

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

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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- κ B and AP-1 were assessed by ELISA or RT-PCR. Anti-Ox-AT mAb (3F4, 10 μ g/ml) and N-acetylcysteine (NAC, 10⁻⁵M) 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- κ B (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- κ B and AP-1 (p<0.001 for all). 3F4 selectively inhibited Ox-AT, IL-8, MCP-1, NF- κ B 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

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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 fiberoptic 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- κ B, 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- κ B 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

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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