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.

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-α 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-α 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-α treatment. We have performed the same experiments in non CF and CF parafinn 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. 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-α 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 H2O2 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 H2O2 induced oxidative stress with some strains showing increased resistance upon Q22 treatment. An increased response in pro inflammatory activity elicited 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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intracellular IL-17A. Infected animals also developed peribronchial B220+ cellular foci.

In mediastinal LNs following infection, PA-specific responses were dominated by B220+ CD19+ CD43+ CD23-B5+ cells expressing and producing IL-17A and IL-22 as well as PA-specific IgM but not IgG. This PA-specific B1 response was not seen in the thoracic lymph nodes of sterile-head treated animals. In splenocytes, there was a pre-existing B cell response to PA with identical features. Peritoneal B1a cells isolated from untreated controls also produced IL-17A, IL-22 and anti-PA IgM following infection, confirming the existence of pre-existing B1 cells that can respond to PA. In μMT animals, chronic colonisation rates, bacterial burden and neutrophil inflammation did not differ from WT littersmates. However, classical PA-specific Th17 responses dominated following infection in μMT animals, suggesting alternative compensatory IL-17 sources acting in the absence of B cells.

**Conclusions**

In chronic pulmonary PA infection, innate-like B1 cells migrate to the site of infection and are a novel source of pro-inflammatory IL-17 cytokines.