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

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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*.

aqueous layer was recovered into a fresh tube and mixed with a half volume of 100% ethanol. Samples were applied to RNeasy Mini spin columns. RT-PCR, using Taqman low-density arrays, was used to determine gene expression.

Results The mean (SD) age and forced expiratory volume in 1 second of asthmatics was 36 (13.4) years and 101.16 (15.47)% predicted respectively and for healthy volunteers was 36 (7.2) years and 92.16 (17.43)% predicted. *MMP25* expression was significantly ($p = 0.04$) higher and *MMP15* expression was significantly ($p = 0.04$) lower is asthmatics compared to healthy volunteers. *ADAM28* was significantly ($p = 0.03$) higher and *ADAM17* and *ADAMTS15* expression were significantly lower in asthma ($p = 0.007$ & 0.008 respectively). *TIMP2* expression was significantly ($p = 0.007$) lower in the asthma. There were no significant changes in expression of any of the metalloproteinases or their inhibitors after montelukast therapy.

Conclusion We have studied a wide range of known MMPs, ADAMs, ADAMTSs and inhibitors with a refined technique and successfully increased the yield from induced sputum samples. Significant differences were found between healthy volunteers and asthmatic patients for gene expression of some metalloproteinases/TIMPs. This technique could be used in the future when evaluating gene expression in asthma.

P188 PREVALENCE OF SERUM EOSINOPHILIA AT TIME OF ADMISSION WITH AN EXACERBATION OF COPD

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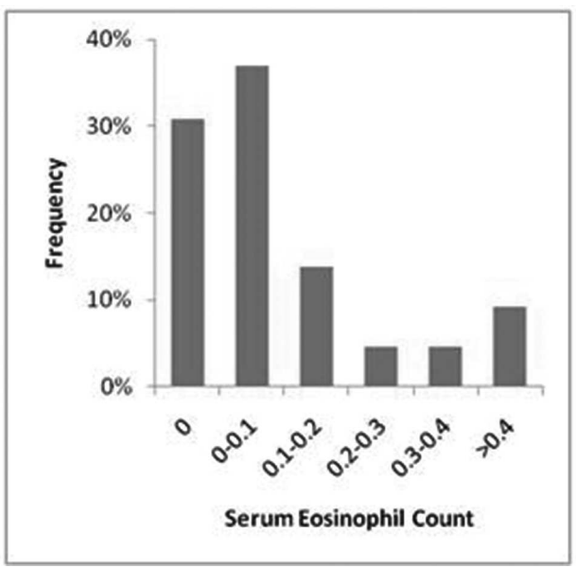
COPD is generally viewed as a disease driven by neutrophillic inflammation but up to 40% of COPD patients have an inflammatory pattern that includes elevated eosinophils (Saha, 2006) and there has been recent interest in the role of eosinophils in the aetiology and pathophysiology of exacerbations of COPD. Increased eosinophilic airway inflammation has been reported during exacerbations of COPD and peripheral eosinophils levels have been used as a surrogate to predict response to corticosteroid therapy. Treatment strategies with oral and inhaled steroids to reduce sputum eosinophils in COPD reduce exacerbation rates compared to a conventional care and there has been interest in using anti-eosinophil therapy to modify the clinical course of exacerbations.



Abstract P188 Figure 1.

Data were collected on 66 patients admitted with an acute exacerbation of COPD between Nov 2011 and Feb 2012 as part of an assessment of a discharge bundle. The mean age of the patients was 72 (range 49–91) and mean FEV1 33% predicted.

Serum eosinophils were measured routinely in full blood counts performed at the time of admission. 20 patients (30%) had no detectable eosinophils, and 6 (9%) had raised eosinophil counts (normal range 0.04–0.40 x 10^9/l). The median eosinophil count was 0.07. One patient had an eosinophil count of 15.24 on admission, having previously had intermittently mildly elevated counts (up to 1.57) since at least 2000. Excluding this patient, the mean (SE) eosinophil count was 0.37 (0.23). The distribution of serum eosinophil counts is shown in fig 1.

In this group of patients, serum eosinophilia (>0.4) was seen in only 9% of patients at the time of admission. This may have been affected by prior self management with oral steroids.

REFERENCES

1. Saha S, Brightling CE. *Int J Chron Obstruct Pulmon Dis*. 2006;1(1):39–47.

P189 MITOCHONDRIAL DYSFUNCTION IN MUSCLE AND AIRWAY COMPARTMENTS IN COPD: PRELIMINARY FINDINGS

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Introduction & Rationale Oxidative stress may underlie both pulmonary and non-pulmonary manifestations of COPD and may result from mitochondrial dysfunction. We hypothesised that if oxidative stress arose from the lung and 'spilled over' to cause non-pulmonary disease (e.g. skeletal muscle weakness) then greater evidence of mitochondrial dysfunction should be evident in the lung.

Objectives We measured mitochondrial function in endobronchial and skeletal muscle biopsies from COPD patients and healthy smokers matched for smoking history, age and sex.

Methods and Measurements We have so far biopsied 4 control smokers with normal FEV_1 and FEV_1/FVC ratio, and 4 GOLD II COPD patients out of a planned total of 40. Bronchoscopy with endobronchial biopsies (EB) and percutaneous muscle biopsy of the vastus lateralis (VL) were obtained on the same day; additional phenotypic measurements included lung function, quadriceps strength and 6-minute walk (6MW) distance. Mitochondria were isolated and mitochondrial reactive oxygen species (ROS) and membrane potential (MP) were measured using MitoSOX Red and the carbocyanine dye JC-1 respectively and intracellular ROS determined by 2'-7'-dichlorofluorescin diacetate (DCF) staining.

Results

Mean ± SEM	COPD		Controls	
Age (years)	63 ± 1		61 ± 3	
FEV_1 (% predicted)	72 ± 2		99 ± 6	
QMVC (kg)	33 ± 8		49 ± 6	
Smoking (pack-years)	42 ± 10		34 ± 6	
6MW (m)	490 ± 47		635 ± 50	
	VL	EB	VL	EB
Mitochondrial ROS (RFU)	42196 ± 9819	244947 ± 78170	11983 ± 1408	93621 ± 17981
Mitochondrial MP (units)	4.6 ± 1.0	10.3 ± 2.5	4.9 ± 1.3	13.6 ± 1.5
Intracellular ROS (RFU)	22131 ± 7195	14820 ± 5014	727 ± 297	752 ± 165