P104 T HELPER 1 AND T HELPER 22 CELL RESPONSES AGAINST PSEUDOMONAS AERUGINOSA INFECTIONS

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Introduction *Pseudomonas aeruginosa* is an important opportunistic respiratory pathogen responsible for ventilator-associated pneumonia, acute lower respiratory tract infections in immunocompromised patients and chronic respiratory infections in cystic fibrosis. Antibody-mediated adaptive immune responses exist in cystic fibrosis patients but these lack protective opsonic activity. The role of $CD4^+$T cell responses to Pseudomonas remains unclear. Novel T helper (Th) cell subsets have also recently been defined; Th22 cells produce IL-22, whereas Th17 cells produce IL-17 cytokines in addition to IL-21 and IL-22. The properties of IL-22-producing $CD4^+$ cells in humans remains to be defined, but include an important respiratory anti-microbial defense function. Immunopathological roles of Th17/Th22 cells may also exist. We assessed T-helper cell responses to Pseudomonas in healthy adults.
Methods $CD14^+$ monocytes and memory $CD4^+CD45RO^+$T cells were isolated from peripheral blood using magnetic-activated cell sorting. Monocyte-derived dendritic cells (DCs) were generated by culture in the presence of IL-4 and GM-CSF. DCs were stimulated with live Pseudomonas aeruginosa strain PA103. DC activation markers, CD40 and CD86, were measured via flow cytometry. Autologous T cells were co-cultured with activated DCs, or their supernatants, for 6-days. T cell phenotype was analysed via flow cytometry and ELISA of supernatants for secreted cytokines. T cell incorporation of carboxyfluorescein-succinimidyl-ester was utilised to demonstrate cell multiplication.
Results We demonstrate that monocyte-derived DCs from healthy adults are readily activated by Pseudomonas. Co-culture of autologous memory $CD4^+$T cells with Pseudomonas-activated DCs resulted in $CD4^+$ T cells primarily producing IFN-gamma, as well as T cell subsets expressing both IFN-gamma and IL-22, or IL-22 alone. No Th17 response to Pseudomonas was evident. The response was MHC-restricted and specific T cell proliferation was demonstrated. Reproducibility of the response within individuals was also shown.
Conclusions These data demonstrate that healthy adults possess Th1 and Th22 memory cell responses to *Pseudomonas aeruginosa*. The results may have important implications for patients with deficits in cellular immunity and the pathogenesis of chronic pseudomonas infection in cystic fibrosis. Further work is required to ascertain cross-reactivity of response, antigenic triggers, and the role these cellular responses play in protection vs pathogenesis in Pseudomonal pulmonary infections.

P105 BURKHOLDERIA LATENS—A NEW INFECTION IN CF PATIENTS

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Background Burkholderia latens was first recognised as a novel species of the Burkholderia cepacia complex (Bcc) in 2008. To date, there are no reports of pulmonary infection. Here, we report a cluster of three CF patients all with chronic infection by *B latens*, and all geographically linked.
Patient details Three patients have been identified as chronically infected with *B latens*: a 23-year female (FEV_1 23% predicted), a 23-year M (FEV_1 55%), and a 26-year female (FEV_1 37%). The first two live within 1 mile of each other, and had been known to be infected with Bcc since childhood, though the organism was only finally identified as *B latens* in 2009. The third patient has been infected since May 2008, but had lived in the same town as the other patients until 2 years previously, and had attended the same paediatric centre.
Microbiology All *B latens* isolates were confirmed by recA sequencing at the national reference laboratory (Colindale), and PFGE profiles showed >90% similarity. Multiple isolates have been obtained from each of the three patients, and they are all considered chronically infected. Formal eradication has not been attempted.
Clinical impact Over the preceding 3 years, annual decline in FEV_1 was 6.5% and 4.5% for the first two patients, though this deterioration was not attributed to Bcc. Annual decline in FEV_1 for patient 3 was 1.5%, with no significant change in spirometry, rate of decline in spirometry or weight following isolation of *B latens*. There have been no cases of cepacia syndrome sepsis secondary to *B latens*.
Summary This is the first report of chronic pulmonary infection by *B latens*. All three subjects are geographically linked to the same town. The organism may come from a common environmental source, but cross infection cannot be ruled out. In this small sample there do not appear to have been any short-term unexpected adverse effects on lung health (patient 3), but it is not yet possible to infer the longer term effects of chronic *B latens* infection.

P106 INFLAMMATORY MARKERS: DATA FROM THE UK CF GENE THERAPY CONSORTIUM RUN-IN STUDY

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Inflammatory markers in sputum and serum have been used with variable success as outcome measures in interventional studies. Limited data are available on reproducibility of such assays in cystic fibrosis (CF) particularly over a long time period. This study was designed to address this; stable patients (FEV_1>40, >10 years age) were recruited into the study which ran over an 18-month period with up to four hospital visits. Patients provided a 24 h sputum collection, for weighing at each visit. Spontaneous sputum was collected at the beginning of each visit; if insufficient sample was obtained, sputum was induced with hypertonic saline. Inflammatory markers were measured in dithiothreitol-processed sputum (total and differential cell counts, IL8 (and other cytokines), calprotectin, neutrophil elastase, myeloperoxidase and extracellular DNA). Blood was collected at each visit for cytokines (IL1β, IL6, IL8, IL10, IL12 (p40) and TNFα) and calprotectin. Data are available from 189 patients at 655 visits. Adequate sputum for analysis was obtained at only 60% of visits. Sputum induction accounted for only 16% of adequate samples. Median and range for each detectable assay are shown below. Serum cytokines IL1β, IL10, IL12 (p40) and TNFα were undetectable at each visit, and IL6 was only detectable in 17% of samples. To assess intra-individual variability the coefficient of variation of results across each visit for each patient is presented. Both sputum and serum assays showed a large range of results at each visit, but the variation for each individual was much higher than the ideal 10%. Serum assays were not able to discriminate between CF and non-CF, apart from calprotectin (CF 10.1 (0.8–72.5) vs non-CF 0.40 (0.2–1.12) $p<0.001$). Due to the difficulty in obtaining sputum samples reliably and the large variability of results between visits in these stable patients, we consider it unlikely that a change due to a new therapy would be detectable. As such, we are not considering sputum inflammatory markers as primary or secondary efficacy endpoints in our multidose gene

therapy trial. Serum markers also appear to be of limited use is assessing efficacy but will still be useful for toxicology and safety studies.

Abstract P106 Table 1

Assay	Visit 1 (median (range))	Visit 2 (median (range))	Visit 3 (median (range))	Visit 4 (median (range))	Intra-individual CV (%)(median (range))
Total cell counts (cells/g sputum)	1.0 (0.1 -7.6)	1.1 (0.1-15.2)	1.0 (0.2-7.4)	1.1 (0.2-7.2)	56 (1-129)
Sputum Neutrophil (%)	97.3 (36-100)	98.3 (71-100)	99.0 (77.3-100)	98.3 (90.7-100)	1.3 (0-12.8)
Sputum IL8 (ng/ml)	14.9 (2.3-57.2)	14.6 (3.2-37.2)	13.2 (2.4-49.5)	15.5 (2.3- 48.0)	30 (5-80)
Sputum Calprotectin (mg/ml)	1.48 (0.19-4.77)	1.63 (0.11-5.29)	1.26 (0.20-4.38)	1.69 (0.20-5.25)	30 (4-86)
Sputum Neutrophil Elastase (mU)	868 (32-3788)	884 (32-4104)	947 (32-4104)	1010 (95-4261)	34 (0-97)
Sputum Myeloperoxidase (µg/ml)	19.9 (3.6-78.8)	18.7 (1.8-69.4)	20.9 (3.3-53.3)	22.4 (5.2 -73.4)	32 (2-85)
Sputum Extracellular DNA (µg/ml)	29.8 (1.8-128.8)	28.0 (2.5-154.4)	26.9 (1.6-96.9)	34.1 (0.6-171.2)	33 (5-101)
24hr sputum weight (g)	7.65 (0.10-143.3)	6.16 (0.53-127.99)	7.07 (0.04-128.64)	8.33 (0.19-117.48)	45 (4-134)
Serum IL8 (pg/ml)	2.9 (0.6-40.3)	2.7 (0.6-49.0)	2.5 (0.5-44.7)	2.8 (0.8-44.5)	26 (2-95)
Serum calprotectin (µg/ml)	12.1 (1.9-72.5)	10.8 (1.6-60.1)	8.4 (0.8-61.8)	8.3 (1.1-56.2)	36 (2-153)

P107 PULMONARY IMAGING TECHNIQUES TO IDENTIFY SUITABLE PATIENTS AND ACT AS OUTCOME MEASURES IN THE UK CF GENE THERAPY CONSORTIUM CLINICAL PROGRAMME

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We are conducting a large, longitudinal study to assess outcome measures and identify optimal patients for a multidose trial of CF gene therapy. Two imaging modalities are being employed: radioisotope deposition scans and high resolution CT. Subjects have undergone these scans on a single occasion, whilst clinically stable. The purpose was: *Deposition scan*—to determine which patients would be most optimal for topical drug delivery and *CT*—to assess the suitability of various parameters as efficacy measures. Following inhalation of ^{99m}Tc-labelled human serum albumin, planar gamma camera images and SPECT were used to assess 3-D deposition. Images were scored both digitally and visually (I- no defects; II- patchy deposition; III- patchy deposition with large defects; IV- grossly abnormal). HRCT scans were scored by two radiologists on a lobar basis for the following: bronchiectasis (extent/severity), airway wall thickening, mucus plugging and gas trapping. 147 deposition scan were available; digital indices (DI) ranged from 34 (best) to 150 (severely abnormal). Visual scores correlated well with DI (R2 0.63; $p<0.001$) and both were significantly negatively correlated with FEV_1% ($p<0.01$). Nine Grade IV subjects had a mean (SD) FEV_1 of 43.9(4.3)%, significantly lower than groups I-III ($p<0.01$). On the basis of very poor deposition, this group is considered unsuitable to progress to the trial. Others have deposition scans which suggest that the gene therapy product could be delivered at least moderately well; they will be filtered through other inclusion/exclusion criteria. Potentially reversible components of the HRCT scores are being considered as efficacy outcome measures. As an example, power calculations suggest that our anticipated group size (n=100) would have 80% power to detect a change in wall thickness half that seen with intravenous antibiotics in a previous study. In conclusion, lung imaging techniques have both aided us in the identification of patients to take through into our multi-dose trial and are currently under consideration as efficacy outcomes.

P108 PHENOTYPIC CHANGES IN PSEUDOMONAS AERUGINOSA (PSA) POPULATIONS DURING EXACERBATIONS IN ADULT CYSTIC FIBROSIS PATIENTS INFECTED WITH NON-EPIDEMIC STRAINS

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Background Chronic infection with Psa in most CF patients is due to single unique strains, which over time accumulate mutations resulting in mucoid conversion, loss of motility, auxotrophy and increased antibiotic resistance, in turn leading to multiple phenotypes. However, changes in Psa population morphology related to short term pressures (eg, during IV antibiotic-treated exacerbations), have not previously been studied. We therefore looked at Psa population structure during exacerbations.

Methods Sputum samples from four CF patients chronically infected (for at least 4 years) with unique single Psa strains were analysed at the beginning and end of an intravenous antibiotic-treated exacerbation, where every patient had subjective and spirometric improvement. From each sample, 40 single Psa colonies were selected (with every morphological type proportionately represented), and colony morphology, susceptibility to six antibiotics (ciprofloxacin, ceftazidime, colomycin, meropenem, tobramycin, tazocin), hypermutability (rate of spontaneous mutation to rifampicin resistance) and auxotrophy (ability to grow on glucose M9 media) determined. Additionally, the density of Psa (colony forming units (CFU) per ml) in sputum samples was measured.

Results Although the predominant colony morphology changed from green, non-mucoid and smooth (mean 67%, range 43–98) to straw-coloured, non-mucoid and smooth (57%, 5–95) ($p=0.001$), there was no change in mean antibiotic resistance to all antibiotics (21.5% vs 20.3%, $p=0.9$), prevalence of hypermutable isolates (38% vs 25%, $p=0.48$) and auxotrophic mutants (66% vs 98%, $p=0.17$). However, there was an increase in Psa sputum density (mean CFU/ml 1.3×10^5 vs 2.0×10^6, $p<0.001$), despite the use of relevant antimicrobial therapy.

Conclusion The changes in prevalent population composition following antibiotic pressure, associated with clinical improvement, might suggest that some morphotypes alone are responsible for the adverse clinical features. Conversely, the increase in sputum density of Psa despite objective clinical improvement implies that the exacerbation has occurred independently of the presence of the organism, supporting the observation that clinical improvement is often seen in CF patients even where the Psa seems resistant to the administered antibiotics. Further work need to be done to tease out the role of Psa in clinical exacerbations of CF patients with chronic infection.

P109 EXERCISE CAPACITY AND PHYSICAL ACTIVITY IN PATIENTS WITH CF: DATA FROM THE UK CF GENE THERAPY CONSORTIUM (UKCFGTC) 'RUN-IN' STUDY

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Introduction Exercise capacity is predictive of mortality in CF. Objective measurement of daily physical activity may be related to exercise capacity and both may be useful outcome measures in