Conclusion HIV-1 gp120 influences key airway cell interactions to disturb redox homeostasis and inflammatory responses at concentrations equivalent to those found in the lungs of individuals receiving long-term HAART.

P53 PHOSPHOINOSITIDE-3 KINASE AND MEK INHIBITION PREVENTS UPTAKE OF BACTERIA BY AIRWAY EPITHELIAL CELLS

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Introduction Bacteria are associated with COPD exacerbations with *Haemophilus influenzae*, and *Streptococcus pneumoniae* being predominant. Airway epithelium can protect pathogens from host defences and antibiotic treatment by internalisation. The mechanisms of this are unclear but may involve phosphoinositide-3- kinase (PI3K), p38, and ERK pathways. This requires many primary cells and therefore a validated cell line model would be of benefit. Therefore, we compared uptake of pathogenic bacteria by airway epithelial cells from non-smokers and COPD patients with A549 and BEAS-2B cell lines and the effects of pathway inhibitors were examined.
Methods Non-smoker (n=7) and COPD (n=8) primary airway epithelial cells, and cell lines (BEAS-2B and A549 n=5) were incubated with fluorescently-labelled, heat-killed, *H. influenzae*, *S. pneumoniae*, or *E. coli* for up to 72 hours and uptake fluorimetrically. Confocal microscopy was used to confirm internalisation of bacteria. CXCL8 release was measured using ELISA. Effects of pathway inhibitors were determined by pre-treating cells with increasing concentrations of LY294002 (PI3K inhibitor), VX745 (p38 inhibitor), or PD98059 (ERK pathway inhibitor). Cell viability was assessed by MTT assay.
Results Primary airway epithelial cells internalised respiratory bacteria *spp.* but not *E. coli*, in a time-dependent manner, with COPD cells internalising more *H. influenzae* but not *S. pneumoniae* compared to non-smokers at 48 hour (*H. influenzae* Non-smoker: 0.97±0.23 *vs*. COPD: 2.49±0.7 RFU χ 10^3, $p<0.05$) and 72 hour (*H. influenzae* Non-smoker: 1.44±0.35 *vs*. COPD: 3.01±2.2 RFU χ 10^3, $p<0.05$). A549 cells engulfed more bacteria than primary cells but the responses of BEAS-2B cells were similar to COPD cells and were used for subsequent experiments. Uptake was inhibited by LY294002 and PD98059, but not VX745. Conversely VX745, but not LY294002 or PD98059 inhibited CXCL8 release (Table 1). None of the treatments affected cell viability.
Conclusion COPD airway epithelial cells engulf more *H. influenzae* than cells from non-smokers and this can be modelled by BEAS-2B cells. Uptake appears to require PI3K and ERK pathways but not p38 although, p38 is required for cytokine release. These data suggest that PI3K or MEK inhibitors in combination with antibiotics might be a good therapeutic strategy to treat bacterial exacerbations and recolonisation in COPD.

P54 EOSINOPHIL MIGRATION IS ENHANCED TOWARDS IL-5 AND EOTAXIN IN COPD

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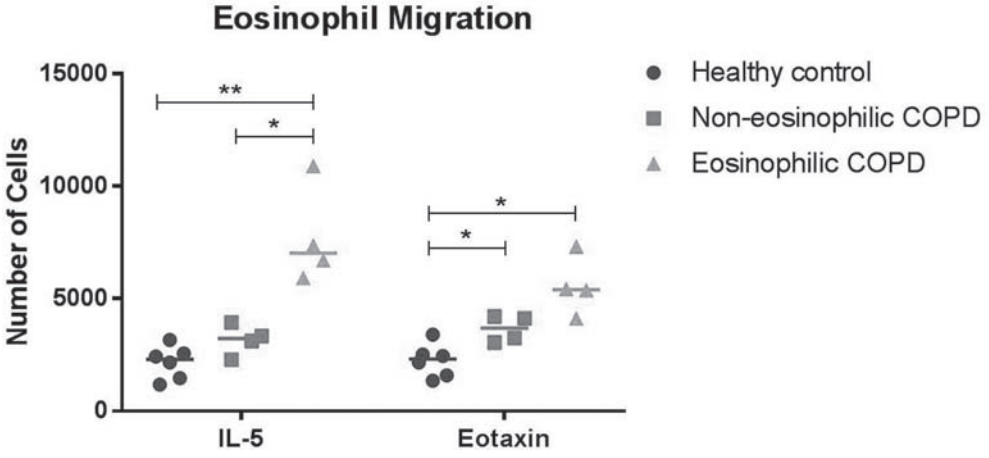
Introduction Eosinophilic COPD is an important inflammatory phenotype, but the mechanism is unknown. In this study we examine the migration of eosinophils in different inflammatory COPD phenotypes towards IL-5 and eotaxin which was unelucidated.
Methods Whole blood from 4 eosinophilic COPD patients, 4 non-eosinophilic COPD patients and 6 healthy controls was collected, with eosinophils isolated by ficoll-paque and dextran Methods Eosinophils were assessed for chemotactic ability. Briefly, eosinophils re-suspended at 1 χ 10^5 cells/ml were placed on a 3 µm pore membrane above solutions of IL-5 and eotaxin (50 ng/ml and 10 ng/ml respectively for one hour). Cells which passed through the membrane were treated with Cell-Titer-Glo solution to allow their detection by plate reader. Eosinophilic COPD was defined as patients with a peripheral blood eosinophil count of ≥2% of white blood cells.
Results Eosinophils from eosinophilic COPD patients showed significantly greater migration than both healthy controls towards IL-5 (median: 7021 cells, IQR: 6098–10 013 v median: 2288 cells, IQR: 1389–2702, p=0.0095) and non-eosinophilic COPD patients, (median: 3219 cells, IQR: 2488–3785, p=0.0286). Eosinophils from eosinophilic and non-eosinophilic patients (median: 5363 cells, IQR: 4101–5420 and median: 3683, IQR: 3091–4179 respectively) showed significantly greater migration towards eotaxin than healthy controls



Abstract P54 Figure 1 Eosinophil migration towards IL-5 and eotaxin.

(median: 2304, IQR: 1519–2723. p=0.0238, p=0.038 respectively) (figure 1).
Conclusion Eosinophils in COPD show enhanced migration towards IL-5 and eotaxin. Whether this is a mechanism underlying T2 high COPD requires further investigation.

P55 EXPLORING RHINOVIRUS-INDUCED ER STRESS IN BRONCHIAL AIRWAY EPITHELIAL CELLS

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Rational and Hypothesis Human Rhinovirus (HRV) infections are major contributors to the increased morbidity burden associated with asthma and COPD acute exacerbations. There are currently no effective treatments or vaccines targeting exacerbations, therefore understanding the host-virus interactions that drive cellular damage will help identify potential therapeutic targets. Viral infections alter the airway environment through increased production of inflammatory mediators, defensive factors and viral proteins. This Results in the upregulation of cellular processes such as the unfolded protein response (UPR), an ER (endoplasmic reticulum) stress pathway that acts to alleviate ER stress caused by increased demands on protein synthesis. In the event that UPR fails to restore cellular homeostasis, pro-apoptotic pathways are activated. Many viruses induce ER stress and have evolved mechanisms to modify UPR to promote their own replication. Interestingly, the mechanisms and consequences of HRV-induced ER stress in bronchial epithelial cells have yet to be explored. We therefore hypothesised that HRV infection induces and manipulates ER stress processes within bronchial epithelial cells.
Objectives To explore the mechanisms and consequences of HRV-induced ER stress within bronchial epithelial cells.
Methods The immortalised bronchial epithelial cell line, BEAS-2B was infected with HRV for 1 hour at MOI 1.5. Induction and subcellular localisation of ER stress markers (GRP78 and ATF4) were measured at various time points by western blotting and confocal microscopy. Tunicamycin (a known ER stress inducer) and filtered HRV were included as positive and negative controls respectively.
Findings Virally infected BEAS-2B cells induced ER stress as evidenced by the significant induction of the UPR chaperone protein, GRP78 at 24 hour. ATF4, a transcriptional activator of UPR target genes, redistributed from a cytoplasmic location to perinuclear regions, as assessed by immunofluorescence and confocal microscopy. Translocation was seen from as early as 1 hour following treatment with Tunicamycin, but this response was relatively delayed in HRV-infected BEAS-2B cells, with ATF4 redistributing to perinuclear regions from 8 hour post infection.
Conclusion Our data demonstrate for the first time HRV-induced ER stress within bronchial epithelial cells, and suggest that HRV may manipulate ER stress pathways to facilitate its own replication.

P56 HUMAN RHINOVIRUS IMPAIRS PHAGOCYTOSIS OF HAEMOPHILUS INFLUENZAE IN ALVEOLAR MACROPHAGES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Introduction COPD exacerbations are the main cause of hospital admission and death in COPD. Respiratory viruses are identified in over half COPD exacerbations with human rhinovirus (HRV) being the most commonly detected. Secondary bacterial infection is associated with prolonged exacerbations, higher rates of hospital admission and increased symptom severity. Our group have previously shown that secondary bacterial infection in HRV induced COPD exacerbations is driven by an outgrowth of *Haemophilus influenzae*.
Hypothesis We hypothesised that HRV may impair phagocytosis of bacteria by alveolar macrophages which may lead to secondary bacterial outgrowth in COPD exacerbations.
Methods Bronchoscopy was performed on participants of the London COPD cohort and healthy controls. Alveolar macrophages were obtained by bronchoalveolar lavage. Alveolar macrophages were incubated with HRV at a multiplicity of infection (MOI) of 5 for 24 hours or media control. Phagocytic capacity was assessed by incubating with fluorescently labelled heat killed *Haemophilus influenzae* or *Streptococcus pneumoniae* for 4 hours. Uptake was measured in Relative Fluorescent Units (RFU) using a fluorimeter.
Results Alveolar macrophages were obtained from 14 COPD patients and 9 healthy controls. HRV significantly impaired phagocytosis of *H. influenzae* by alveolar macrophages in patients with COPD (HRV median 0.97 (0.50–2.17 interquartile range) RFU $x10^3$ vs media control median 1.38 (0.70–2.50 interquartile range) RFU $x10^3$ $p<0.05$) but did not impair phagocytosis *of S. pneumoniae*. HRV did not impair phagocytosis in alveolar macrophages from healthy controls. Baseline phagocytic capacity of H. influenzae was impaired in COPD patients compared to healthy controls (COPD 1.59+/-1.31 RFU $x10^3$ vs healthy control 3.81+/-1.82 RFU $x10^3$). Phagocytosis of H. influenzae correlated with worsening FEV1 percent predicted in COPD ($R^2=0.452$ $p<0.05$).
Conclusions The presence of HRV impaired phagocytosis of *H. influenzae* in alveolar macrophages from patients with COPD but not healthy controls. This may contribute to secondary bacterial infection in COPD exacerbations.

P57 SOLUBLE RECEPTOR FOR ADVANCED GLYCATION END-PRODUCTS (SRAGE) IN PATIENTS WITH COPD: THE ERICA STUDY

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