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±505MFI) compared to healthy controls (1517±253MFI; p=0.048) and COPD patients (1560±515MFI; 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 (1360±315MFI; p=0.046). 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=0.001) in the presence of PiZZ, compared to PiMM (1921MFI) compared to PiMM (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

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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 antibody was used to probe the effect of Ox-AT. CSE-induced TNF-α and IL-6 mRNA were determined by western blotting.

CONCLUSIONS Ox-AT induced inflammatory cytokine release from A549 cells, but not from NHBE cells. Ox-AT induced expression of TNF-α, IL-6, IL-8, MCP-1, NF-kb and AP-1 in A549 cells, but not in NHBE cells. Ox-AT may therefore have potential as a therapeutic target for A1ATD.

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

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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. Cells were treated with CSE or PA LPS, either with or without pre-treatment with CSE for 24 h. CSE and LPS are 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.

Results Constitutive release of IL-6 and IL-8 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-kb activation were reduced only in COPD cultures. 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 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