cocktail to which the organisms were sensitive suggests that Pa was cleared earlier and more effectively in the phage-treated animals; this was confirmed by significant differences in bacterial load at the earlier, 24 hour time point. Further work is underway to explore the therapeutic potential of bacteriophage in pulmonary Pa infection.

## S104 EXPOSURE TO WELDING FUME AND ADHESION OF STREPTOCOCCUS PNEUMONIAE TO A549 ALVEOLAR CELLS

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**Background and Objectives** Epidemiological studies suggest that that occupational exposure to welding fumes (WF), increases susceptibility to invasive pneumococcal disease.[1] The alveoli are a site for invasive disease caused by *Streptococcus pneumoniae* (Sp). To date the mechanism whereby welding fumes increases susceptibility to pneumococcal pneumonia is unknown. Platelet activating factor receptor (PAFR) is a host entry receptor for Sp in airway cells exposed to particulate matter air pollution.[2] We assessed the hypothesis that welding fumes increase adhesion of Sp to airway cells via PAFR.

**Methods** Fume from a mild steel hyperbaric welding operation (WF) were collected from filtres and resuspended in PBS. Monolayers of the alveolar epithelial cell line, A549, were exposed to either WF alone or WF + a PAFR blocker (CV3988) for 3 h. Cells were then exposed to Sp for 2 h. Adherent and internalised bacteria were assessed by quantitative culture. Data were expressed as a mean±SEM of >4 replicates and were compared by t test.

**Results** WF increased pneumococcal adhesion to A549 cells (Fig 1A) in a dose-dependent manner (**p<0.01 vs. control). Blocking PAFR with CV3988 attenuated WF-stimulated adhesion (** p<0.005, Fig 1B).

**Conclusion** Increased PAFR-medicated pneumococcaladhesion to lower airway cells is a putative mechanism for the association between exposure to WF and vulnerability to pneumococcal pneumonia.

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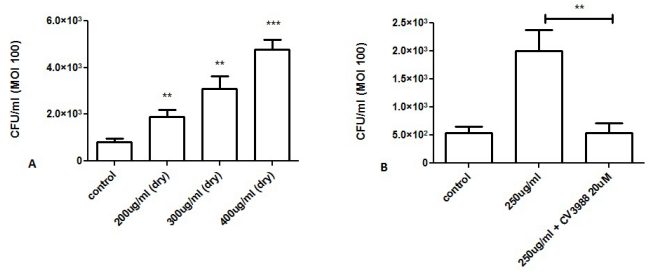


Abstract S104 Figure 1

## S105 ENDOTOXIN SPECIFIC IGG2 ANTIBODIES IMPAIR BACTERIAL KILLING IN NON CYSTIC FIBROSIS BRONCHIECTASIS PATIENTS COLONISED WITH PSEUDOMONAS AERUGINOSA

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**Introduction** We have previously identified patients colonised with *Pseudomonas aeruginosa* (PA), in whom strains isolated from their sputum cannot be killed by their serum, yet are fully sensitive to healthy control serum (HCS). Addition of inhibitory patient serum to HCS also impaired killing of PA.

Fractionation of patient serum identified that IgG was responsible for the inhibition. FACS demonstrated excessive binding of IgG2 from patient serum to autologous strains.

Visualisation of lipopolysaccharide (LPS) isolated from bacterial strains by silver staining, showed that strains from patients with inhibitory serum demonstrated detectable O antigen expression – a component of the LPS cell wall of PA.

We aimed to confirm this relationship between inhibitory IgG2 and LPS expression by PA.

**Methods** Anti-LPS antibodies were removed from inhibitory serum (S4) by binding LPS from PA isolated from patient sputum, to polymyxin-B agarose overnight. Inhibitory serum was then passed over the LPS bound column. Antibodies specific for LPS bound to the column, and the flow through fractions of serum were collected. Bound antibody was subsequently buffer exchanged into PBS.

LPS was isolated from a PA strain resistant to patient sera (B4) and attached to a 96 well plate. ELISA was performed by adding dilutions of patient or HCS to the plate followed by anti-human IgG2 conjugated to alkaline phosphotase. Results were derived 30 minutes after addition of developer.

**Results** LPS removal of IgG from the patient's serum restored bacterial killing. Adding the eluted antibody to HCS impaired killing. This confirmed that inhibitory IgG2 is LPS specific. The patients' serum that blocked bacterial killing had high titres of IgG2 to LPS compared to those from patients with bactericidal serum (fig. 1)

**Conclusion** We have established that PA strains deficient in LPS O antigen are generally sensitive to killing by patients' serum, whilst the presence of O antigen leads to serum resistance mediated by IgG2.

**Results** indicate that PA LPS O antigen repeats are central to serum resistance of autologous strains but not normal HCS. Current data indicates that bacterial killing is impaired in subjects colonised with PA expressing O antigen due to overproduction of LPS specific IgG2.

## S106 INFLUENZA INFECTION OF HUMAN LUNG MACROPHAGES INCREASES PDL1 EXPRESSION

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**Background & Objective** Influenza infection has recently been shown to cause rapid functional impairment of CD8+ T cell responses in a murine infection model via the PD1/PDL1 pathway.[1] In this mouse model, it was the induction of PDL1 that was required for this impairment of CD8+ function. A previous study suggested that the anti-inflammatory cytokine, IL-10, was the principal driver of human macrophage PDL1 expression in response to HIV infection.[2] The aim of this study was to investigate how human lung macrophages regulate their PDL1 expression in response to influenza infection.

**Methods** Alveolar macrophages washed from resected human lung tissue and purified by plate adherence or human positively-isolated CD14+ monocyte-derived macrophages (MDMs) were cultured with H3N2 X31 influenza virus or a UV-irradiated aliquot of virus (UVX31) for 2 h, after which the cultures were washed and media replaced and incubation continued for a further 22 h. Virally infected cells and expression of cell surface markers were identified using flow cytometry. Gene expression was measured using RT-PCR.

**Results** No increase in MDM infection was seen using the UVX31 but incubation with X31 resulted in an average infection rate of 9.1%. Infection with X31 significantly increased cell surface expression of HLA-DR and PDL1 (p<0.05), but not of PDL2 by MDMs as

Figure 1. Patient serum IgG titre specific for LPS isolated from B4 determined by ELISA.

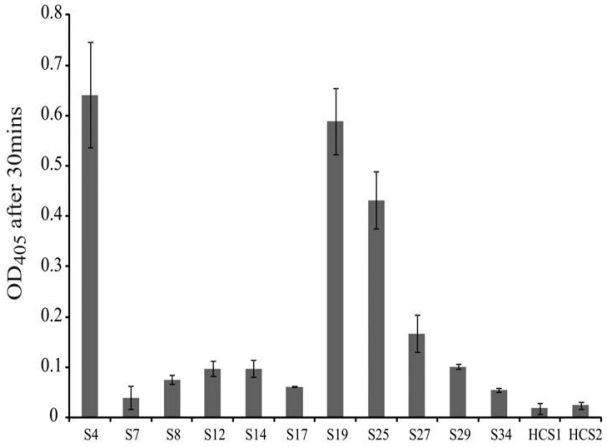


Figure 1: Only the 3 patients who had impaired serum killing (S4, S19 and S25) had high antibody titres to the LPS indicating that the LPS in strains isolated from these patients was responsible for high levels of IgG2 that impairs killing of the PA strain.

Abstract S105 Figure 1

measured by flow cytometry. Using RT-PCR, we observed an increase of PDL1 mRNA after X31 infection suggesting that the expression of this protein is transcriptionally regulated. In addition, we saw an increase in type I interferon expression by MDMs in response to X31 infection, but no expression of IFNγ. In contrast we observed a trend towards decreased expression of IL-10 mRNA. In further experiments, infection of alveolar macrophages with X31 also caused significant increases in HLA-DR and PDL1 cell surface expression.

**Conclusions** These data indicate that, in contrast to HIV infection of macrophages[2] influenza-induced expression of PDL1 may not be regulated by IL-10 in human macrophages.

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## S107 EFFECTS OF EXPOSURE TO CIGARETTE SMOKE CONDENSATE ON PNEUMOCOCCAL GENE EXPRESSION IN RELATION TO BIOFILM FORMATION

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Although cigarette smoking is well-recognised as being the strongest independent risk factor for development of invasive pneumococcal disease, little is known about its direct effects on the expression of virulence factors by the pneumococcus. The primary objectives of the current study were to investigate the effects on gene expression in relation to biofilm formation following exposure of the pneumococcus to cigarette smoke condensate (CSC). Strain 172 (serotype 2–3F) of the pneumococcus was exposed to CSC (20–160 μg/ml) for 16 hours at 37°C in 6-well tissue culture plates to facilitate adherence and biofilm formation. Following incubation, biofilm associated with the adherent bacteria was stained with 0.1% crystal violet, extracted and assayed spectrophotometrically. In the case of gene expression, the bacteria (2 x $10^8$ colony forming units/ml) were exposed to CSC (160 μg/ml) or solvent for 60 min at 37°C, after which RNA was extracted and converted to cDNA by reverse transcriptase-PCR (RT-PCR) and whole genome gene expression profiles determined using the *Streptococcus pneumoniae* TIGR4 DNA Microarray Chip. Six microarrays were performed (in triplicate for the control and CSC-treated systems). Exposure of the pneumococcus to CSC resulted in dose-related augmentation of biofilm formation which attained statistical significance ($P<0.05$) at concentrations of 80 and 160 μg/ml. CSC-mediated augmentation of biofilm formation was associated with selective and significant up-regulation of the genes encoding the two-component 11 system (TCS11), consisting of the genes *hk11* (histidine kinase) and its cognate response regulator, *rr11*, which has been implicated in biofilm formation by *S. mutans*. Relative to the non-exposed control system, the respective levels of up-regulation of each gene were 19.7- and 22.5-fold ($P<0.001$ and $P<0.0006$). Induction of biofilm formation, probably as a stress response resulting in activation of TCS11, may underpin cigarette smoke-mediated colonisation of the respiratory tract by the pneumococcus.

# Evaluating impact in pulmonary rehabilitation

## S108 THE EFFECT OF AN INTERDISCIPLINARY REHABILITATION PROGRAMME ON DAILY PHYSICAL ACTIVITY FOR PATIENTS WITH LESS ADVANCED COPD IN A PRIMARY CARE SETTING: A SYSTEMATIC REVIEW

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