracy (Chemotactic Index - a vector analysis of migratory tracks) compared to cells from healthy age-matched controls (HC) in the presence of IL-8 and f-MLP, replicating previous work. For example, Chemotactic Index: IL8; HC, 0.42CU (0.03), COPD 0.22CU (0.05), p = 0.002: fMLP; HC, 0.34CU (0.05), COPD, 0.18CU (0.03) p = 0.014).

Treatment with Simvastatin significantly improved the chemotactic ability of COPD neutrophils in a dose response with greatest improvement seen at the highest concentration (e.g. Chemotaxis to IL8, Carrier control 0.8um/min (0.2), 1nM Simvastatin 1.3um/min (0.2), p = 0.04; 1uM Simvastatin 1.4um/min (0.2), p = 0.004). A similar improvement was seen in Chemotactic Accuracy (e.g. Chemotactic Index to fMLP, Carrier control 0.17CU (0.03), 1nM Simvastatin 0.26CU (0.02), p = 0.018; 1uM Simvastatin 0.31CU (0.03), p = 0.002).

Conclusions Migratory accuracy of circulating neutrophils is reduced in COPD patients compared with healthy, smoking, age-matched controls but can be restored by treatment with therapeutic concentrations of Simvastatin *in vitro*. Our data suggest statin therapy might be an adjuvant intervention in COPD, modulating neutrophil responses.

S115 THE EFFECTS OF HYPOXIA ON NEUTROPHIL-MEDIATED TISSUE DAMAGE IN THE LUNG

[1]K Hoenderdos, [2]RA Hirst, [1]L Porter, [1]C Chen, [1]K Lodge, [3]C O'Callaghan, [1]ER Chilvers, [1]AM Condliffe; *[1]University of Cambride, Cambridge, Cambridgeshire; [2]University of Leicester, Leicester, Leicestershire; [3]University College London, London, London*

10.1136/thoraxjnl-2013-204457.122

Sites of infection and inflammation are profoundly hypoxic, requiring neutrophils to function under low oxygen tensions. Although neutrophils are well adapted and can rely on glycolytic metabolism, hypoxia still impairs the neutrophil oxidative burst, reduces bacterial killing and delays apoptosis.[1] As neutrophil proteases have been implicated in lung diseases such as COPD, we hypothesised that hypoxia might also promote neutrophil degranulation, with an enhanced potential for neutrophil-mediated tissue injury.

Neutrophils isolated from healthy volunteers were subjected to normoxia (20 kPa) or hypoxia (3 kPa) and subsequently activated with GM-CSF (10 ng/ml) and the bacterial tri-peptide fMLP (100 nM). A549 cells and ciliated human primary bronchial epithelial (NHBE) cells were exposed to neutrophil supernatants, the extent of cellular damage was determined by MTT assay (A549 cells), EM ultrastructure and cleaved caspase 3 staining. Ciliary function was also investigated using video microscopy in the ciliated NHBE cells.

Hypoxic incubation for 4 hours resulted in a 3–5 fold increase in neutrophil degranulation; this was evident for active elastase, MPO, MMP-9 and lactoferrin and hence occurred from all granule sub-types. Supernatants from hypoxic neutrophils induced 50% more cell death in A549 cells compared to supernatants from normoxic neutrophils. NHBE cells exposed to supernatants from hypoxic versus normoxic neutrophils suffered more cellular damage (EM; images were scored for cytoplasmic blebbing, vacuole formation and dead cells), an increase in LDH activity (from 35.7 ± 6 to 50.2 ± 0.7 nmol/min/ml, was indicative of cell death), increased cleaved caspase 3 staining was shown to be an indicator of apoptosis and there was a substantial increase in the proportion of dyskinetic and immotile cilia.

In conclusion; hypoxia induced a destructive neutrophil phenotype with delayed apoptosis, impaired bacterial killing and increased release of histo-cytotoxic proteases. This phenotype may be important for understanding the mechanisms of chronic inflammatory diseases like COPD.

Funded by the British Lung Foundation and NIHR Cambridge BRC.

REFERENCES

1. McGovern, N. N. *et al*. Hypoxia selectively inhibits respiratory burst activity and killing of Staphylococcus aureus in human neutrophils. *J. Immunol. Baltim. Md 1950* 186, 453–463 (2011).

Physiological measurement of breathlessness and breathing

S116 BREATHLESSNESS IN COPD IS ASSOCIATED WITH ALTERED COGNITIVE PROCESSING IN THE MEDIAL PREFRONTAL CORTEX

M Herigstad, A Hayen, E Evans, R Davies, M Hardinge, K Wiech, KT Pattinson; *University of Oxford, Oxford, UK*

10.1136/thoraxjnl-2013-204457.123

Introduction Breathlessness is the main cause of suffering in COPD. Its brain mechanisms remain poorly understood, yet may represent a novel therapeutic avenue. Until now, functional magnetic resonance imaging (FMRI) studies of breathlessness have been limited to experiments in healthy volunteers. FMRI demonstrates that imagination of painful events engages the same brain networks responsible for perception of physical pain. We