S101 INTENSIVE CARE UNIT ADMISSION SHOULD PLAY A ROLE IN THE MANAGEMENT OF SELECTED PATIENTS WITH INTERSTITIAL LUNG DISEASE

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Introduction and Objectives Little is known about outcomes of patients with interstitial lung disease (ILD) requiring emergency admission to the intensive care unit (ICU). Historically, based on data for patients with idiopathic pulmonary fibrosis (IPF), in whom outcome is poor, there has been a reluctance to provide advanced support for individuals with ILD. The aim of this study was to evaluate outcomes of patients with ILD admitted to ICU.

Methods A retrospective review of electronic patient records, ICU database and clinical notes was undertaken for all patients with confirmed ILD admitted to ICU between January 2006 and June 2012 in a single tertiary ILD referral centre. Patients admitted electively for bronchoscopy in a high-dependency environment (n=10, no complications) were not included.

Results We identified 35 patients (21 male), mean age 48 (range 18–76) years. Of these, 20 were inpatients referred by the ILD team at the same hospital, 6 were admitted following deterioration after a thoracic surgical procedure and 9 were directly transferred from other hospital ICUs.

The most common diagnoses were connective tissue disease -associated ILD (6), pulmonary alveolar proteinosis, PAP (4), sarcoidosis (4), IPF (4) and hypersensitivity pneumonitis (3).

Twenty-seven (77%) patients received invasive mechanical ventilation, 4 of whom also required extracorporeal support for gas exchange. The majority of patients received immunosuppressive therapy with corticosteroids, and over one-third received cyclophosphamide and/or rituximab whilst in ICU.

Overall, 21 (60%) survived to ICU discharge, only 1 of whom died before hospital discharge. Sixteen (47%) patients survived to 6 months. All 4 patients with IPF died before hospital discharge. All patients with PAP survived to 6 months. ICU length of stay for survivors was 27 (range 1–127) days and for non-survivors was 17 (1–48) days.

Conclusions Our series suggests that aggressive supportive and disease-targeted management of selected patients with ILD is appropriate, and such patients should therefore be considered for transfer to a specialist unit.

Lung infection mechanisms

S102 THE EFFECT OF INFLUENZA INFECTION ON BLEOMYCIN INDUCED PULMONARY FIBROSIS

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Introduction Transforming Growth Factor-beta (TGFβ) promotes anti-proliferative and pro-apoptotic pathways in lung epithelial cells, both of which have been implicated in the pathogenesis of IPF. TGFβ must be activated before it can mediate these events. Acute exacerbations of IPF are characterised by widespread epithelial cell apoptosis. The precise cause of these exacerbations is not known. The Influenza A virus is a single-stranded segmented RNA virus that infects epithelial cells leading to cell death and injury, and can also activate TGFβ. The role of infection in acute exacerbations of IPF is unclear. The aim of this study is to investigate the effect of influenza infection on bleomycin-induced pulmonary fibrosis and TGFβ activation in vivo.

Materials and Methods 60 U of bleomycin was instilled into the lungs of 6–8 week old male C57Bl/6 mice. After 28 days mice were exposed intranasally with 10, 20 Units of influenza virus 'x31' or PBS, and the lungs harvested 5 days later. Bronchoalveolar lavage (BAL) was performed and lung tissue harvested for mRNA analysis, histology and hydroxyproline levels. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

Results Mice exposed to bleomycin and infected with influenza lost less weight compared with saline-exposed influenza-infected animals. However, the lungs from bleomycin-exposed, influenza-infected mice showed increased lung damage with more matrix deposition on trichrome staining than saline-exposed, influenza-infected mice. Saline-exposed, influenza-infected mice demonstrated the anticipated dose dependent increase in BAL lymphocytosis as well as apoptosis staining in histological TUNEL assessment. However, in bleomycin-exposed mice, influenza infection did not promote enhanced BAL lymphocytosis or apoptosis. However, influenza appeared to enhance the fibrotic response demonstrated by an increase in matrix deposition on masson's trichrome and increased lung hydroxyproline levels in influenza infected bleomycin exposed mice, as early as 5 days post infection.

Conclusions These data suggest that influenza infection may exacerbate lung fibrosis by promoting epithelial apoptosis.

S103 ANTI-PSEUDOMONAL BACTERIOPHAGE COCKTAIL REDUCES INFLAMMATORY RESPONSES IN THE MURINE LUNG

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Bacteriophages are naturally occurring viruses that specifically target and infect bacteria and, unlike antibiotics, are able to multiply at infection sites and adapt to resistant bacteria. We hypothesise that bacteriophage cocktails may be useful against *P. aeruginosa* (Pa) in cystic fibrosis and tested this in a murine infection model.

Two strains of Pa were assessed: a) a clinical strain from an adult CF patient, b) a laboratory strain. Both strains were shown to be sensitive to a novel anti-Pseudomonal bacteriophage cocktail on a standard plaque assay.

Adult BALB/c mice were inoculated intranasally with 50ul of Pa followed by 20ul of bacteriophage cocktail (treated, n=21) or SM buffer (control, n=21). Twelve mice were sacrificed at 24hrs after infection and the others at 48hr. Bronchoalveolar lavage was serially log diluted, cultured at 37°C and the remainder centrifuged and supernatant stored at –80°C for future analysis of soluble inflammatory markers. Total cell counts were determined using a haemocytometer. Non-quantitative splenic cultures were performed.

Results All mice treated with bacteriophage (n=6) had cleared infection at 24hrs compared with none of the controls (n=6) (median [range] CFU/ml 0 [0–0] vs. 1305 [190–4700], $p<0.01$); inflammatory cell counts did not differ. At the 48hr time point most mice had cleared the infection, with no phage-related differences. However, treated mice demonstrated significantly fewer inflammatory cells in BAL compared with controls (median [range] 4.50 [2.84–5.86] × 10^4/ml vs. 9.12 [6.93–13.86], $p<0.01$ for the clinical strain; median [range] 6.04 [5.56–10.60] × 10^4/ml vs. 9.72 [8.56–15.28], $p<0.01$ for the laboratory strain). Differential cell counts and measurements of soluble inflammatory mediators are underway.

BALB/c mice successfully cle ared this dose of Pa by 48hrs, with an accompanying acute cellular inflammatory response. The reduction in cell number following co-administration of a bacteriophage

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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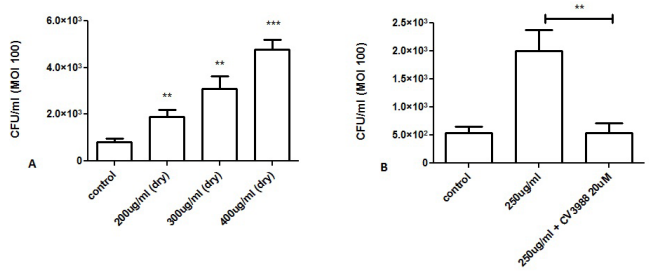

Abstract S104 Figure 1

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