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 $\times 10^3$, $p < 0.05$) and 72 hour (*H. influenzae* Non-smoker: 1.44 ± 0.35 vs. COPD: 3.01 ± 2.2 RFU $\times 10^3$, $p < 0.05$). 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 1×10^5 cells/ml were placed on a 3 μ m 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 $\geq 2\%$ 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.