

Cell-dissociated *Haemophilus influenzae* and bacteria-associated inflammatory mediators in the airways of patients with chronic obstructive pulmonary disease.

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Background

Patients with COPD have a susceptibility to respiratory tract infections associated with increased pulmonary inflammation. Bacteria can reside within the host as cell-associated (attached to host cells via adhesins, pili or biofilm formation) or cell-dissociated bacteria. It is unclear how bacteria-to-cell interactions affect pulmonary inflammation and whether these levels differ over an exacerbation time course. We sought to investigate the effects of *Haemophilus influenzae* cell-interaction upon airway inflammation and whether the levels of *H. influenzae* bacteria and cell-dissociated bacteria differ over an exacerbation time course.

Methods

Cell differential counts were carried out on sputum samples as per standard protocol. Bacterial DNA was extracted and *H.influenzae* was quantified using qPCR from the sputum plug (contains cell-associated and dissociated bacteria) and the sputum cell-free supernatant (cell-dissociated bacteria only). Inflammatory mediators (IL-1 α , TNF- α , IL-8 and neutrophil elastase (NE)) were measured in the sputum supernatant using commercial assays.

Results

63 patients (77% male; average age of 69 (45-88); FEV₁ percentage predicted of 53%; mean percentage neutrophil count in sputum of 65%) at stable state were analysed. Levels of *H. influenzae* in the supernatant only correlated with the sputum total cell count ($r=0.38$; $p=0.03$). Levels of *H. influenzae* in the plug correlated with inflammatory mediators (sputum neutrophil percentage $r=0.42$, $p=0.01$; sputum macrophage percentage $r=-0.35$, $p=0.04$; IL-1 α $r=0.36$, $p=0.03$; IL-8 $r=0.49$, $p<0.01$; NE $r=0.40$, $p=0.02$). The exacerbation time course in 10 paired COPD subjects was examined. There was no significant difference in *H. influenzae* levels in the plug ($p=0.89$)(Fig 1a). However, there was a significant increase in levels in the supernatant over the exacerbation time course ($p=0.05$) (Fig 1b).

Conclusion

H. influenzae levels in the sputum plug appear to have much more of an effect on airway inflammation than levels of cell-dissociated *H. influenzae* suggesting that cell-associated

bacteria may be a driver of airway inflammation in COPD. Further investigation into this highly complicated relationship needs to be conducted.

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