WS11.1 Role of short chain fatty acids produced by anaerobic bacteria in cystic fibrosis

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Rationale: Chronic bacterial infection leading to an irreversible decline in lung function is the main cause of mortality and morbidity in people with cystic fibrosis (CF). Anaerobic bacteria have been recently shown to be present in sputum in high numbers in CF; however their role in infection and inflammation needs further investigation. Short chain fatty acids (SCFA) are known to be produced as metabolic by-products of anaerobic bacteria and we investigated if their role in initiating the pro-inflammatory cascade.
Objectives: To investigate the role SCFAs play in the CF lung and if they are pro-inflammatory mediators.
Methods and Results: We investigated the protein and mRNA expression levels of GPR41 and GPR 43, G protein coupled receptors for SCFAs, using Western blot analysis and reverse transcriptase-polymerase chain reaction. We found that GPR41 and GPR 43 protein and mRNA are highly expressed in cystic fibrosis bronchial epithelial cells, (CFBE 41o) as compared with normal human bronchial epithelial cells (16HBE14o) and this was shown to be significant ($p < 0.001$).
We evaluated the role of IL-8 production by quantifying the most known prevalent SCFA, sodium butyrate on CFBE 41o and 16HBE14o using enzyme-linked-linked immunosorbent assay (ELISA). We found increased IL-8 production in CFBE cells compared to HBE cells.
Conclusion: We found differentiated increased expression of GPR 41 in CFBE 41o cells compared with 16HBE14o. We also found that sodium butyrate, a SCFA, increases IL-8 production in CFBE cells.

WS11.2 Novel LTTR plays a key role in biofilm formation and pathogenicity in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is the predominant cause of mortality in cystic fibrosis (CF) patients. Once established, eradication of this pathogen from the lungs is almost impossible due to its natural resistance to antibiotics and its biofilm-like mode of growth. Biofilms are dynamic, three-dimensional communities of bacteria living in an extracellular polysaccharide matrix that is relatively impenetrable to antibiotics. In this study, a mutant library for the clinical *P. aeruginosa* strain PA14 was used to identify genes involved in biofilm formation. From the >5200 mutants screened, 30 genes involved in the disruption of biofilm formation in artificial sputum media were identified. One of these genes, a LysR-type transcriptional regulator (LTTR) was further characterised. Interestingly, Type Three Secretion and motility were affected by this LTTR, but more importantly it also appeared to play a key role in pathogenicity in the *C. elegans* model of infection. Extensive analysis, including transcriptome, and whole proteome analysis using the LTQ Orbitrap mass spectrometer have been performed. The data generated from these studies is currently being interrogated in order to identify the molecular mechanisms underpinning these key virulence traits. Mechanisms identified could be exploited to develop novel therapeutic strategies to combat chronic *P. aeruginosa* infection in the CF lung.

WS11.3 Role of pulmonary cytosolic phospholipase A2 in mouse mortality by *Pseudomonas aeruginosa* infection

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Pseudomonas aeruginosa induces lung injury in numerous diseases including cystic fibrosis (CF). The host cytosolic phospholipase A2α (cPLA2α) releases arachidonic acid (AA), the precursor of prostaglandin E2 (PGE2), but its role in lung injury by *P. aeruginosa* infection is unknown. Here, we examined this role in a mouse model of lung infection by a clinical strain (CHA) of *P. aeruginosa*. We showed that CHA increased mouse mortality, PMN extravasation and cytokine levels in bronchoalveolar lavage fluids (BALFs). This was accompanied by increased protein and LDH levels in BALFs. cPLA2α-null mutation reduced both animal mortality, protein and LDH levels without interfering with PMN and cytokine levels. CHA triggered cPLA2α phosphorylation in parallel to AA, PGE2 and LDH release in lung epithelial cell line A549 through an activation of the p38-MAPK. Pharmacological inhibition of cPLA2α attenuated CHA-induced AA, PGE2 and LDH release, but had no effect on IL-8 secretion by A549 cells. Finally, we examined the role of cPLA2α in CHA-induced mortality in CF mice previously shown to display increased pulmonary cPLA2α activity. CHA induced higher mortality in CF compared to non-CF mice. Inhibition of cPLA2α attenuated CHA-induced CF mice mortality without interfering with lung inflammation. We conclude that cPLA2α plays a role in *P. aeruginosa*-induced lethality independently from lung inflammation and in part via a cPLA2α-induced toxicity. These findings might have pathophysiological relevance for CF or other *P. aeruginosa* lung infections.

WS11.4 Host response to *Pseudomonas aeruginosa* adaptation during airway chronic infection

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Cystic fibrosis (CF) lung disease is characterized by transient airway *P. aeruginosa* infections and excessive neutrophil-dominated inflammation early in life followed by permanent chronic infection leading to the decline in lung functions. Here we aimed to dissect the host response to *P. aeruginosa* patho-adaptive strains during acute and chronic infection.
Sequential clonal strains isolated from a CF patient during a period of up to 7.5 years were tested in IB3–1 CF and C38 isogenic wt cells, macrophage-like cells THP-1 and mouse models. Microarray analysis and ELISA of infected cells showed a decreased inflammatory response, including down-regulation of leukocyte receptors and adhesion molecules, and an increased damage mediated by tissue remodelling, due to late strains compared to early strains.
In murine model of acute infection, the early *P. aeruginosa* strain induced higher mortality than late clonal strains. Although attenuated in mortality, *P. aeruginosa* late strains retained their capacity to persist in mouse models of chronic infection, induced leukocytes recruitment, and stimulated pro-inflammatory cytokines and chemokines production (MIP-2, KC, TNF-α, IL-17, IFN-γ, MCP-1, TGF-β) up to one month from challenged. H&E, PAS- and MTS-staining, and Tunnel assay of *P. aeruginosa*-infected lungs showed that late strains increased numbers of mucin-positive goblet cells, collagen deposition and apoptotic cells, typical hallmarks of damage in the airway chronic diseases.
Our findings suggest that *P. aeruginosa* patho-adaptive variants, although less inflammatory or lethal, can promote persistence while causing damage and fibrosis in the airway of chronic disease.