



Abstract S46 Figure 1 Phagocytosis of unopsonised SA by blood neutrophils over 60 min

20), or *Escherichia coli* bioparticles (EC, $n = 10$) and fluorescently labelled disease-relevant bacteria, *Haemophilus influenzae* (HI, $n = 10$) and *Streptococcus pneumoniae* (SP, $n = 10$) was assessed, at regular intervals over 60 min, using flow-cytometry. Results were confirmed using time-lapse video microscopy.

Results Peak phagocytosis was achieved at 60 min for unopsonised bacteria and 30 min for opsonised bacteria. There were no differences in time to peak phagocytosis between bacterial species. Blood neutrophils from patients with COPD and HC displayed similar phagocytic ability, in both percentage of neutrophils with phagocytic activity and the amount of SA, EC, HI or SP ingested (as indicated by MFI) (COPD vs. HC, $p > 0.05$ for all). This was ubiquitous to both opsonin independent and opsonin-dependant phagocytosis, and was consistent across all time points measured. A typical comparison is shown in figure one, with unopsonised SA data.

Conclusions Phagocytic ability of blood neutrophils from patients with COPD to ingest *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Haemophilus influenzae* is not altered compared to age-matched healthy controls. This should be replicated in lung neutrophils to assess whether transmigration to the tissues affects function.

S47 ENHANCED IL-6/CCL3 SIGNALLING IN THE PLASMA OF PATIENTS WITH COPD

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Rationale IL-6 is a pro-inflammatory cytokine that signals through soluble (sIL-6R/sgp80) and membrane bound (gp80) receptors to promote recruitment of mononuclear cells. IL-6 induces expression of CCL3, a monocytic chemokine. Monocytes are precursors of macrophages and dendritic cells. They can be classified into three subtypes according to surface expression of CD14 (LPS receptor) and CD16 (FcγRIII): CD14++CD16-, CD14+CD16+, CD14-CD16++. We measured plasma levels of IL-6, sIL-6R and CCL3 and determined the chemokine receptor expression profile of circulating monocytes in COPD.

Methods 70 COPD patients and 30 healthy controls comprising 15 smokers (S) and 15 healthy non-smokers (HNS) underwent plasma sampling. Levels of IL-6, sIL-6R and CCL3 were

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	COPD	S	HNS	ANOVA
IL-6 (pg/ml)	33.7 (190.3)	3.8 (1.9)	0*	$p < 0.0001$
sIL-6R (pg/ml)	5338 (850.3)	4453 (613.2)	4853 (856.8)	$p = 0.0005$
CCL3 (pg/ml)	74.8 (111.9)**	0*	0*	-

Data expressed as mean (SD) * < lower limit of quantification of the assay **CCL3 levels registered above the assay's lower limit of quantification in 7/70 COPD patients.

determined by multiplex analysis (MSD) of plasma. Multi-colour flow cytometry was performed on whole blood obtained from 32 COPD patients, 8 S and 8 HNS to measure surface expression levels of chemokine receptors CCR1, CCR2, CCR7, CXCR1 and CX3CR1 on CD14++CD16-, CD14+CD16+ and CD14-CD16++ monocytes.

Results COPD patients had the greatest levels of IL-6 and sIL-6R. CCL3 was not detected in any controls, but was present in a subset of COPD patients. % surface expression of the CCL3 receptor CCR1 measured on CD14++CD16- monocytes of COPD patients was greater than those of HNS ($p = 0.04$). There were no significant differences in expression levels of other chemokine receptors.

Conclusion We report evidence of enhanced IL-6 signalling in the plasma of COPD patients and increased plasma CCL3 in a subset of individuals from this disease group. Furthermore, there was increased CCR1 expression on COPD monocytes. Enhanced IL-6 may co-ordinate the mononuclear component of the inflammatory response in COPD.

S48 AIR POLLUTION PARTICULATE MATTER PROMOTES DC MATURATION AND ENHANCES THEIR STIMULATION OF CD8 LYMPHOCYTE RESPONSES

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Background High levels of ambient urban particulate matter (UPM), a component of air pollution, are associated with respiratory tract infections and exacerbations of airways diseases. Dendritic cells (DCs) exposed to inhaled UPM orchestrate the resulting immune response. We have previously shown that UPM-stimulation of DCs results in enhanced proliferation of naïve CD4 lymphocytes but decreased priming of IFN γ -producing CD4 lymphocytes. These CD4 lymphocytes are important in anti-viral immune responses; however, Tc1 CD8 lymphocytes have more direct anti-viral action. In this research we have studied the effect of UPM on DC priming of CD8 lymphocytes.

Methods CD1c peripheral blood DCs were isolated, cultured in the presence/absence of UPM stimulation, with GM-CSF or in medium alone. DC expression of CD83, CCR7, CD40 and MHC Class I were measured by flow-cytometry at 24 h. Pre-treated DCs were also cultured with naïve CD8 lymphocytes in

Abstract S48 Table 1 Effect of UPM stimulation of DCs upon naïve CD8 lymphocyte response in MLR at day 5. Median (Inter-Quartile Range) TNF α , IFN γ and IL-13 production

	Control	UPM	GM-CSF	UPM + GM-CSF
TNF α (pg/ml)	77.6 (60.2–256)	149 (72.3–853)	904 (148–1425)	2411 (434–4869)
IFN γ (pg/ml)	84.5 (44.9–195)	225 (73.6–1537)	1009 (66.25–1477)	2343 (189–8726)
IL-13 (pg/ml)	25.9 (5.88–106)	59.0 (25.3–335)	939 (45.8–984)	638 (74.6–1266)

an allogeneic mixed-lymphocyte reaction (MLR). Lymphocyte proliferation (flow-cytometric measurement of CFSE) and cytokine production (multiplex bead array) were assessed at day 5. The proportion of lymphocytes primed to produce IFN γ was measured at day 7 (intracellular staining).

Results UPM-stimulation increased DC expression of the maturation marker CD83 ($p = 0.0038$) and chemokine receptor CCR7 ($p = 0.0018$). It had no effect on CD40 or MHC Class I expression. UPM-stimulation of DCs also significantly increased CD8 lymphocyte proliferation ($p = 0.020$), and the production of IFN γ , TNF α and IL-13 by CD8 lymphocytes in MLR at day 5 (all $p < 0.05$; Table 1). The proportion of CD8 lymphocytes primed to produce IFN γ was also increased by UPM-stimulation of DCs ($p = 0.034$).

Conclusion No evidence of an impaired Tc1 response was seen with UPM-stimulated DCs, in contrast to our previous findings with CD4 T lymphocytes. This may be because CD8 lymphocytes are more primed to respond and produce cytokines at baseline. However, UPM-treatment of DCs did significantly increase DC expression of CCR7, which directs DCs to lymph nodes, and increased the priming of Tc1 and Tc2 responses in the absence of any other stimulation. Inhalation of UPM may give rise to pathological CD8 responses to otherwise innocuous novel antigens.

S49 TELOMERE ATTRITION IN CIRCULATING WHITE BLOOD CELLS IN COPD RELATES TO LUNG FUNCTION AND OUTCOMES

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Introduction Increasing evidence suggests accelerated ageing as a pathogenic mechanism in COPD.

Methods and results Telomere length in circulating WBC, a marker of biological ageing, was assessed in 200 ex-smoker COPD patients (108 male, age 61.5 ± 6.4 years, FEV₁ $45.6 \pm 17.1\%$ predicted), 50 ex-smokers with normal lung function (27 male, age 59.9 ± 7.3 years, FEV₁ $109.1 \pm 13.4\%$ predicted) and 50 non-smoker healthy subjects (27 male, age 59.3 ± 8.3 years, FEV₁ $113.2 \pm 13.1\%$ predicted). TL was assessed by qPCR and expressed as relative T/S ratio.

TL was shorter in COPD (0.77 ± 0.2 relative T/S ratio) than in both ex-smokers (0.83 ± 0.2 relative T/S ratio) and non-smokers (0.84 ± 0.2 relative T/S ratio) ($p < 0.05$). Furthermore TL correlated negatively with age ($r -0.17$, $p 0.007$), emphysema score ($r -0.217$, $p 0.001$), number of exacerbations in the previous year to inclusion in the study ($r -0.129$, $p 0.04$), number of hospitalisations over 3 years follow-up ($r -0.167$, $p 0.004$) and positively with FEV₁ ($r 0.135$, $p = 0.03$) and arterial oxygen saturation ($r 0.161$, $p 0.01$).

Conclusion COPD patients have evidence of premature ageing (shortened TL) compared to normal subjects irrespective of their smoking history. TL relates to FEV₁, SatO₂, exacerbation rate and hospitalisations.

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S50 AIRWAY SMOOTH MUSCLE INFLAMMATION IS CONTROLLED BY MICRORNA-145 TARGETING OF SMAD3 IN COPD

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Introduction and objectives Airway smooth muscle cells (ASMCs) may contribute to the pathological airway inflammation and remodelling in COPD through the secretion of inflammatory cytokines and increased proliferation. Our previous work demonstrated that ASMCs from patients with COPD release greater amounts of IL-6 and CXCL8 compared to those from healthy subjects and are in a state of hyperproliferation. MicroRNAs (miRNAs) have recently emerged as important homeostatic regulatory molecules in COPD, and we have previously demonstrated the role of these in controlling ASMC proliferation in asthma. We hypothesise that microRNA-145 (miR-145) controls the aberrant phenotype observed in ASMCs from patients with COPD by targeting SMAD3, an important downstream signalling molecule of the TGF- β pathway.

Methods Human primary ASMCs were grown from individuals classified as being healthy non-smokers, healthy smokers, or those with COPD ($n = 9$ per group). Cells were stimulated with TGF- β and foetal calf serum, and miRNA and mRNA expression levels were measured by RT-PCR. IL-6 and CXCL8 release was measured by ELISA. Transfection of miR-145 mimics and inhibitors were used to model the effects of miR-145 over-expression and knock-down, respectively.

Results Low concentrations of TGF- β significantly upregulated SMAD3 expression in ASMCs from patients with COPD. Higher concentrations of TGF- β led to a suppression of SMAD3 expression, with a concomitant increase in miR-145 expression in these cells, to a greater degree than in healthy subjects.

Inhibiting miR-145 in ASMCs from COPD patients reduced the increased IL-6 and CXCL8 release and proliferation back to levels comparable to that of healthy individuals.

Conclusions This is the first time that miR-145 has been demonstrated to be important in controlling the increased inflammatory state of ASM cells from COPD patients. This miRNA may not only act as a novel biomarker for COPD, but may also be a novel target for treatment.

S51 CIRCULATING DESMOSINE RELATES TO CARDIOVASCULAR COMORBIDITY, CORONARY ARTERY CALCIFICATION SCORE (CACS), SYSTEMIC INFLAMMATION AND MORTALITY IN PATIENTS WITH COPD

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