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

1AK Ravi, 5 Khurana, 1A Banyard, 1G Booth, 1M Catley, 1H Healy, 3E Smith, 3J Vealbo, 3D Singh. 1The University of Manchester, Manchester Academic Health Science Centre, University Hospital of South Manchester NHS Foundation Trust, NIHR South Manchester Respiratory and Allergy Clinical Research Facility, Manchester, UK; 2UCB, Slough, UK

Abstract S47 Table 1

<table>
<thead>
<tr>
<th></th>
<th>COPD</th>
<th>S</th>
<th>HNS</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>33.7 (19.3)</td>
<td>3.8 (1.9)</td>
<td>0*</td>
<td>p = 0.0001</td>
</tr>
<tr>
<td>sIL-6R (pg/ml)</td>
<td>5338 (950.3)</td>
<td>4453 (613.2)</td>
<td>4853 (856.8)</td>
<td>p = 0.0005</td>
</tr>
<tr>
<td>CCL3 (pg/ml)</td>
<td>74.8 (111.9)*</td>
<td>0*</td>
<td>0*</td>
<td>-</td>
</tr>
</tbody>
</table>

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.

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 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 was measured on CD14+CD16+ and 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.

Conclusions 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.

Rationale IL-6 is a pro-inflammatory cytokine that signals through soluble (sIL-6R/gp80) 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 (FcegammaRIII): CD14++CD16-, CD14+CD16+ and 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 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 was measured on CD14+CD16+ and 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.

Conclusions 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.
an autologous mixed-lymphocyte reaction (MLR). Lymphocyte proliferation (flow-cytometric measurement of CFSE) and cytokine production (multiplex bead array) were assessed at day 7. The proportion of lymphocytes primed to produce IFNγ 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 IFNγ, TNFα 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 IFNγ 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 ATTENTION IN CIRCULATING WHITE BLOOD CELLS IN COPD RELATES TO LUNG FUNCTION AND OUTCOMES**

1Roberto A Rabinovich, 2Gourab Choudhury, 3Ranil Lakhdar, 4Ellen M Drost, 5Liane McGlynn, 6Jing Bai, 7Paul G Miller, 8Bruce E Miller, 9Alvar Agusti, 10William MacNee.

**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 ± 17.1% predicted), 50 ex-smokers with normal lung function (27 male, age 59.9 ± 7.3 years, FEV₁ 113.2 ± 13.1% predicted). TL was assessed by qPCR and expression 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.

The ECLIPSE study (GSK Study No. SCO104960, NCT00292352) was sponsored by GlaxoSmithKline.