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±310MFI) 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 (1852MFI), 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 ANTI-TRYPSIN 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 mAb (3F4, 10 µg/ml) and N-acetyl cysteine (NAC, 10-3M) 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 (584.7±58.4pg/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-kB (OD at 405nm, 1.325 vs. 0.315, p<0.001) and AP-1 (OD at 405nm, 0.982 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±162.8pg/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-kB and AP-1 (p<0.001 for all). SF4 selectively inhibited Ox-AT, IL-8, MCP-1, NF-kB 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.

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