Results Healthy individuals andpatients with cystic fibrosis had robust antigen-specific memory CD4$^+$ T cell responses to *Pseudomonas aeruginosa* that not only contained a Th1 and Th17 component but also Th22 cells. In contrast to previous descriptions of human Th22 cells, these Pseudomonal-specific Th22 cells lacked the skin homing markers CCR4 or CCR10, although they did express the chemokine receptor CCR6 that would direct migration to damaged epithelial surfaces. Furthermore, IL-22 production was evident in the lungs of CF patients colonised with PA. Healthy individuals and patients with cystic fibrosis had similar levels of Th22 and Th1 cells, but the patient group had significantly fewer Th17 cells in peripheral blood.

Conclusions MemoryTh22 cells specific to *Pseudomonas aeruginosa* are induced in both healthy individuals and patients with CF, with IL-22 secretion being demonstrated in the CF lung. These Th22 cells do not express tissue specificity for gut or skin sites and we thus hypothesise may have a role in respiratory defense. Along with Th17 cells, they may play an important role in the pathogenesis of pulmonary infection with this microbe in patients with cystic fibrosis.

S101 PATHOGEN ASSOCIATED MOLECULAR PATTERNS IN CYSTIC FIBROSIS PATHOGENS: ANALYSIS OF PEPTIDOGLYCAN STRUCTURE

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Background Pathogen associated molecular patterns (PAMPS) are key virulence determinants in triggering immune mediated damage in cystic fibrosis. The lipopolysaccharide (LPS) in *Pseudomonas aeruginosa* strains isolated from CF-lungs has "CF specific" structural motifs not seen in other clinical isolates nor environmental strains. We have shown these motifs are also present in *Burkholderia* cepacia complex (BCC) species. We hypothesised that another key virulence determinant, peptidoglycan (PG), may show similar CF specific changes. PG is essential for bacterial cell wall stability and is the principle target for many antibiotics and for antimicrobial hydrolases of the innate immune system. Soluble PG fragments show biological activity interacting with NOD receptors.

Methods *Burkholderia* cepacia complex strains of interest were selected from the BCC international reference panel (n = 8), *P. aeruginosa* from a novel international reference panel and local repository (n = 6) and *Achromobacter xylosoxidans* from a local repository (n = 6). After growth in LB media and SDS extraction the PG was digested and the resulting muropeptides were analysed by HPLC and MS. The profiles were compared to the well characterised *E. coli* PG.

Results *P. aeruginosa* PG was similar to the archetypical PG from *E. coli*. Strikingly, BCC spp. PG contained amidated muropeptides that have previously been described only in some Gram-positive bacteria. The extent of amidation varied between strains and did not map to either CF, other clinical or environmental origins. It was not linked to LPS chemotype nor BCC genomovars. *A. xylosoxidans* PG also contained novel muropeptide structures different to those seen in BCC.

Conclusions We describe for the first time novel and unexpected modifications in the PG of BCC and *Achromobacter*, which were present in all strains tested so far. These modifications may offer either a biological advantage against PG hydrolases or may be linked with modulating the host immune responses. Immunostimulatory data are presently being collected to investigate this aspect.

S102 THE EFFECT OF RESPIRATORY VIRUSES ON THE LUNG MICROBIOME OF ADULTS WITH CYSTIC FIBROSIS

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Introduction Viral respiratory infection (VRI) has been implicated in the pathogenesis of cystic fibrosis (CF) lung disease and it has been hypothesised that respiratory viruses may predispose CF patients to acquisition of bacterial pathogens. We performed a prospective observational study to determine whether VRI leads to a change in the respiratory microbiome of adults with CF.

Methods Participants provided sequential paired sputum samples over a ten month period. One sputum sample from each visit was processed using conventional culture. The second sample was analysed with 16S rRNA gene pyrosequencing and ribosomal intergenic spacer analysis (RISA) to examine bacterial diversity. Polymerase chain reaction assays for nine respiratory viruses were also performed on the second sample. Study visits were classified as "stable," "viral" or "non-viral exacerbation." Generalised estimating equation models were used to examine differences between these groups of study visits. Statistical analysis accounted for repeated observations from individual patients.

Results Eighteen patients provided a total of 77 paired sputum samples over a median follow-up period of 290 days (range 62–359). 23/46 (50%) study visits were positive for a respiratory virus with rhinovirus accounting for 56% of cases. 19/46 (41%) study visits met the pre-specified criteria for pulmonary exacerbation of which 11 (58%) were virus-positive and 8 (42%) were virus-negative.

Conventional culture did not identify any new bacterial species during the study period. A mean of 5453 (SD 2847) reads per sample were detected by 16S rRNA gene pyrosequencing. The mean Shannon Index was 0.59 (SD 0.58) with a mean richness of 15.1 (SD 7.9) genera. No consistent change in bacterial diversity indices or relative abundance of individual genera was seen in response to either VRI or non-viral exacerbations. The majority of patients had highly variable RISA and 16S rRNA gene profiles during follow-up. A subset of four patients (22%) had a stable respiratory microbiome which was heavily dominated by *Pseudomonas aeruginosa* throughout despite episodes of VRI, pulmonary exacerbation and antibiotic therapy.

Conclusions Longitudinal change of the respiratory microbiome varies considerably among adults with CF. No consistent effect of either VRI or pulmonary exacerbation on the lung microbiome was observed.

S103 NEUTROPHIL TRANS-EPITHELIAL MIGRATION IN CYSTIC FIBROSIS AIRWAYS

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Background Lung injury in cystic fibrosis (CF) is caused by recurrent airway infection and inflammation partially due to the massive infiltration of neutrophils in airways. The processes regulating neutrophil migration across the bronchial and the alveolar epithelia are poorly understood especially in CF. The aim of this study is to analyse the adhesion molecules expressed by neutrophils and epithelial cells during the neutrophil trans-epithelial migration through the bronchial epithelium. We have already shown that ICAM-2, previously thought to be present only on endothelial cells, is also expressed on the bronchial epithelium and plays a key role in T cell migration[1].

Objectives We investigated whether ICAM-2 regulates neutrophil trans-epithelial migration through the bronchial barrier.

Methods We have used human bronchial epithelial cell lines and primary human bronchial epithelial cells (HBECs) from non CF and CF patients, at baseline and on TNF- exposure for 24h.

Results We have shown a constitutive expression of ICAM-2 at the basal side of the primary HBECs grown at air-liquid interface for 21 days. A significant 4-fold increase in ICAM-2 mRNA expression was observed 24h after TNF- treatment in non CF cell line and primary HBECs. Moreover, from confocal microscopy and immunoblots, we have found that ICAM-2 protein expression is statistically up-regulated 24h after TNF- treatment. We have performed the same experiments in non CF and CF paraffin embedded lung sections and we demonstrated a significant increase in ICAM-2 expression in CF. It has previously been pointed out that in CF cells there is actin disorganisation and disruption of the tight junctions leading to an increase in the neutrophil migration[2]. Our preliminary data showed that interaction neutrophil-epithelium provokes an actin remodelling that we can avoid using an ICAM-2 blocking antibody prior the contact with neutrophils.

Conclusions ICAM-2 mRNA and protein levels are higher in CF lung sections and in non CF cells treated with TNF- than in controls. Understanding the interactions neutrophil-epithelium in CF could prevent neutrophil accumulation in airways and attenuate lung injury.

REFERENCES
1. Porter & Hall, *FASEB J.*, 2009
2. Castellani *et al.*, *Lab Invest.*, 2012

S104 | **TARGETING THE BACTERIAL CYTOSKELETON OF CF PATHOGENS FOR ANTIMICROBIAL DEVELOPMENT–A CAUTIONARY TALE?**

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Background *Burkholderia cepacia* complex (BCC) bacteria are opportunistic pathogens that cause severe lung infections in cystic fibrosis (CF). Treatment of BCC infections is difficult due to the inherent multidrug resistance of BCC. There is a pressing need to find new bacterial targets for antimicrobials. We have previously shown that the novel compound Q22, which is related to A22 and inhibits the bacterial cytoskeletal protein MreB, inhibits growth of BCC bacteria.

Aims We aimed to further analyse the phenotypic effects of Q22 treatment on BCC virulence traits to assess its feasibility as an antimicrobial.

Methods BCC bacteria were grown in the presence of Q22 and a broad phenotypic analysis was performed, including resistance to H_2O_2 induced oxidative stress, changes in inflammatory potential of cell surface components and *in vivo* drug toxicity studies. The influence of Q22 treatment on inflammatory potential was measured by monitoring the cytokine responses of BCC whole cell lysates, purified lipopolysaccharide and purified peptidoglycan extracted from bacterial cultures grown in the presence or absence of Q22 in differentiated THP-1 cells. Compound Q22 was also assessed for toxicity in both zebrafish and mouse infection models.

Results BCC bacteria grown in the presence of Q22 displayed varying levels of resistance to H_2O_2 induced oxidative stress with some strains showing increased resistance upon Q22 treatment. An increased response in pro inflammatory activity elected by whole Q22 treated bacterial lysate was observed for cytokines TNFa and IL-1b but this was variable between strains. Further dissection of this response is under investigation. Despite minimal toxicity previously shown *in vitro* with primary CF cell lines, *in vivo* studies demonstrated Q22 toxicity in both zebrafish and mouse infection models.

Conclusions In the case of BCC bacteria destabilisation of the bacterial cytoskeleton using compounds such as Q22 can lead to unexpected increases of *in vitro* virulence-related traits. These changes appear to vary depending on strain and species. Future development of antimicrobials targeting the BCC bacterial cytoskeleton may be hampered if such effects translate into the *in vivo* environment of CF infection.

S105 | **INNATE B1 CELLS ARE A NOVEL SOURCE OF IL-17 IN CHRONIC PULMONARY PSEUDOMONAS AERUGINOSA INFECTION**

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Introduction and Objectives *Pseudomonas aeruginosa* (PA) is an important respiratory pathogen resulting in damaging neutrophilic responses. The cytokine IL-17 is important in orchestrating such inflammation. Cells producing IL-17, such as Th17 cells, have been shown to be important in host defense in chronic pulmonary PA infection. We set out to determine the origin and role of IL-17 in a model of chronic pulmonary PA infection.

Methods Experimental chronic pulmonary infection in mice was produced by intra-tracheal instillation of mucoid PA strains embedded in agar-beads; sterile beads were utilised as controls. Thoracic lymph nodes (LNs), splenocytes and peritoneal B1a cells were restimulated with PA followed by cytokine assay and immunostaining to define responding cell subsets. PA-specific immunoglobulins were measured in sera and culture supernatants. Intrapulmonary B cells were identified via immunohistochemistry. Mice genetically engineered to lack B cells (MT strain) were utilised to examine the effect on pathogenesis in the absence of B cell responses.

Results Chronic PA infection developed in 43% (SD: 25%) of infected animals at 14-days.

Following infection, the pulmonary LN B cell compartment expanded, with a large B1 population (B220^{+} CD19^{+} CD43^{+} IgM hi IgD lo and predominantly CD5^{+}) that expressed