WS2.5 Airway epithelial regeneration is abnormal in CF in absence of endogenous infection and inflammation

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In CF, the airway epithelium is frequently remodeled and has to rapidly regenerate its structure. Whether these alterations are related to infection and/or inflammation or to a dysregulated regeneration process remains to be elucidated. Previously, we showed in an aseptic xenograft model that CF epithelial regeneration is delayed and reconstitute a remodeled epithelium. The aim of the present study was to determine if CF airway epithelial regeneration is abnormal in absence of endogenous inflammation.

CF and non-CF epithelial cells collected from aseptic nasal polyps were cultured at the air-liquid interface. Histology and cell proliferation were examined on culture sections after histological or immunological staining. Epithelial functionality was assessed in terms of electrical properties and bactericidal activity of epithelial secretions.

In absence of endogenous inflammation, the CF epithelial regeneration is delayed. The regenerated epithelium is remodeled, exhibits basal cell hyperplasia and is significantly thicker compared to non-CF. However, a delay in cell proliferation, rather than hyperproliferation, is observed. Finally, the CF regenerated epithelium is not functional.

We show here that in absence of exogenous infection and inflammation, the CF regenerated epithelium is a remodeled. This strongly suggests the involvement of the basic CFTR default in this phenomenon. To verify this hypothesis, we will examine the regeneration process of cells after treatment with CFTR correctors or CFTR-encoding lentiviral vectors to correct CF cells, as well as CFTR siRNAs to silence CFTR in non-CF cells.

We thank Vaincre la Mucoviscidose, Région Champagne-Ardenne and INSERM.

WS2.6 Potassium channel currents induced by lipoxin stimulate airway epithelial repair

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The main cause of morbidity and mortality in cystic fibrosis (CF) is due to lung damage resulting from chronic bacterial infection and inflammation and a reduced ability of the epithelium to repair. Reduced levels of the anti-inflammatory mediator LipoxinA4 (LXA_4) have been reported in the bronchoalveolar lavages of patients with CF. We investigated the LXA_4 ability to trigger epithelial repair in CF airways. Spontaneous epithelial repair was significantly slower in CF epithelial cultures (Cufi-1 homozygous F508del) compared to controls (Nuli-1). LXA_4 stimulated a dose-dependent increase in cell proliferation (MTT) and repair (Scratch Assay) in NuLi-1 cells but also to a greater extent in CuFi-1 cells. The levels of the lipoxin receptor, FPR2 (FACS) were found to be significantly lower in CF cells. LXA_4 induced cell proliferation was inhibited by the FPR2 receptor antagonist, Boc-2, the MAP kinase (ERK1/2) inhibitor, PD98059, and the K_{ATP} channel inhibitor, glybenclamide. The K_{ATP} channel opener, pinacidil, alone significantly stimulated cell proliferation and showed a slight additive effect in combination with LXA_4. Although LXA_4 did not affect K^+ channel mRNA expression, LXA_4 increased the Barium (K^+ channel inhibitor)-sensitive whole-cell currents.

Taken together our results indicated that LXA_4 triggers epithelial repair and proliferation of CF cells, through stimulation of the FPR2 receptor followed by ERK and K_{ATP} potassium channel activation. These results suggest that the decrease of the endogenous LXA_4 reported in CF contributes to the reduced ability of CF airway epithelium to repair and highlights the use of exogenous LXA4 as a potential therapeutic in CF.

WS2.7 Rapid effect of 17β-estradiol on airway surface liquid hydration of normal and cystic fibrosis epithelia

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Cystic Fibrosis (CF) males survive 9 years longer than females ("CF gender gap") and lung exacerbations in CF females vary during the estrous cycle. 17β-estradiol (E2) has been reported to reduce airway surface liquid (ASL) height in female CF bronchial epithelium. Here we investigated the effect of estrogen on ASL height and ion transport in normal (NuLi-1) and CF (CuFi-1) bronchial epithelium monolayers. Confocal fluorescence microscopy experiments revealed that ASL height was significantly higher in the non-CF cell line compared to CF cells. E2 (0.1 to 10 nM) reduced ASL height in both non-CF and CF cell lines after 30 min treatment. Treatment with the Cl^- transport inhibitor bumetanide (10 μM) decreased ASL height significantly in both cell lines. However, E2 had no additive effect on ASL height in the presence of this ion transporter inhibitor. E2 decreased bumetanide-sensitive $Cl^{*minus;}$ current in normal cells and produced an increase in amiloride (10 μM) sensitive current in CF cells. Treatment with the nuclear-impeded Estrogen Dendrimer Conjugate (0.1–1 nM E2 equivalent concentration) produced a reduction in ASL height in CF and non-CF whereas the empty dendrimer did not show any effect. These results demonstrate that E2 dehydrates CF and normal ASL via a membrane-initiated rather than the classical nuclear receptor signal transduction pathway. The ion transporter inhibitor data indicate that E2 acts on ASL by inhibiting Cl^- secretion in non-CF cells and increasing Na^+ absorption in CF cells.

Supported by EU FP7 Marie Curie CEMP Fellowship (to VS-C), Higher Education Authority of Ireland (PRTLI4 NBIP to BJH) and the National Institutes of Health (R37 DK015556 to JAK.).

WS2.8 Slc26a9-mediated Cl^- secretion is enhanced in allergic airway inflammation and prevents mucus obstruction in mouse airways

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Recent studies have shown that allergic airway disease induces enhanced epithelial Cl^- secretion in mouse airways. However, the molecular identity of the underlying Cl^- conductance remains unknown. Slc26a9 is a recently identified Cl^- channel. To determine its role in allergic airway disease, we induced Th2-mediated airway inflammation by intratracheal instillation of IL-13 in mice and compared effects on ion transport, morphology and mucus content in airways from wild-type and Slc26a9-deficient mice. We show that IL-13 enhanced constitutive Cl^- secretion in airways from wild-type, but not Slc26a9-deficient mice. While IL-13 induced mucus overproduction was similar in both genotypes, lack of Slc26a9-mediated Cl^- secretion caused significant airway mucus obstruction in IL-13 treated Slc26a9-deficient mice that was not seen in wild-type controls. Our data demonstrate that the Slc26a9 Cl^- channel is activated in airway inflammation and suggest that Slc26a9-mediated Cl^- secretion is essential to maintain airway surface liquid homeostasis and mucus clearance thus preventing airway obstruction in allergic airway disease. These results indicate that Slc26a9 may serve as a novel therapeutic target in airway diseases associated with mucus plugging, such as CF.

Support: DFG (MA2081/3–3, SE460/13–4, SE460/9–6), BMBF (DZL)