

Oral Presentations

Workshop 2. Interactions between microbes and the host

S3

WS2.1 Liposomal clarithromycin effect on bacterial adhesion to epithelia of cystic fibrosis patients

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Objective: Cystic fibrosis (CF) lungs infected with antibiotic resistant bacteria is very difficult to treat. Our aim is to improve the effectiveness of existing anti-pseudomonal drugs through the use of liposomal antibiotic formulations.

Methods: The effect of sub-inhibitory concentrations of liposomal clarithromycin on *P. aeruginosa* (PA) adherence to human lung epithelial cell (A549) in culture and the mechanisms of anti-adherence property of the formulation were investigated by comparing outer membrane protein profiles of antibiotic treated cultures to that of controls using 2D gel electrophoresis. The release kinetics in CF sputum, the antibacterial activity against biofilm forms of PA and the mucus penetrating ability of liposomal clarithromycin in cystic fibrosis sputum were assessed by HPLC, fluorescence and microbiological assay. The nebulized liposomal clarithromycin was prepared using a (PARI) nebulizer and the physicochemical properties of nebulized were analyzed using scanning electron microscopy.

Results: The sub-inhibitory concentrations of liposomal clarithromycin were found to diminish the ability of PA to bind to human lung epithelial cell (A549). The liposomal formulation penetrated into deep mucus layers and kept it up longer. The antibacterial activity liposomal clarithromycin against biofilm forms of PA was increased by several folds compared to clarithromycin alone.

Conclusion: These liposomal formulation played an important role in preventing the attachment of PA to cell surfaces and their administration by Inhalation is achievable. This strategy could prevent PA from causing infections and damaging the lung in CF patients.

WS2.3 Multifaceted iron acquisition mechanisms of *Burkholderia cenocepacia*

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The *Burkholderia cepacia* complex (Bcc) is a group of antibiotic resistant, opportunistic pathogens associated with a high mortality rate in cystic fibrosis (CF). *Burkholderia cenocepacia*, one of the more clinically relevant members of the Bcc, produces iron chelating molecules known as siderophores to acquire iron in the host. This study demonstrated that in iron-depleted cultures (0 μ M and 1 μ M) siderophore production by *B. cenocepacia* was significantly upregulated by 4 h ($P < 0.001$) with maximal production by 5 h. At 2 μ M and 3 μ M iron, siderophore responses were significantly upregulated by 6 h ($P < 0.001$) and were maximal by 7 h. We examined the ability of *B. cenocepacia* to acquire iron from other sources such as host iron-binding proteins and xenosiderophores. Ornibactin deficient mutants demonstrated a 7 fold growth increase with ferritin ($P < 0.001$) and a 6 fold increase with hemin ($P < 0.001$). *B. cenocepacia* wild type strains showed a 16 and 10 fold increase in growth in the presence of ferritin and hemin, respectively, indicating that these mechanisms are siderophore-independent. Lactoferrin and transferrin had no effect on *B. cenocepacia* growth. Both wild type and mutant strains of *B. cenocepacia* demonstrated significantly enhanced growth in the presence of *Aspergillus fumigatus* siderophores, triacetylfulvarinine C and fusarinine C, demonstrating the potential for inter-kingdom competition between these pathogens. The multifaceted iron acquisition strategies employed by *B. cenocepacia* facilitate colonisation of the iron-restricted niche of the CF lung and survival in a polymicrobial community and thus warrant further investigation as potential therapeutic targets.

WS2.2 Identification of two novel immunogenic *Burkholderia cepacia* complex proteins involved in lung cell attachment

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Burkholderia cepacia complex (Bcc) infection can result in a fatal necrotising pneumonia and bacteraemia in a subgroup of CF patients. While several virulence factors have been identified, the pathogenesis of Bcc has not been fully elucidated. We previously identified immunogenic proteins from *B. cenocepacia* and *B. multivorans* using immunoproteomics. Two-dimensional blots of Bcc proteins were probed with serum from Bcc colonised CF patients. Twelve *B. cenocepacia* and 14 *B. multivorans* immunogenic proteins were identified using MALDI-Tof MS, of which six proteins were common to both species. An OmpA family lipoprotein (BCAL3204) and a hypothetical protein located on a pathogenicity island (BCAS0292), were chosen to investigate their role in virulence.

Targeted deletion mutants were developed and examined in the *Galleria mellonella* virulence model. The virulence of the BCAL3204 mutant was significantly reduced ($p < 0.001$) in comparison to the wild-type (WT), confirming its pathogenic role. In contrast, larvae injected with supernatant from this mutant displayed lower survival compared to the WT supernatant suggesting a structural role for this protein. Confocal microscopy showed that, in comparison to the WT, the BCAS0292 and BCAL3204 mutants displayed a three-fold and two-fold reduction in adhesion to CF epithelial cells, respectively, demonstrating that both proteins may play a role in host lung cell attachment.

Both proteins have been recombinantly expressed and their characterisation will provide a clearer understanding of their roles in Bcc pathogenesis, which may lead to their development as potential vaccine antigens or targets for anti-virulence therapies.

WS2.4 *Pseudomonas aeruginosa* capability to colonize the CF lung may be favored by its remarkable ability to recruit zinc under conditions of limited metal availability

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Objectives: Calprotectin (CP) is likely the most abundant protein present in the sputum from CF patients. Although CP is usually considered just as a marker of inflammation, it is a zinc and manganese sequestering antimicrobial protein, released by neutrophils at sites of infection to contain microbial proliferation. It has been recently shown that the ability of several pathogens to withstand the antimicrobial activity of CP relies on their ability to produce metal transporters characterized by high affinity for the Zn ion. On this ground, we hypothesize that the ability *P. aeruginosa* to colonize the inflamed CF lung must be supported by effective strategies to counteract CP-induced metal starvation. Therefore, in the light of recent studies showing that it is possible to pharmacologically target Zn homeostasis in pathogenic microorganisms, we have undertaken a characterization of the Zn import apparatus of *P. aeruginosa* and of its contribution to bacterial ability to colonize the CF lung.

Methods: We have generated strains lacking the two known ATP-dependent Zn importers ZnuABC and HmtA and carried out experiments to evaluate their contribution to bacterial growth in Zn-limiting conditions and in infected mice.

Results: Although the mutants strains lacking *znuABC* show some defects in motility and alginate production, the mutant strains display modest growth defects in Zn-limited environments, elevated intracellular Zn content and a limited reduction of virulence in mice.

Conclusions: *P. aeruginosa* is equipped with redundant mechanisms for the acquisition of Zn which may contribute to colonization of the CF lung by favoring the evasion of the antimicrobial action of CP.