nose- and throat-swabs for the diagnosis of respiratory viruses in adults with CF.

Methods 100 adults with CF provided sputum, nose- and throat-swabs every 2 months between December 2010 and June 2011 within a prospective observational study. Samples were repeated if new respiratory symptoms developed between regular visits. Specimens were analysed using PCR assays for adenovirus, influenza, metapneumovirus, parainfluenza, respiratory syncytial virus and rhinovirus. Positive viral identification rates for each specimen type were compared. FEV₁, inflammatory markers and symptom scores for upper respiratory tract infection (URTI) and pulmonary exacerbation (PE_x) were recorded at each visit.

Results 210 sets of samples were collected. A respiratory virus was identified at 52 (24.8%) visits of which rhinovirus accounted for 63%, influenza A 10.5%, metapneumovirus 9% and influenza B 7%. Among virus-positive cases, sputum was positive in 34 (65%), nose swab in 25 (48%) and throat swab in 21 (40%). A single specimen type was positive in 32 (62%) cases; two specimens in 12 (23%) and all three specimens in only 8 (15%). Sputum alone was positive in 17 (33%) cases, nose-swab alone in 8 (15%) and throat-swab alone in 7 (13%). An increasing number of positive specimens was associated with higher mean (SD) URTI scores (4.9 (5.0) vs 6.7 (6.6) vs 10.3 (5.5) for 0, 1 and =2 positive specimens respectively; $p=0.046$ for =2 vs 1 specimens) and higher PE_x scores (2.8 (2.8) vs 3.2 (2.2) vs 5.1 (1.5); $p=0.002$). FEV₁, CRP and WCC were similar between these groups. There were no significant differences in lung function, symptoms or inflammatory markers when viruses were detected in sputum compared with the upper airways.

Conclusions Sputum is superior to nose- and throat-swabs for the diagnosis of respiratory viruses in adults with CF but all three are required for optimal identification rates. Viral positivity in =2 specimens is associated with higher upper and lower respiratory symptom scores.

S47 PSEUDOMONAS AERUGINOSA INDUCES APOPTOSIS IN HUMAN DENDRITIC CELLS: A POTENTIAL MECHANISM TO EVADE PULMONARY IMMUNE RESPONSES

doi:10.1136/thoraxjnl-2011-201054b.47

H K Bayes, T Evans. *Institute of Infection, Immunology & Inflammation, University of Glasgow, Glasgow, UK*

Introduction Airway colonisation with *Pseudomonas aeruginosa* (PA) represents a hallmark of cystic fibrosis (CF). Mechanisms by which PA establishes pulmonary infection are undoubtedly complex and, in part, will reflect the capacity to interfere with host defense mechanisms. Dendritic cells (DCs) represent the most potent antigen-presenting cells in the lungs, with the unique ability to prime naïve T cells. PA-induced apoptosis has been demonstrated in epithelial cells and macrophages. We examined the capacity of PA to induce cell death in human dendritic cells, the cytotoxicity of clinical PA isolates, and the impact on antigen-presenting capacity.

Methods CD14⁺ monocytes were isolated from peripheral blood of healthy controls ($n=11$) and individuals with CF ($n=5$). Monocyte-derived DCs were generated by culture in the presence of IL-4 and GM-CSF. DCs were infected with live PA including isogenic laboratory strains of PA103 and PA isolates derived from the sputum of patients with CF. Heat-inactivated PA were utilised to evaluate the role of bacterial membrane components. Presence of early apoptosis and established cell death was analysed via annexin-V and 7-AAD incorporation, respectively. Cytotoxicity was further demonstrated via LDH release into the supernatant. Co-stimulatory molecules, CD40 and CD86, were measured via flow cytometry.

Results PA readily induced apoptosis and cell death in human DCs, with cytotoxicity seen within 3 h of infection. Induction of apoptosis by PA was an active process requiring live organisms, but was not dependent on a functional type III secretion system. A significant decrease in viable DCs was seen in response to infection with clinical PA strains at 3 h and 20 h compared with laboratory PA103 strains ($p<0.05$ and $p<0.001$, respectively). Due to increased cytotoxicity of clinical PA isolates, post-infection DCs demonstrated no increase in co-stimulatory molecule expression compared with uninfected DCs ($p>0.05$).

Conclusions These data demonstrate that human dendritic cells are susceptible to apoptosis induced by *P. aeruginosa*, with clinical isolates of PA demonstrating high levels of cytotoxicity, and a subsequent reduction in DC antigen-presenting capacity. Elimination of these important antigen-presenting cells could lead to impairment of immune responses and thus a factor in the establishment of chronic PA colonisation in the CF lung.

S48 MUCOIDY AND THE MICROBIOME: COMMUNITY COMPOSITION IN RELATION TO THE PRESENCE OF CULTURABLE, MUCOID PSEUDOMONAS AERUGINOSA

doi:10.1136/thoraxjnl-2011-201054b.48

¹M J Cox, ²A L Jones, ²M R Loebinger, ¹R M Duff, ²N J Simmonds, ²J C Davies, ²R Wilson, ²E W Alton, ¹W O C M Cookson, ²D Bilton, ¹M F Moffatt. ¹National Heart and Lung Institute, Imperial College, London, UK; ²Royal Brompton and Harefield NHS Foundation Trust, London, UK

Introduction and Objectives Cystic fibrosis (CF) and non-cystic fibrosis bronchiectasis (BX) are chronic airway diseases with significant microbial involvement. The presence of mucoid *Pseudomonas aeruginosa* (mPA) is associated with worse outcomes in these patients. We hypothesised that the presence of this organism would also influence the microbial community structure. We used high throughput sequencing to identify microorganisms present in sputum from these patients, and to associate culture data of mPA with this analysis.

Methods Expecterated sputum was collected from 16 patients (9 CF, 7 BX) and DNA extracted using standard protocols. PCR of the bacterial 16S rRNA gene and subsequent sequencing using a Roche 454 GS Junior sequencer was compared with standard clinical culture. Non-parametric t test was used to identify significant differential abundance of taxa in the molecular data (Bonferroni-corrected).

Results In the pilot study, 35 000 high quality 16S rRNA sequences were generated and could be assigned to 240 different bacterial taxa. Both CF and BX samples were dominated by the γ -proteobacteria. The presence of culturable *Paeruginosa* in a sample had no significant effect on either community composition or structure. However, Principal Coordinate Analysis indicated that the presence of mPA in clinical culture was associated with a different community structure. Though there were no significant differences between the diversity of mPA positive samples ($p>0.15$), there were significant differences in the abundance of particular species; *Achromobacter* spp. and *Pseudomonas* spp. increased in relative abundance ($p<0.001$ in each case) and *Haemophilus* spp., *Stenotrophomonas* spp. and *Staphylococcus* spp. decreased in mPA positive vs mPA negative samples ($p<0.001$ in each case).

Conclusions Deep sequencing of sputum samples from CF and BX patients revealed a relationship between culture positivity for mPA and the presence of other known pathogens such as *Achromobacter* spp., suggesting an alternative mechanism for worse outcomes in these patients. This pilot has been extended to a larger cohort of 120 patients in order to confirm the result and data will be presented at the meeting.