115* Azithromycin affects the processing of tight junction proteins and ENaC in human airway epithelia in vitro

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The macrolide antibiotic azithromycin improves lung function and prognosis among patients with cystic fibrosis, independent of bacterial eradication. Anti-inflammatory effects have been implicated but the link between abnormal CFTR and bronchial infections remains uncertain. We hypothesized that azithromycin might affect transepithelial electrolyte transport in human airway epithelia.

To explore this possibility we treated human airway epithelia on filter supports with azithromycin and monitored transepithelial electrical resistance. We found that azithromycin increased transepithelial electrical resistance of airway epithelia in a dose dependent manner from 1234 ± 29 (control) to $2920\pm195\,\Omega\,cm^2\pm SEM$ ($P<0.05$, $n=24$). Immunocytochemistry and western blot analysis showed that addition of azithromycin changed protein location in cell cultures and induced processing of tight juction proteins; claudin-1 and –4, occludin and junctional adhesion molecule-A. These effects were reversible and neither produced by penicillin nor erythromycin. In addition, immunocytochemistry and western blot analysis indicated that azithromycin decreased the expression of ENaC in human airway epithelia.

Conclusions: The data indicate that azithromycin may affect transepithelial electrolyte transport in human airway epithelia *in vitro* by affecting the processing of tight junction proteins and ENaC. The results are novel and may help explain the beneficial effects of azithromycin in CF.

116 Characterisation of bacterial community composition in Cystic Fibrosis lung infection using biofilm models

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Many studies have considered that the bacterial community present in cystic fibrosis (CF) lung infections exists as a biofilm. Further, the ability of key CF pathogens such as *Pseudomonas aeruginosa* to resist antibiotic therapies may well largely be due to poor drug penetration of these biofilms. Current models for determining antibiotic susceptibility are based on non-biofilm-based modes of single species growth. However, this fails to represent the complex bacterial community now known to exist in the CF lung. In this study, we describe the successful generation of an in vitro biofilm model resembling the bacterial community sampled from CF lungs. These studies analysed the bacterial composition of biofilms generated directly from six adult CF sputum samples, during both early formation and maturation. The bacterial communities in both sputa and the biofilm models were characterised by using terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA gene PCR products amplified from directly extracted nucleic acids. Although some loss of diversity was observed during biofilm maturation, the data generated showed that the bacterial community in sputa was well reproduced in the biofilm models. Typically, sputa contained 4.61 ± 0.85 bacterial species, with species number decreasing to 2.83 ± 0.35 over 5 days. The generation of biofilm models directly from sputum represents a potentially powerful tool for the characterization of bacterial behavior in lung infections. The ability to screen responses to conventional therapies in vitro using biofilms will greatly increase the likelihood of novel treatments being identified.

117 DNA release in *Pseudomonas aeruginosa* biofilms

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Biofilm formation in *Pseudomonas aeruginosa* has been shown to play a significant role in chronic infection of cystic fibrosis patients. Extracellular DNA exists as one of the major matrix components in *P. aeruginosa* biofilms. In a microtiter based assay employing the florescent DNA stain propidium iodide, it was shown that extracellular DNA was mainly released in the stationary growth phase. By comparing *P. aeruginosa* PAO1 with different quorum sensing mutants we found that DNA release was under control of quorum sensing. The extracellular DNA was shown to originate from cell lysis by measuring the β-galactosidase activity in the supernatant of a culture with a lacZ-containing *P. aeruginosa* strain. Extracellular DNA has been shown to stabilize *P. aeruginosa* biofilms. DNaseI treated flow cell biofilms were more sensitive to SDS treatment than untreated biofilms. We also tested DNA release from clinical isolates. The results showed that clinical isolates have large variation in the ability to release DNA and that this is related to their biofilm formation potential. These results show that DNA release in bacterial biofilms by cell lysis has an important physiological role for bacterial survival as a biofilm community.

118* Development of tolerance to the antimicrobial peptide Colistin in *Pseudomonas aeruginosa* biofilms

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The biofilm mode of growth and development of subpopulations within these biofilms of *P. aeruginosa* is believed to be responsible for persistent lung infections in patients afflicted with cystic fibrosis.

P. aeruginosa wild type develops mushroom-shaped biofilms on a glass surface in presence of glucose as carbon source. The mushroom-cap is formed by cells which colonize the top of a microcolony via a process dependent on type IV pili. This cap-forming subpopulation is tolerant to treatment with the antimicrobial peptide Colistin, whereas the non-motile subpopulation, forming the stalk of the mushroom-like structure, is sensitive to Colistin, indicating that tolerance to antimicrobial treatment might be linked to a process dependent on type IV pili. To get more detailed information about the regulation of tolerance development, we investigated the role of the pilGHIJchpABCDE chemosensory gene cluster regulating type IV pili in *P. aeruginosa*, using flow cell technology and enhanced confocal laser scanning microscopy.

Our data show, that all chemosensory mutants, develop mushroom-shaped biofilms, composed of two subpopulations. However, type IV pili-driven motility deficient strains develop an irregular cap structure, compared to the smooth cap formed by the wild type strain. Interestingly, the cap-forming subpopulation of all biofilms including the type IV pili-driven motility deficient strains, were tolerant to Colistin. Intriguingly, the stalk forming subpopulation formed by pilG, pilJ and pilK mutants remained tolerant, whereas wild type stalks are killed by Colistin.

These data indicate that the chemosensory gene cluster might be involved in regulating tolerance development in subpopulations of *P. aeruginosa* biofilms.