months when vitamin D levels are lowest. The purpose of this study was to investigate the local and systemic effects of vitamin D deficiency in a murine model of sepsis-induced lung injury where we can predictably time the initiating insult.

Methods We fed 8 wild-type C57Bl/6 mice a diet completely devoid of vitamin D for 6 weeks to induce severe vitamin D deficiency (9 nmol/l) and compared to 7 mice fed a vitamin D sufficient diet (42 nmol/l). Caecal ligation and puncture (CLP) was used to establish sepsis. Animals were culled 16h after CLP and blood, peritoneal lavage fluid (PLF) and bronchoalveloar lavage fluid (BALF) were collected. Cell infiltrates were assessed by flow cytometry. Fluid protein levels were measured and tissue protein permeability index (PPI) was calculated as the ratio between fluid and serum protein. Bacterial load was evaluated as colony-forming units (CFU) after 24h incubation on appropriate media.

Results Vitamin D deficient mice had increased bacterial load in BALF, blood and PLF compared to dietary sufficient mice. BALF protein permeability index was higher in deficient compared to sufficient mice but there was no difference in cell numbers recruited to the lung. PLF protein permeability index was also increased in the deficient group compared to sufficient mice with an associated significant increase in neutrophils recruited to the peritoneum. (See Table 1)

Conclusion Vitamin D deficiency significantly increases the bacterial load both systemically, locally and within the lung in a murine model of peritonitis. This is associated with an increase in tissue permeability locally and within the lung. These data support pre-existing vitamin D deficiency as a determinant of the severity of bacteraemic sepsis and may account for some of the seasonal variations observed in the incidence of sepsis.

Abstract S98 Table 1. Differences between dietary deficient and sufficient mice post CLP induced sepsis. Data is expressed as median values.

	Sufficient (n = 7)	Deficient (n = 8)	p-value
Bacteria (CFU x10^3)	0.51	2.51	0.038
BALF	0.28	66.1	0.019
Blood	8.22	336.6	0.005
PLF			
PPI (x1000)	1.82	3.30	0.0003
BALF	27.2	46.9	0.05
PLF			
Neutrophil Number	64.1	27.9	0.183
BALF	2.11	4.60	0.04
PLF (x10^6)			

S99 A FUNCTIONAL VARIANT OF ELAFIN WITH IMPROVED ANTI-INFLAMMATORY ACTIVITY

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Introduction and Objectives Elafin is a well-known serine protease inhibitor produced by epithelial and inflammatory cells with anti-inflammatory properties. Previous work has shown that unregulated protease activity can cause the proteolytic cleavage of elafin, therefore impairing the innate immune function of the protein. Consequently, the aim of this study was to generate a variant of elafin that would demonstrate increased protease resistance whilst retaining many of the beneficial characteristics of the parent molecule.

Methods Two elafin variants (GG-elafin and QQ-elafin) were recombinantly synthesised in a yeast-based expression system and subsequently tested for antiprotease, transglutaminase and protease susceptibility. In addition, the LPS neutralisation activity of the GG-variant was evaluated in *in vitro* based assays and an *in vivo* mouse model of LPS-induced acute lung inflammation.

Results GG- and QQ-elafin retained similar antiprotease and transglutaminase activity compared to wild-type elafin (WT-elafin). When incubated with diseased bronchoalveolar lavage fluid (BALF), the elafin variants displayed significantly enhanced resistance to degradation when compared to WT-elafin. Intriguingly, both variants, but particularly GG-elafin, demonstrated improved LPS neutralisation by inhibiting cytokine expression in monocytic cells. Moreover, the GG-elafin showed improved anti-inflammatory properties in a mouse model of LPS-induced acute lung inflammation with significantly decreased inflammatory cell counts, namely neutrophils ($p = 0.0362$). Furthermore, total BAL protein levels were significantly decreased ($p = 0.0336$) and a reduction in pro-inflammatory cytokine/chemokine levels was observed in mice treated with GG-elafin compared to those treated with WT-elafin.

Conclusions Site-specific mutants of elafin, particularly GG-elafin, showed increased functionality when compared to WT-elafin and may be of future therapeutic relevance in the treatment of lung diseases, particularly acute lung injury (ALI).

Mechanisms of cystic fibrosis

S100 NOVEL T HELPER CELL RESPONSES AGAINST PSEUDOMONAS AERUGINOSA IN HEALTHY INDIVIDUALS AND PATIENTS WITH CYSTIC FIBROSIS

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Introduction and Objectives *Pseudomonas aeruginosa* (PA) colonisation is a hallmark of cystic fibrosis (CF) resulting in damaging neutrophilic inflammation. Patients with CF produce anti-pseudomonal antibodies but the role of $CD4^+$ T cell responses to PA remains unclear. Novel T helper cell subsets, Th17 and Th22 cells, have important roles in host defense but may also enhance tissue damage. We aimed to define the antigen-specific memory T helper cell responses to *Pseudomonas aeruginosa* in healthy humans and patients with cystic fibrosis.

Methods $CD14^+$ monocytes and memory $CD4^+$ $CD45RO^+$ T cells were isolated from peripheral blood of CF patients with PA colonisation (n = 8) and healthy controls (n = 10). Monocyte-derived dendritic cells (DCs) were stimulated with live Pseudomonas strain PA103 and PA isolates derived from CF patients. Autologous T cells were co-cultured with activated DCs. The resultant T helper response was determined by measuring proliferation, immunoassay of cytokine output, and immunostaining of intracellular cytokines. Lavage samples from explanted CF lungs were assayed for IL-22 secretion.

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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