COPD and asthma: mechanisms of airways inflammation and treatment

P184 MACROPHAGE PHAGOCYTOSIS IN COPD PATIENTS AT EXACERBATION COMPARED TO STABLE STATE

[1]R Singh, [2]K Such, [1]BS Kowlessar, [1]ARC Patel, [1]AJ Mackay, [1]SE Brill, [1]JP Allinson, [1]GC Donaldson, [2]PJ Barnes, [1]JA Wedzicha, [2]LE Donnelly; *[1]Centre for Respiratory Medicine, UCL Medical School, London, UK; [2]Airway Disease Section, NHLI, Imperial College, London, UK*

10.1136/thoraxjnl-2013-204457.336

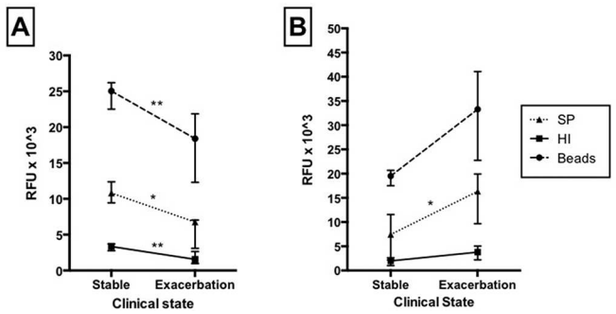
Introduction COPD exacerbations are clinically important events, commonly triggered by bacterial infection. Defective phagocytosis of potentially pathogenic microorganisms (PPMs) in stable COPD has been demonstrated in both alveolar and monocyte-derived macrophages (MDMs). We hypothesised that phagocytosis may be suppressed further during an acute COPD exacerbation and relate to bacterial aetiology of the exacerbation.

Methods Whole blood was collected from patients in the London COPD cohort at both stable state and at exacerbation, as defined by prospectively completed daily symptom diary cards (Seemungal *et al*., 1998). Monocytes were isolated and cultured with GM-CSF (2ng/ml) for 12 days to generate MDMs. MDM phagocytosis of fluorescently-labelled polystyrene beads, *Haemophilus influenzae* (HI) and *Streptococcus pneumoniae* (SP) was measured by fluorimetry. Diary card data was used to determine prodromal symptoms, exacerbation duration and exacerbation symptom intensity. Sputa collected at exacerbation were cultured for PPMs.

Results MDMs were cultured from 13 COPD patients at paired stable and exacerbation states. 54% were male, mean age 72.8 years (SD 6.2), FEV_1 predicted 55.7% (20.5) and 38% current smokers. The median time between stable and exacerbation states was 147 days [IQR 75–212].

Two distinct patterns of change in phagocytic ability were seen between the stable and exacerbation state. Eight patients significantly decreased their phagocytic capacity compared to stable state for beads, HI and SP ($p = 0.004$, $p = 0.008$ and $p = 0.020$ respectively, Figure 1A). Five of these eight patients (63%) had a PPM isolated in their exacerbation sputum sample. Five of 13 patients increased phagocytosis at exacerbation (Figure 1B), but only SP reached statistical significance ($p = 0.031$). Only one of these five (20%) had a PPM in their exacerbation sputum sample.

There was no significant difference between the presence of prodromal symptoms, exacerbation duration or symptom intensity between the two patterns.



Abstract P184 Figure 1(A). Increased MDM Phagocytosis at exacerbation, n = 8; (B) Decreased MDM phagocytosis at exacerbation, n = 5.

Conclusion Phagocytosis of bacteria is suppressed further during some COPD exacerbations. This may contribute to bacterial aetiology of these exacerbations. Phagocytosis appears partially more effective in other exacerbations. Further work is needed to understand these apparently dichotomous changes and their impact on clinical outcomes.

P185 PULMONARY INFECTION IN PIZZ ANTITRYPSIN INDIVIDUALS IS ASSOCIATED WITH INCREASED OXIDATIVE AND NITROSATIVE STRESS

S Alam, J Parmar, K Santhanakrishnan, R Mahadeva; *University of Cambridge, Cambridge, UK*

10.1136/thoraxjnl-2013-204457.337

Severe deficiency *of* the major anti-elastase $_1$-antitrypsin (AT) due to the Z (Glu342Lys) variant is associated with early-onset emphysema which can require lung transplantation. AT is mainly produced by the liver and a lesser proportion (around 10%) is produced in the lung. Therefore individuals with Z-AT post-lung transplantation remain severely deficient in AT. We studied patients with and without AT deficiency with infection (post lung transplantation) to examine the relationship between deficiency of AT and oxidative/nitrosative stress.

BALF was obtained at scheduled surveillance, and when clinically indicated to assess for infection, rejection and airway injury. 25 patients post-transplant were evaluated; 12/26 samples from 12 Z-AT patients had infective tracheobronchitis, and 7/29 samples from 13 M(normal)-AT had infective tracheobronchitis. The level of oxidative stress (F_2-Isoprostane), TNF- , IL-1 , IFN- was quantified using respective ELISA kits and for total glutathione (GSH) and oxidised GSH (GSSG) using OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit and nitrosative stress using Griess reagent.

BALF of Z-AT patients with infection had increased oxidative stress compared to infected M-AT patients; F_2-Isoprostane, 773SEM ± 79.5 pg/ml vs. 425.8 ± 53.2, $P < 0.001$ respectively, oxidised glutathione (0.112 ± 0.1μM/ml vs. 0.155 ± 0.1, $P = 0.027$). However, total glutathione was unchanged (0.885 ± 13 μM/ml vs. 1.17 ± 0.141, $P = 0.117$). There was increased nitrosative stress in Z-AT vs. M-AT patients (nitrite release as a measure of nitric oxide (NO) production (261 ± 47 pg/ml vs. 117 ± 41.2, $P < 0.001$), and increased levels of TNF- (91 ± 16.3 pg/ml vs. 59 ± 10.5, $P = 0.002$), IL-1 (121.2 ± 17.4 pg/ml vs. 69.6 ± 5.8, $P = 0.047$) and IFN- (481.3 ± 74.8 pg/ml vs. 201.2 ± 28.5, $P = 0.025$). Infected Z-AT BALF had increased free HLE compared to infected M-AT patients (167 ± 16 ng/ml vs. 42.7 ± 16, $P < 0.001$).

In conclusion, Z-AT lungs had increased oxidative and nitrosative stress, and furthermore had increased levels of cytokines that are associated with induction of the inducible NO synthase (iNOS) gene. This suggests that excess production of iNOS-derived NO is likely to contribute to exaggerated inflammation in the PiZZ individuals lungs during episodes of infection which may contribute to progression of their lung disease.

P186 MAST CELL MEDIATORS STIMULATE HUMAN AIRWAY SMOOTH MUSCLE GROWTH, A FEATURE OF AIRWAY REMODELLING IN ASTHMA VIA MATRIX METALLOPROTEINASE (MMP-1) ACTIVITY

S Naveed, D Shaw, SR Johnson; *University of Nottingham, Nottingham, UK*

10.1136/thoraxjnl-2013-204457.338

Introduction and Objective : Increased airway smooth muscle (ASM) mass and infiltration by mast cells are key features of airway remodelling in asthma. We tested a hypothesis to investigate the relationship between ASM growth, mast cell mediators and the matrix metalloproteinase MMP-1 activity.

Methods : Primary ASM cultures were derived from a healthy subject. ASM cells were cultured for up to 2 days firstly in the presence and then in absence of serum and treated with conditioned media either collected from activated mast cells cultures or inactive/ unstimulated mast cells cultures. Mast cells were grown in suspension and activated using phorbol 12-myristate 13-acetate (PMA) and calcium ionophore (A23187) to release serine proteases such as histamine, beta-tryptase and chymase etc. We also performed western blots to determine MMP-1 activity in the supernatants of ASM cultures.

Results ASM cells treated with stimulated mast cells conditioned media showed increased cell proliferation by almost 2 folds after 48 hours of incubation under serum free conditions confirmed by cell counting and MTT assay in comparison with untreated airway smooth muscle cells or ASM cells treated with inactive/ unstimulated mast cells culture media.

Furthermore our experiments showed that matrix metalloproteinase (MMP -1) levels and activity was significantly increased in ASM cultures treated with activated mast cells as compared to other two control conditions as mentioned earlier.

Conclusion These findings clearly indicate role of mast cell proteases in ASM proliferation and therefore airway remodeling in asthma, a mechanism that perhaps is modulated by MMP-1 activity. We further suggest that the pathway will prove susceptible to pharmacological intervention for treatment of chronic asthma.

P187 MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN SPUTUM OF ASTHMATICS

P Sankaran, O Jupp, M Jefferson, D Sexton, C Brockwell, I Clark, AM Wilson; *Univeristy of East Anglia, Norwich, Norfolk*

10.1136/thoraxjnl-2013-204457.339

Introduction and Objectives Metalloproteinases are implicated in the development of airway remodelling in asthma due to their ability to cleave collagen and elastin with extracellular matrix. We optimised a method to purify messenger Ribonucleic acid (mRNA) sputum samples.

Methods The mRNA expression of a wide range of pertinent Matrix Metalloproteinases (MMP), A Disintegrin And Metalloproteinases (ADAM), A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS) and Tissue Inhibitors of Metalloproteinases (TIMP) was measured from induced sputum with hypertonic saline using Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) in 17 (11 male) non-smoking adults with steroid naive asthma and 12 (6 male) healthy controls. Ten patients with asthma completed open labelled montelukast therapy 10mg per day for 8 weeks. Total mRNA was extracted from the cellular content of the induced sputum plug using a combination of Trizol extraction and Qiagen RNeasy spin columns. To each 0.5ml Trizol extract, 300 l of chloroform was added. The

Abstract P187 Table 1. Changes in m-RNA levels for metalloproteinases and their inhibitors.

Metalloproteinase	Healthy Volunteer N	Median (IQR) Relative Quantification (RQ)	Asthma N	Median (IQR) Relative Quantification (RQ)	P value
MMP 1	9	0.0015(0.0021)	16	0.0018 (0.0054)	0.890
MMP 2	9	0.0065(0.0075)	17	0.0091(0.0122)	0.367
MMP 7	9	0.0016(0.0029)	17	0.0040(0.0074)	0.07*
MMP 8	9	0.00006(0.00007)	15	0.00011(0.00016)	0.238
MMP 9	8	0.0163 (0.0157)	16	0.0296(0.0657)	0.928
MMP 10	9	0.0002(0.0001)	16	0.0006(0.0050)	0.461
MMP 11	5	0.00002(0.00009)	9	0.00006(0.00005)	0.112
MMP 12	9	0.0122 (0.0116)	16	0.3348(0.0468)	0.07*
MMP 14	9	0.1260(0.0348)	17	0.1001(0.1018)	0.306
MMP 15	9	0.0312 (0.0012)	16	0.0019(0.0015)	0.04**
MMP 17	9	0.0002(0.00021)	13	0.0001(0.0002)	0.40
MMP 19	9	0.0769 (0.0809)	17	0.1039(0.0662)	0.491
MMP 25	9	0.0213(0.0386)	16	0.0734(0.0950)	0.04**
ADAM 8	9	0.0199(0.0220)	16	0.0530(0.0434)	0.07*
ADAM 9	9	0.2242(0.0352)	17	0.2381(0.0613)	0.228
ADAM 10	9	0.3032(0.0906)	17	0.2415(0.1213)	0.209
ADAM 12	8	0.0006(0.0011)	16	0.0009(0.0007)	1.0
ADAM 17	9	0.1294(0.0502)	17	0.0712(0.0446)	0.007**
ADAM 19	9	0.0078(0.0094)	16	0.0165(0.0179)	0.07*
ADAM 28	9	0.0030(0.0030)	15	0.0106(0.0094)	0.03**
ADAM TS1	8	0.0026(0.0111)	14	0.0007(0.0006)	0.764
ADAM TS15	9	0.0088(0.0077)	17	0.0042(0.0039)	0.008**
TIMP 1	9	0.3072(0.2163)	17	0.6409(0.5125)	0.06*
TIMP 2	9	0.7018(0.1670)	17	0.4360(0.2671)	0.007**
TIMP 3	9	0.009(0.0014)	17	0.0024(0.0011)	0.164
TIMP 4	9	0.0012(0.0006)	16	0.0018(0.0047)	0.357

p <0.05, p=0.05–0.07***

Values represent gene of interest expressed as relative quantification (RQ) relative to a housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) using a $2^{-\Delta Ct}$ transformations, ΔCt is the threshold cycle (Ct) of the target gene–Ct of *GAPDH*.