**Data supplement**

**Table E1.** Bronchoalveolar lavage total cell counts for healthy controls (n= 16) and COPD participants (n= 20) undergoing bronchoscopy. Analysis was performed using a Mann Whitney U test. All data is presented as median (IQR) unless otherwise stated.

|  |  |  |  |
| --- | --- | --- | --- |
| Bronchoalveolar lavage cells | Healthy | COPD | p value |
| Macrophages x 10^6 | 4.52 (1.70 – 7.05) | 2.48 (0.54 – 4.55) | 0.0908 |
| Neutrophils x10^6 | 0.1 (0 – 0.3) | 0.2 (0.1 – 0.4) | 0.0988 |
| Lymphocytes x10^6 | 1.40 (0.55 – 2.30) | 0.88 (0.41 – 2.58) | 0.5150 |
| Epithelial cells x10^6 | 0.0 (0.0 – 0.0) | 0.05 (0.0 – 0.05) | 0.0515 |
| Macrophages % | 68.5 (62.50 – 73.53) | 67.18 (55.75 – 76.35) | 0.9803 |
| Neutrophils % | 2.36 (0 – 5.09) | 3.75 (1.90 – 10.61) | 0.3075 |
| Lymphocytes % | 29.13 (20.59 – 37.04) | 18.18 (13.82 – 36.45) | 0.3257 |
| Epithelial cells % | 0.0 (0.0 – 0.0) | 0.0 (0.0 – 2.10) | 0.0561 |
| Total cell count x10^6 | 5.9 (2.7 – 10.8) | 4.45 (1.88 – 6.73) | 0.1712 |

**Figure E1. The effect of human rhinovirus 16 (HRV) on cell viability and phagocytosis of latex beads by monocyte derived macrophages (MDM) from COPD patients.** Monocytes were obtained by adherence and cultured in media supplemented with GM-CSF (panel A and C) or M-CSF (panel B and D) for 12 days prior infection. MDM were infected with HRV at increasing multiplicity of infection (MOI) for 24 hours before exposure to *H. influenzae* (panel A and B) or fluorescently labelled latex beads (panel C and D). Phagocytosis was assessed by fluorimetry. Cell viability was assessed using a thiazolyl blue tetrazolium bromide (MTT) assay and calculated as percent viable cells compared to unstimulated cells. Data are presented in relative fluorescent units (RFU) or percent viable cells where each point represents an individual subject with median and interquartile range.



A

B

C

D

**Figure E2. The effect of human rhinovirus on phagocytosis of bacteria by monocyte derived macrophage from healthy controls.** Monocytes were obtained by adherence and cultured in media supplemented with GM-CSF for 12 days. Monocyte derived macrophages were exposed to HRV16 at a multiplicity of infection (MOI 5) for 24 hours before exposure to *H. influenzae* (panel A) or *S. pneumoniae* (panel B) Phagocytosis was measured using fluorimetry. Data are presented are presented in relative fluorescent units (RFU) where each point represents an individual subject. Analysis was performed using a Wilcoxon paired signed rank test



B

A



**Figure E3. The effect of inhaled corticosteroid treatment on reduction of phagocytosis of bacteria by human rhinovirus 16 (HRV) in alveolar macrophages from COPD patients.** Alveolar macrophages were exposed to HRV16 at a multiplicity of infection (MOI) 5 for 24 hours before exposing to *H. influenzae* (panel A) or *S. pneumoniae* (panel B) for 4 hours. Phagocytosis was measured using fluorimetry. Data are presented are presented in relative fluorescent units (RFU) where each point represents an individual subject. Analysis was performed using a Mann Whitney U test.



A

B

**Figure E4. A Comparison of phagocytosis of bacterial pathogens by alveolar macrophages and MDM from healthy controls and COPD patients.** Alveolar macrophages (Panel A, B) and MDM (Panel C, D) were exposed to fluorescently labelled *H. influenzae* (panel A, C) or *S. pneumoniae* (panel B, D). Phagocytosis was measured by fluorimetry. Data are presented in relative fluorescent units (RFU) where each point represents individual subjects with median and interquartile range. Analysis performed using a Mann Whitney U test where \*= p<0.05 and \*\* = p<0.01.



A



B



C

D



**Figure E5. Analysis of phagocytosis of bacterial pathogens by alveolar macrophages and lung function parameters in COPD patients** Alveolar macrophages were exposed to fluorescently labelled *H. influenzae* (panel A, C and E) or *S. pneumoniae* (panel B, D and F). Phagocytosis was measured using fluorimetry. Data are presented in relative fluorescent units (RFU) where each point represents an individual subject. Analysis was performed using spearman’s rank test.



A

B

C

D



E

F



**Figure E6. The effect of inhaled corticosteroid treatment and smoking status on phagocytosis of bacteria by alveolar macrophages in patients with COPD.** Alveolar macrophages were exposed to fluorescently labelled *H. influenzae* (panel A and C) or *S. pneumoniae* (panel B and D). Phagocytosis was measured using fluorimetry. Data are presented in relative fluorescent units (RFU) where each point represents an individual subject. Analysis was performed using a Mann Whitney U test.



A

B

C

D

**Figure E7. The effect of human rhinovirus 16 (HRV16), Poly I:C, interferon β and interferon γ on phagocytosis of *Haemophilus influenzae* and cytokine response to *Haemophilus influenzae* in monocyte derived macrophages from healthy control participants.** Monocyte derived macrophages were exposed to HRV16 (MOI 5), poly I:C (300 μg/ml), interferon β (10 μg/ml), interferon γ (100 μg/ml) or media control for 24 hours followed by fluorescently labelled *H. influenzae* for 4 hours*.* Phagocytosis was measured by fluorimetry (panel A). IL10 (panel B), CXCL8 (panel C), TNFα (panel D) and IL-6 (panel E) were measured by ELISA. Analysis was performed using Friedman’s test with Dunn’s post-test where \*\*=p<0.01



A

B

D

C

E