Aims

1. To compare neutrophil surface expression of PR3 and NE in patients with A1ATD, usual COPD and healthy controls.
2. To determine the influence of the local concentration of A1AT on neutrophil surface expression of PR3 and NE.

Methods Clinically stable patients with A1ATD (n=9), COPD (n=6) and healthy controls (n=9) were recruited. Neutrophils were isolated from blood. Half were stimulated with fMLP and half were unstimulated. Membrane expression of NE and PR3 was measured by flow cytometry.

Neutrophils isolated from six further healthy controls were stimulated in the presence of either normal (PiMM) or A1ATD plasma (PiZZ). Membrane expression of NE and PR3 was measured.

Results PR3 expression on the surface of unstimulated neutrophils was greater in A1ATD patients (2365±305MFI) compared to healthy controls (1517±253MFI; p=0.048) and COPD patients (1360±315MFI; p=0.046). NE expression was similar between groups.

PR3 expression on stimulated neutrophils was greater in A1ATD patients (5112±547MFI) compared to healthy controls (3411±541MFI; p=0.042), but not different to COPD patients (4723±1509MFI; p=0.78). NE expression was similar between groups.

When neutrophils from healthy controls were stimulated in the presence of plasma, the surface expression of PR3 (but not NE) was greater (p=0.031) in the presence of PiZZ plasma (1921MFI) compared to PiMM plasma (1352MFI), but less than that observed without plasma.

Conclusions Baseline neutrophil surface expression of PR3 is greater in A1ATD patients compared to healthy controls. Neutrophils express more PR3 when stimulated in an environment with low concentrations of A1AT, suggesting that membrane binding is dependent on the ability of A1AT to bind released PR3 but not NE.

This may have clinical significance for A1ATD emphysema since active membrane-bound PR3 is resistant to inhibitors and can replicate the pathological features associated classically with NE.

These findings may explain the association of Wegener's granulomatosis (where PR3 is an autoantigen) with A1ATD.

S86 FORMATION OF OXIDISED ALPHA-1 ANTITRYPSIN INDUCES INFLAMMATORY RESPONSE IN HUMAN BRONCHIAL EPITHELIAL CELLS

doi:10.1136/thoraxjnl-2012-202678.092

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Alpha-1 antitrypsin (AT) is a major anti-elastase and protects the lung from uncontrolled elastolysis. AT is highly susceptible to oxidation in vivo. We investigated the role of Ox-AT in the inflammatory response.

Lung epithelial (A549 and NHBE) cells were exposed to 25% CSE. Ox-AT, TNF-α, IL-6, IL-8, MCP-1, NF-kB and AP-1 were assessed by ELISA or RT-PCR. Anti-Ox-AT mAb (3F4, 10μg/ml) and N-acetylcysteine (NAC, 10^{-3}M) were used to probe the effect of Ox-AT.

CSE (compared to PBS) significantly induced TNF-α (440.4±76.8pg/ml vs. 17.5±2.6, p<0.001) at 24h and induced IL-6 at 72h (354.7±38.4pg/ml vs. 17.6±2, p<0.001). CSE induced TNF-α mRNA at 0.5h (p<0.001) and IL-6 mRNA at 3h (p<0.001). CSE (compared to PBS) activated NF-κB (OD at 405nm, 1.325 vs. 0.315, p<0.001) and AP-1 (OD at 405nm, 0.988 vs. 0.296, p<0.001) at 0.5h. At 24h CSE (compared to PBS) resulted in significant level of Ox-AT (mean±SEM, 1372.8±162.8ng/ml vs. undetectable, p<0.001) and induced IL-8 (1415.7±92.5pg/ml vs. 57.2±10, p<0.001) and MCP-1 (15683±713pg/ml vs. 2208±157, p<0.001). At 24h Ox-AT (compared to PBS) significantly induced IL-8 (1168±9pg/ml vs. 110±8, p=0.008) and MCP-1 (14500±424pg/ml vs. 4225±470, p=0.005) in A549 cells. NAC inhibited Ox-AT, TNF-α, IL-6, IL-8, MCP-1, NF-κB and AP-1 (p<0.001 for all). 3F4 selectively inhibited Ox-AT, IL-8, MCP-1, NF-κB and AP-1 (p<0.001 for all). These findings were confirmed with NHBE cells.

In conclusion, Ox-AT generated in the airway interacts directly with epithelial cells to release MCP-1 and IL-8, so enhancing lung inflammation. This mechanism could potentially contribute to the abnormal inflammatory response seen in COPD independent of CSE. Anti-oxidant treatment inhibited both CSE and Ox-AT induced inflammatory response further supporting a role for these agents in COPD.

S87 DIFFERENTIAL INFLAMMATORY RESPONSES OF PRIMARY BRONCHIAL EPITHELIAL CELLS FROM SUBJECTS WITH COPD, HEALTHY SMOKERS AND NEVER SMOKERS

doi:10.1136/thoraxjnl-2012-202678.093

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Introduction We investigated the responses of primary bronchial epithelial cells (PBECs) obtained from subjects with COPD, healthy smokers (HS), and non-smokers (NS) to cigarette smoke extract (CSE) treatment. We hypothesised that PBECs from subjects with COPD respond differently to CSE and *Pseudomonas aeruginosa* lipopolysaccharide (PA LPS) stimulation than PBECs obtained from HS and NS.

Methods PBECs from 16 COPD subjects, 11 HS and 10 NS were obtained at fibreoptic bronchoscopy and cultured up to air-liquid interface and stimulated with PA LPS, either with or without pre-treatment with CSE for 24 h. COPD patients and HS were similar for smoking history in pack years and all 3 groups were matched for age. Apoptosis was evaluated using Annexin-V staining and the terminal transferase-mediated dUTP nick end-labelling (TUNEL) method. IL-6 and IL-8 were measured by ELISA and Toll-like receptor 4 expression by flow cytometry. CSE was prepared by combusting 1 Marlboro cigarette through 25 ml of media. Activation of NF-κB, mitogen-activated protein kinase (MAPK), and caspase-3 were determined by western blotting.

Results Constitutive release of IL-8 and IL-6 was greatest from the COPD cultures. 5% CSE pre-treatment followed by PA LPS stimulation reduced cytokine release from COPD PBECs, but increased the release from HS and NS cultures. Constitutive TLR-4 expression, MAPK and NF-κB activation were reduced only in COPD cultures after 5% CSE treatment. After treatment with CSE for 24 h, 44% of the COPD cells were apoptotic and 9% necrotic, whereas only 18% of the healthy smoker's cells and 6% of the non-smokers cells were apoptotic, with no cells in the latter 2 groups becoming necrotic. COPD cultures had the highest levels of cleaved caspase-3 after CSE treatment.

Conclusions 5% CSE attenuates inflammatory responses to LPS in cells from people with COPD but not from NS or HS. COPD epithelial cells have an increased susceptibility to apoptosis. Research funded by NI RDO.

S88 HIGH SENSITIVITY ERK AND AKT PHOSPHOSTATUS ASSAYS IN LUNG CANCER AND EMPHYSEMA

doi:10.1136/thoraxjnl-2012-202678.094

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Introduction Aberrant expression of oncogenic signalling proteins and their activation by phosphorylation is a key feature of malignancy. Current methodologies do not allow detailed analysis of the

Abstract S87 Table 1

	A Media	BL Media	A CSE	BL CSE	A LPS	BL LPS	A CSE & LPS	BL CSE & LPS
NS IL-8 [pg/ml]	24060±2514	34490±3260	36193±3755	29417±2352	34166±3347	36969±5019	43031±3211*	43890±5257
HS IL-8 [pg/ml]	28728±2422	22903±3500	41908±3269	27995±4233	37298±3052	33298±5856	39952±3131	34095±5634
COPD IL-8 [pg/ml]	32163±2474	43768±8730	24376±2474*	21994±4176*	44092±3247	54604±11363	20866±1564*	27180±4680*
NS IL-6 [pg/ml]	227±32	735±98	253±35	678±106	391±53	633±68	469±48*	714±107
HS IL-6 [pg/ml]	289±48	518±102	285±53	418±86	447±77	487±125	536±55*	884±169*
COPD IL-6 [pg/ml]	432±128	846±235	300±89	728±113	680±139	1012±186	324±43	578±92

IL-8 and IL-6 release from well differentiated bronchial epithelial cell cultures into apical and basolateral compartments after stimulation with PA LPS±pre-treatment with 5% CSE for 24 h. NS: Non-smoker; HS: Healthy Smoker; A: Apical; BL: Basolateral.
Media: No stimulation; CSE: Stimulation with 5% CSE 24 h; LPS: Stimulation with PA LPS 24 h; CSE & LPS: Pre-treatment with 5% CSE 24 h, stimulation with PA LPS 24 h.
* Statistically significant difference for soluble mediator release into a given compartment within each study group relative to the equivalent compartment without stimulation; $p<0.05$.

phosphorylation patterns of oncoproteins in small clinical samples. Therefore we developed an exquisitely sensitive capillary is oelectric focusing immunoassay (cIEF) method for discriminating phosphorylated isoforms of ERK and AKT in lung tissue.

Methods Participants undergoing curative resection for early stage NSCLC were recruited. Samples from normal lung and tumour tissue were taken shortly after resection and analysed using cIEF.

Results Twenty patients were recruited (12 male, 8 female), mean age 67.3 years (SD 7.5), 10 current and 10 former smokers with a mean smoking exposure of 48.4 pack years (SD 24.4). Tumour tissue (9 squamous and 11 adenocarcinoma) was collected and paired macroscopically normal lung that was histologically normal (n=7) or showed emphysema (n=13), graded pathologically as mild (n=7), moderate (n=3) or severe (n=3). The coefficient of variance was 7.6% for ERK and 4.2% for the AKT assay. The limits of detection and quantification were 8ng and 16ng of total protein respectively.

Diphosphorylated ERK (active form) was nearly three-fold higher in emphysematous lung tissue than normal lung tissue ($p=0.002$) and was associated with pathological severity of emphysema. In contrast, diphosphorylated ERK did not differ between paired normal lung and tumour ($p=0.45$) and there was no correlation of ERK status with age, gender, smoking status, tumour site, histology or stage. AKT was significantly more phosphorylated in tumour than matched normal lung. There was a trend for more phosphorylated AKT with poor differentiation but no correlation with tumour histology, size, site or stage of disease. Current smokers had more phosphorylated AKT than ex-smokers. There was no difference in the phosphorylation patterns of AKT according to age, gender or emphysema status.

Conclusions We found a strong relationship between diphosphorylated ERK and emphysema. By contrast, AKT phosphostatus was associated with lung cancer but not with emphysema. The requirement for just nanograms of protein defines the potential of this technique for determining the phosphostatus of oncogenic signalling proteins in tiny clinical samples.

S89 EPITHELIAL MESENCHYMAL TRANSITION (EMT) IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) AIRWAYS IS ATTENUATED BY INHALED CORTICOSTEROIDS (ICS)

doi:10.1136/thoraxjnl-2012-202678.095

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Introduction and Objectives We recently reported[1, 2] that EMT is active in the airways of COPD patients; in this process epithelial cells change shape and become motile, then digest through the reticular basement membrane (Rbm) which becomes fragmented and transition to a mesenchymal fibroblast-like cell. We also demonstrated that the Rbm is hyper-vascular, a combined picture specifically suggesting active EMT-type-III, which is a dangerous, pre-malignant condition. This may well be the link between COPD and lung cancer. In this study, we have assessed the effects of ICS on markers of EMT in endobronchial biopsies (ebb) in COPD.

Methods A double-blinded, randomised, placebo-controlled study assessed the effects of inhaled fluticasone propionate (FP: 500µg twice daily) on EMT in 34 COPD patients. Ebb were assessed for EMT related Rbm fragmentation (core structural marker) and immunostained for the EMT signatures S100A4 (a fibroblast epitope), matrix-metalloproteinase-9 (MMP-9) and the epithelial activation marker, epidermal growth factor receptor (EGFR).

Results Table 1.

Conclusions This is the first study to report the positive effects of ICS on EMT markers in COPD. This may be the mechanistic link between ICS and its reported preventive action against smoking-related lung cancer in COPD.

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Improving lung cancer survival

S90 THE NATIONAL LUNG CANCER AUDIT – NO EVIDENCE OF A "SEVEN-YEAR ITCH"

doi:10.1136/thoraxjnl-2012-202678.096

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Introduction The National Lung Cancer Audit, now in its7th year, is run jointly by the Royal College of Physicians and The Information Centre for health and social care, and is commissioned by the Healthcare Quality Improvement Partnership (HQIP). Its development was driven by the realisation that lung cancer outcomes vary widely across the UK and are poor compared to other western