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.

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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.

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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