this did not reach significance in the NBS group. (NBS CF $p=0.05$, established CF $p<0.01$).

Conclusion Our results demonstrate that inflammation is already present by 4 months of age in asymptomatic infants diagnosed through NBS, although at a lower level than seen in established CF. The results underscore the importance of early surveillance and lend support to the evolving focus on this age group for interventional trials.

Abstract S82 Table 1

Table 1: BALF cell counts $\times 10^3$. Figures given are medians (range)

	NBS CF micro +ve	NBS CF micro -ve	Established CF micro +ve	Established CF micro - ve	Healthy controls
n (absolute cell count)	10	22	33	15	6
Absolute cell count $\times 10^3$	418 (155.0- 2060)	378 (75-1877)	1495 (140-48990)	555 (143- 14780)	143 (70-270)
n (neutrophil Differential)	13	24	22	15	6
Neutrophil differential (%)	17.2 (2.0-73.0)	8.4 (0.3-57.3)	53.2 (15.7-92.0)	19.3 (1.7-87.7)	1.4 (0.3-2.7)

S83 LEVELS OF ANTIMICROBIAL PEPTIDES IN THE AIRWAY OF CHILDREN WITH CYSTIC FIBROSIS ARE NOT RELATED TO SERUM VITAMIN D CONCENTRATION

doi:10.1136/thoraxjnl-2012-202678.089

¹RM Thursfield, ²A Bush, ¹EWFW Alton, ¹JC Davies. ¹National Heart and Lung Institute, Imperial College, London, UK; ²Department of paediatric respiratory medicine, Royal Brompton Hospital, London, UK

Introduction There is convincing evidence of the clinical health benefits of adequate vitamin D in many respiratory diseases but the evidence in cystic fibrosis (CF) is unclear. There are increasing data on the role of vitamin D as an immunomodulatory agent and it is thought, from *in-vitro* data, that this may be via induction of the antimicrobial peptides, LL37 and H β D-2. We hypothesised that antimicrobial peptide levels would be increased in children with adequate vitamin D and this could account for reported improvements in lung function via improved airway defence.

Aims and methods The main aim was to establish if a relationship exists between vitamin D and antimicrobial peptides, LL37 and H β D-2, and whether any clinically beneficial effects of adequate vitamin D exist in children with CF. Bronchoalveolar fluid (BALF) supernatant levels of LL37 and H β D-2 were by measured by ELISA and serum 25(OH)D₂ by mass spectrometry coupled with high-performance liquid chromatography.

Results Samples were collected from 120 children with CF (58% female); median age (range) 6.9 (0.1–17.6) years. One third of patients were vitamin D insufficient (<50 nmol/L). Median 25(OH)D₂ was 57 nmol/L (range 7–191 nmol/L). LL37 ranged from 0.1–21.9 ng/mL with median value of 0.49 ng/mL and H β D-2 from <15.6 – >1000 pg/mL, median 150 pg/mL.

LL37 was significantly correlated with serum neutrophils ($r=0.4$, $P<0.0001$), BALF total cell count ($r=0.7$, $p<0.0001$), and BALF neutrophil differential ($r=0.5$, $p<0.0001$). These relationships were not seen with H β D-2. Contrary to our hypothesis neither LL37 nor H β D-2 correlated with vitamin D and no differences were seen between vitamin D 'adequate' and 'insufficient' patients. There was no association seen between vitamin D and FEV₁ ($r^2=0.01$, $p=0.4$).

Conclusions Our results demonstrate that there is not a relationship between serum 25(OH)D₂ and BALF H β D-2 or LL37. If

vitamin D is involved in the induction of such defence peptides *in-vivo*, the impact of this on protein levels may be limited in the degradative environment of the inflamed airway. In addition, we found no clinical or physiological effects of vitamin D deficiency. If any beneficial effect of vitamin D on respiratory health does exist in CF, it is small and not mediated via the antimicrobial pathway.

Mechanisms of airway injury in COPD

S84 CIGARETTE SMOKE PROMOTES EXAGGERATED INFLAMMATORY RESPONSE IN THE PRESENCE OF Z-AT

doi:10.1136/thoraxjnl-2012-202678.090

¹S Alam, ²S Janciauskiene, ¹R Mahadeva. ¹University of Cambridge, Cambridge, UK; ²Hannover Medical School, Hannover, Germany

Severe deficiency of the major anti-elastase α_1 -antitrypsin (AT) due to the Z (Glu342Lys) variant is the commonest genetic reason for the development of COPD. Cigarette smoke (CS) accelerates decline in lung function in Z-AT homozygotes. We investigated whether Z-AT is associated with an exaggerated inflammatory response compared to normal AT (M-AT).

Lung epithelial (A549 and NHBE) cells transfected with human M-AT or Z-AT (M-AT/Z-AT cells) were exposed to 12.5% CSE generated from 1R3F cigarettes. Supernatants, lysates and inclusion bodies were assessed for total AT to confirm a successful cell-model system. Supernatant was assessed for TNF- α , IL-6, IL-8 and MCP-1, oxidised pZ-AT (Ox-pZ-AT), NF- κ B and AP-1 by ELISA, immunoblot or RT-PCR. N-acetylcysteine (NAC, 10^{-5} M) was used to probe the effect of oxidants.

At 24h CSE in Z-AT (CSE-Z-AT) compared to CSE-M-AT (unless stated) significantly induced TNF- α (212 ± 20.71 pg/ml vs. 37.1 ± 2.7), IL-6 (421.4 ± 20.8 pg/ml vs. 159.3 ± 12.1), IL-8 (8763 ± 497 pg/ml vs. 2593 ± 450) and MCP-1 (23564 ± 1852 pg/ml vs. 5329 ± 706), $p<0.001$ for all. CSE-Z-AT had significantly induced mRNA for TNF- α , IL-6, IL-8 and MCP-1 at 0.5h ($p<0.001$ for all). Development of Ox-pZ-AT were exclusively detected in CSE-Z-AT inclusion (3246 ± 433 ng/ml vs. undetectable, $p<0.001$). CSE-Z-AT had significantly activated NF- κ B ($p<0.001$) and AP-1 ($p=0.001$) at 0.5h. In CSE-Z-AT treatment with NAC significantly inhibited TNF- α , IL-6, IL-8, MCP-1, NF- κ B, AP-1 and Ox-pZ-AT formation ($p<0.001$ for all). These findings were confirmed on NHBE cells.

In conclusion, following CS exposure Z-AT cells had significantly elevated inflammatory mediators compared to M-AT cells, which was inhibited by NAC. We propose that during CS exposed lung inflammation Z-AT monomer undergoes oxidation to form oxidised polymers thereby further reducing the level of protective monomeric AT, which predisposes to increased lung inflammation.

S85 NEUTROPHIL CELL MEMBRANE EXPRESSION OF PROTEINASE 3 AND ITS RELATIONSHIP TO ALPHA-1-ANTITRYPSIN DEFICIENCY (A1ATD)

doi:10.1136/thoraxjnl-2012-202678.091

¹NJ Sinden, ²E Sapay, ²GM Walton, ¹RA Stockley. ¹ADAPT project, Queen Elizabeth Hospital, Birmingham, United Kingdom; ²University of Birmingham, Birmingham, United Kingdom

Introduction Serine proteinases such as proteinase 3 (PR3) and neutrophil elastase (NE) on the surface of activated neutrophils retain their activity, which may be central to the tissue damage in emphysema. However, little is known about this expression in A1ATD when it may be more critical.

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 ± 305 MFI) compared to healthy controls (1517 ± 253 MFI; $p=0.048$) and COPD patients (1360 ± 315 MFI; $p=0.046$). NE expression was similar between groups.

PR3 expression on stimulated neutrophils was greater in A1ATD patients (5112 ± 547 MFI) compared to healthy controls (3411 ± 541 MFI; $p=0.042$), but not different to COPD patients (4723 ± 1509 MFI; $p=0.78$). 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 (1921 MFI) compared to PiMM plasma (1352 MFI), 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

S Alam, Z Li, R Mahadeva. *University of Cambridge, Cambridge, UK*

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, 10μ g/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 ± 76.8 pg/ml vs. 17.5 ± 2.6 , $p<0.001$) at 24h and induced IL-6 at 72h (354.7 ± 38.4 pg/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 $\pm$ SEM, 1372.8 ± 162.8 ng/ml vs. undetectable, $p<0.001$) and induced IL-8 (1415.7 ± 92.5 pg/ml vs. 57.2 ± 10 , $p<0.001$) and MCP-1 (15683 ± 713 pg/ml vs. 2208 ± 157 , $p<0.001$). At 24h Ox-AT (compared to PBS) significantly induced IL-8

(1168 ± 9 pg/ml vs. 110 ± 8 , $p=0.008$) and MCP-1 (14500 ± 424 pg/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

doi:10.1136/thoraxjnl-2012-202678.093

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

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

doi:10.1136/thoraxjnl-2012-202678.094

¹PAJ Crosbie, ²EJ Crosbie, ³M Aspinall-O'Dea, ⁴A Pierce, ¹R Shah, ¹R Booton, ²AW Whetton. *¹University Hospital of South Manchester, Manchester, UK; ²University of Manchester, Manchester, UK*

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