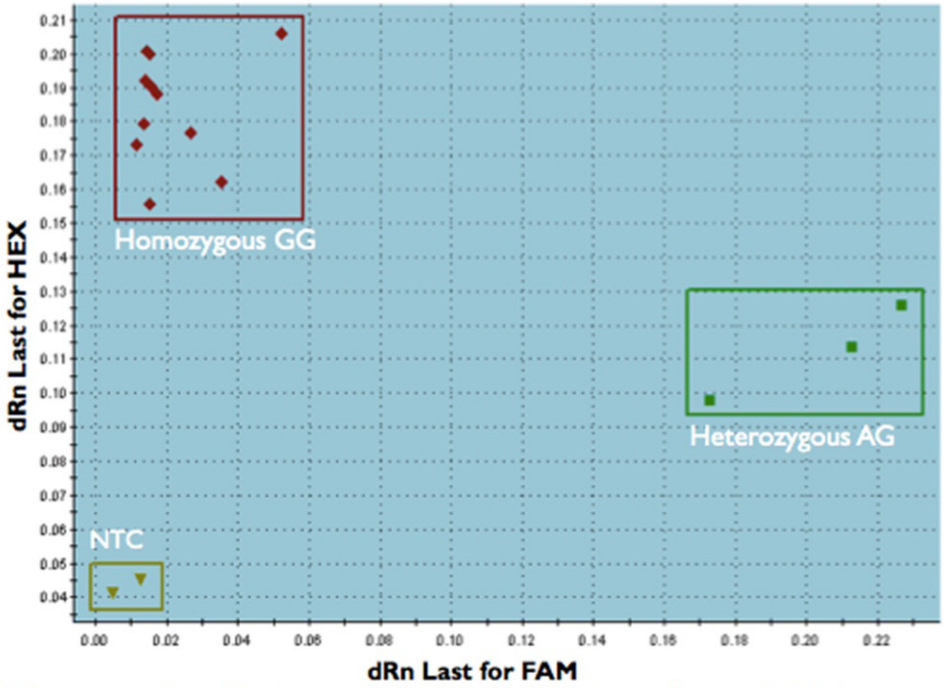

FIGURE 1: A sample TaqMan® assay of 16 samples (including 2 negative controls, NTC). Fluorescence of the different allele-specific fluorophores used (HEX and FAM) allows differentiation between genotypes GG, GA, AA.

Abstract P249 Figure 1 A sample TaqMan® assay of 16 samples (including 2 negative controls, NTC). Fluorescence of the different allele-specific fluorophores used (HEX and FAM) allows differentiation between genotypes GG, GA, AA.

P250 ASSOCIATION BETWEEN PGRN AND AIRWAY INFLAMMATION IN COPD

doi:10.1136/thoraxjnl-2012-202678.311

[1]MJ Ungurs, [1]NJ Sinden, [2]RA Stockley. *[1]University of Birmingham, Birmingham, UK; [2]Queen Elizabeth Hospital, Birmingham, UK*

Background Progranulin (PGRN) is an anti-inflammatory protein, which is converted into pro-inflammatory granulin peptides (GRNs) by neutrophil elastase (NE) and proteinase-3 (PR3) (1). Neutrophilic inflammation is implicated in the pathophysiology of COPD, therefore the influence of PGRN on mechanisms of neutrophilic inflammation may be of great relevance to understanding and treating inflammation in the disease.

Methods Sputum and serum samples were obtained from COPD patients with chronic bronchitis at exacerbation and in the subsequent clinically stable state. Sputum samples were graded by purulence and cultured for quantitative microbiology. PGRN was measured in sputum sol phase together with leukotriene B4 (LTB4), interleukin 8 (IL-8), myeloperoxidase (MPO), tumour necrosis factor α (TNFα), NE and PR3. PGRN and C-reactive protein (CRP) were measured in the serum.

Results PGRN was lower in purulent sputum (median=38.91 ng/ml (IQR=1.55–89.71 ng/ml)) than mucoid (median=79.78 ng/ml (IQR=51.57–111.24 ng/ml)) (p=0.024, n=69). In purulent sputum PGRN correlated negatively with bacterial load and markers of inflammation (Table 1). Serum PGRN did not correlate with CRP, but was higher in stable COPD patients (median=65.71 ng/ml (IQR=0–86.46 ng/ml)) than healthy controls (median=38.55 ng/ml (IQR=36.11–44.82 ng/ml)) (p=<0.001, n=20), and increased further during purulent exacerbations (median=75.43 ng/ml (IQR=64.83–85.28 ng/ml)) (p=0.010, n=25).

Conclusion The concentration of PGRN in the lung is associated with the increased inflammation seen with bacterial infection and exacerbation as assessed by markers of inflammation. The conversion of PGRN to GRNs may provide a mechanism by which neutrophil proteases regulate inflammation in COPD. Elevated circulating PGRN may reflect systemic inflammation associated with COPD.

1. Kessenbrock K, et al. J Clin Invest. 2008 Jul; 118(7):2438–47.

Abstract P250 Table 1 Correlations and their significance between PGRN and markers of inflammation measured in sputum sol phase

	Correlations	n
IL-8 (nM)	r=–0.512, p=<0.001	44
MPO (units/ml)	r=–0.543, p=<0.001	36
LTB4 (nM)	r=–0.438, p=0.003	44
TNF (pM)	r=–0.647, p=0.032	11
NE (nM)	r=–0.614, p=0.002	22
PR3 (nM)	r=–0.737, p=<0.001	22
Bacterial load (CFU/ml)	r=–0.418, p=0.005	44

P251 THE ROLE OF REACTIVE OXIDATIVE SPECIES WITHIN CIGARETTE SMOKE EXTRACT ON APOPTOSIS AND INFLAMMATION IN PRIMARY NASAL EPITHELIAL CELLS

doi:10.1136/thoraxjnl-2012-202678.343

D Comer, JS Elborn, M Ennis. *Centre for Infection and Immunity, Belfast, Northern Ireland*

Introduction The responses of the bronchial epithelium to cigarette smoke (CS) are well characterized, but effects on the nasal epithelium, also important in respiratory disease, are not as well defined. The mechanism of cell death due to cigarette smoke extract (CSE) exposure is controversial. As there is convincing evidence that cigarette smoke decreases levels of protective antioxidants, we hypothesised that reactive oxidative species contained within CSE contribute to its immunomodulatory and cytotoxic properties.

Methods Nasal brushings were obtained from 16 healthy volunteers from the medial aspect of the inferior turbinate as previously

described. CSE was prepared by combusting 1 Marlboro cigarette through 25 ml of media. Cell viability was determined after primary nasal epithelial cells (PNECs) were stimulated with 5% CSE for 24 h (caspase 3 levels determined after 4 h), in the presence or absence of 20 mM N-acetylcysteine (NAC). In separate experiments, cultures were stimulated with *Pseudomonas aeruginosa* lipopolysaccharide (PA LPS) for 24 h (0 – 30 μg/ml), and the effects of pre-incubation with CSE±20 mM NAC for 4 h evaluated in terms of cytokine release. Phospho-NF-κB activity was determined after 1 h PA LPS exposure. Apoptosis was evaluated using annexin-V staining and the terminal transferase-mediated dUTP nick end-labelling (TUNEL) method.

Results 5% CSE (4 h) exposure was immunosuppressive in PNEC cultures for both IL-8 and IL-6 release (0.53 fold reduction in IL-8 and 0.49 fold reduction in IL-6 release after stimulation with 30 μg/ml PA LPS for 24 h). 4 h exposure to CSE heightened active caspase 3 levels, and a 24 h exposure induced both early and late apoptosis established by annexin-V staining (Table 1). Apoptosis was confirmed using the TUNEL assay. All of these effects were mitigated with the addition of 20 mM NAC to the CSE (0.85 fold reduction in IL-8 and 0.73 fold reduction in IL-6 release after stimulation with 30μg/ml PA LPS for 24 h).

Conclusions A 4 h CSE exposure was immunosuppressive in PNEC cultures and induced apoptosis. Reactive oxidative species are at least partially responsible for these observations.

Research funded by NI RDO.

Abstract P251 Table 1 Annexin-V analysis of 5% CSE±20 mM NAC treatment in PNEC Cultures.

	Viable	Early Apoptotic	Late Apoptotic	Necrotic
Control 24 h	99.4%	0.4%	0.1%	0.1%
5% CSE 24 h	29.1%	47.7%	21.2%	2.0%
NAC & 5% CSE 24 h	74.0%	18.3%	4.8%	2.9%

P252 PD-1 EXPRESSION ON HUMAN LUNG T CELLS IN HEALTH AND COPD

doi:10.1136/thoraxjnl-2012-202678.344

RT McKendry, CM Spalluto, KJ Staples, TMA Wilkinson. *The University of Southampton, Southampton, United Kingdom*

Introduction and Objectives Patient s with chronic obstructive pulmonary disease (COPD) are susceptible to the effects of recurrent respiratory infections despite increased numbers of CD8+ T cells in the lungs. We hypothesised that the inability of CD8+ T cells to successfully combat respiratory pathogens in COPD may be due to T cell "exhaustion" - a phenomenon described in chronic infections. Exhausted CD8+ T cells have significantly reduced cytotoxicity and inflammatory cytokine release. Exhaustion is thought to be initiated by the binding of PD-1 on T cells to its ligand (PD-L1) which is expressed on epithelial cells and macrophages. PD-1 expression is upregulated in murine models of acute and chronic viral infection, but this has yet to be elucidated in human cells.

We aimed to identify and quantify PD-1+ CD4+ and CD8+ T cells and cells expressing PD-L1 in the lungs of COPD patients and non-COPD controls.

Methods Lung tissue from patients undergoing surgery was digested using collagenase to form single-cell suspensions. Lung T cells were identified as populations of CD45+CD3+ cells which were either CD4+CD8- or CD4-CD8+. T cells expressing PD-1 were quantified by multi-colour flow cytometry. Patients with a FEV1/FVC ratio <70% were defined as having COPD.

Results The proportion of CD8+ T cells in the COPD lung (mean expression=40.87%, SD=14.67) was significantly higher (p=0.013, students t-test) than in non-COPD (mean expression=26.74%, SD=11.12), reflecting previous findings. PD-1 expression in CD4+ T cells appeared to be lower in COPD (mean expression=39.91%, SD=13.02) than non-COPD (mean expression=50.53%, sd=13.05) but this was not significant. PD-1 expressing CD4+ cells (mean expression=2.17%, SD=1.4) and CD8+ cells (mean expression=6.02%, sd=5.73) were detected in tissue, but not in the blood of the same patients. PD-L1 was undetectable on lung epithelial cells but was expressed on macrophages (mean expression = 2.85%, SD=1.91).

Conclusion Elements of the exhaustion pathway are expressed in the human lung in stable COPD. Further work is needed to clarify if there is an upregulation of this pathway in COPD that may explain the susceptibility of these patients to viral exacerbation. Exhaustion of cells recognising respiratory pathogens may have a significant role in COPD outcomes and requires further elucidation.

P253 ASSOCIATION OF DEFECTIVE MONOCYTE-DERIVED MACROPHAGE PHAGOCYTOSIS WITH CLINICAL PHENOTYPES IN STABLE COPD

doi:10.1136/thoraxjnl-2012-202678.345

[1]R Singh, [2]KK Chana, [3]LL Wright, [1]DS Garcha, [1]BS Kowlessar, [1]ARC Patel, [1]AJ Mackay, [2]PS Fenwick, [1]GC Donaldson, [2]PJ Barnes, [1]JA Wedzicha, [2] LE Donnelly. *[1]Centre for Respiratory Medicine, UCL, London, UK; [2]Airway Disease Section, NHLI, Imperial College London, London, UK; [3]Centre for Clinical Microbiology, UCL, London, UK*

Introduction Macrophages play an important role in clearing inhaled particles and bacteria from the lung, thus maintaining its sterility. Defective phagocytosis of bacteria has been demonstrated in both alveolar and monocyte-derived macrophages (MDMs) from COPD patients and may play a role in the aetiology of the frequent exacerbator phenotype. We hypothesised that defective phagocytosis may also be associated with lower airway bacterial colonisation (LABC) and other clinical parameters in stable COPD.

Methods Whole blood and sputa were collected from stable patients in the London COPD cohort. Stable COPD was defined as no symptom-defined exacerbations recorded on prospectively completed diary cards in the preceding four weeks and subsequent two weeks. Monocytes were isolated from the whole blood and cultured with GM-CSF (2 ng/ml) for 12 days to generate MDMs. MDM phagocytosis of fluorescently-labelled polystyrene beads, *Haemophilus influenzae* (HI) and *Streptococcus pneumoniae* (SP) was measured by fluorimetry. LABC was defined as detection of HI, SP or *Moraxella catarrhalis* (MC) in sputum using quantitative PCR.

Results MDMs were cultured from 26 COPD patients. 54% were male, mean age 70.0 years (SD 8.3), FEV_1 predicted 55.3% (20.3), 46% were current smokers, median daily inhaled corticosteroid (ICS) dose was 1000 (640–2000) mcg (beclomethasone equivalent dose) and median exacerbation frequency per year was 1.8 (1.0–2.9) based on diary card events.

Phagocytosis of HI was significantly less with increasing exacerbation frequency (p=0.002, r=–0.58, Figure 1), although no significant associations were demonstrated between exacerbation frequency and phagocytosis of inert beads or SP (p=0.27 and p=0.22 respectively). 13 patients (50%) with LABC did not demonstrate any significant difference in phagocytosis of either beads (p=0.29), HI (p=0.66) or SP (p=0.88) compared with non-colonised patients. There was no significant association between phagocytosis of beads, HI or SP with age, FEV_1%predicted, smoking pack year history, ICS dose or BMI (all p>0.05).

Conclusion In stable COPD patients, decreasing phagocytosis of HI was associated with increasing exacerbation frequency. Phagocytosis was not related to LABC suggesting that macrophage activity alone may not determine bacteria colonisation. Further work is needed to elucidate the mechanisms of reduced phagocytosis in COPD and its relationship to exacerbation frequency.