Flagellin-induces a hypersecretory phenotype in primary human bronchial epithelial cells

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Introduction

A screen of microbial-derived products using a 3D model of the human airway epithelium (bronchospheres) revealed flagellin (FLG) as a potential regulator of mucociliary structure and function. Flagellin, a TLR5-agonist, is the key protein component of bacterial flagella expressed by a number of organisms including Pseudomonas aeruginosa. Considering that mucus plugs in the airways of many cystic fibrosis (CF) patients would be reasonably expected to contain FLG, the epithelium is likely to be chronically exposed to this bacterial protein.

The aim of the present study was to test the hypothesis that the prolonged exposure of primary cultures of human bronchial epithelial cells (HBEC) at air-liquid interface to FLG would modify both the structure and function of the tissue. Acute exposure of the murine airway to FLG has been previously demonstrated to attenuate ENaC function[1], although to our knowledge, prolonged exposure to the bacterial product has not been examined. A more chronic exposure to FLG might be reasonably expected to model aspects of the environment of the chronically colonised human lung and in particular regions adjacent to infected mucus plugs.

Flagellin increases goblet cell numbers

Wild-type (non-CF) HBEC were seeded onto 12 well Transwell inserts and cultured submerged for the first 7 days followed by 14 days at air-liquid interface[2]. Cells were treated with FLG (0.04 – 1.0 µg/mL; basolateral media) for the 14 days at air-liquid interface. HBEC were fixed in 4% formaldehyde and then stained with antibodies specific for MUC5AC (goblet cells) and acetylated α-tubulin (ciliated cells) together with fluorescent secondary antibodies. Using a motorised stage and protocol written to take 9 images per insert, fluorescent images were acquired and surface areas staining positive for the goblet and ciliated cell markers were measured using Image J.

FLG (1 µg/mL) significantly increased the MUC5AC$^+$ stained area (ANOVA with post-hoc Dunnett's test). There was a trend towards a reduction in ciliated cell staining although this did not reach statistical significance.

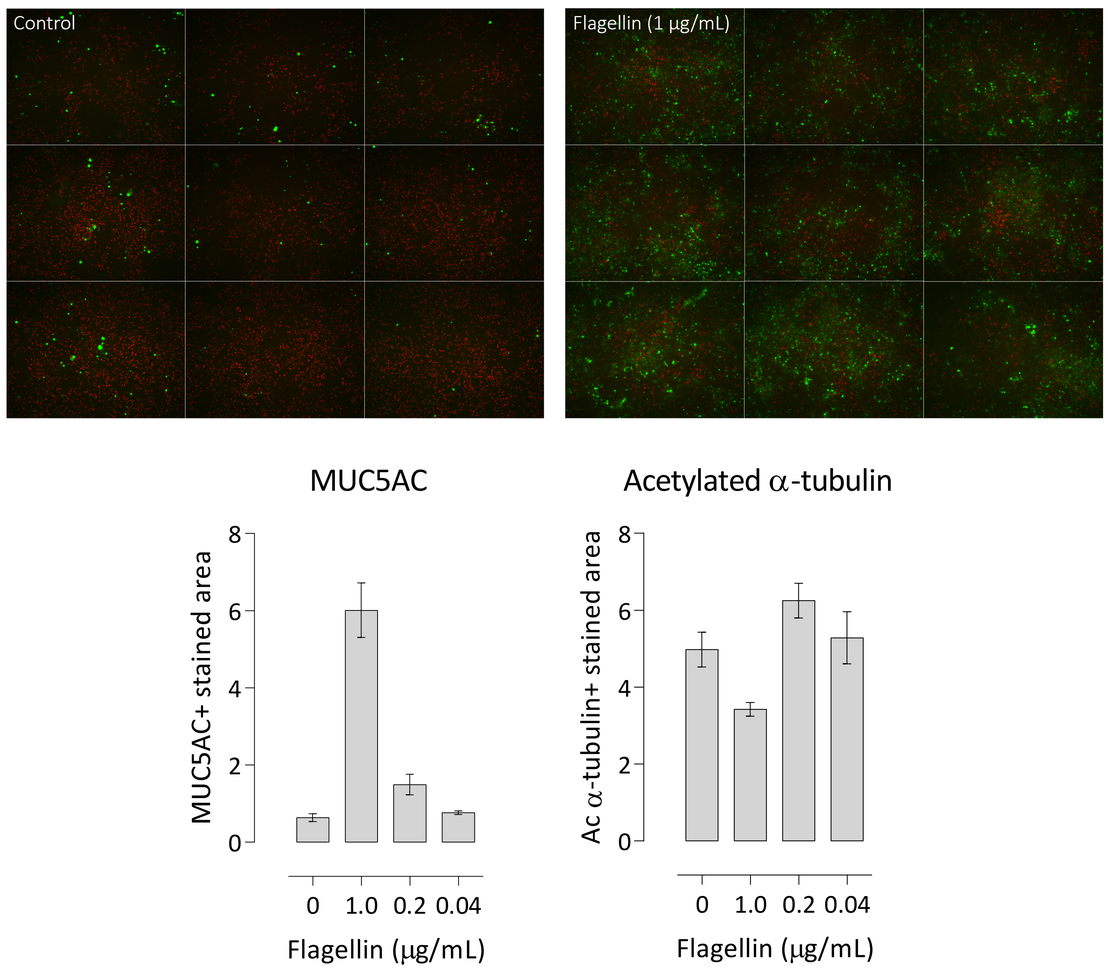

Figure 1.

Sample images illustrating the goblet and ciliated cell distribution in HBEC following 14 days culture at air-liquid interface and the effects of treatment with FLG. A total of 9 images were acquired for each independent insert of HBEC. Green staining = MUC5AC$^+$ goblet cells; red staining = ciliated cells.

Mean ± SD data to quantify the effects of FLG on HBEC (n=3 inserts per group).

Flagellin enhances the ion secretory capacity of HBEC

HBEC were cultured on Snapwell inserts and treated with FLG (1 µg/mL) as per the imaging studies (during 14 days of differentiation at ALI). Cells were bathed in isometric Ringers solution and voltage clamped to 0 mV. The initial baseline short-circuit current (ISC) together with the subsequent responses to amiloride, forskolin, Inh172, UTP and CaCC-A01 were quantified.

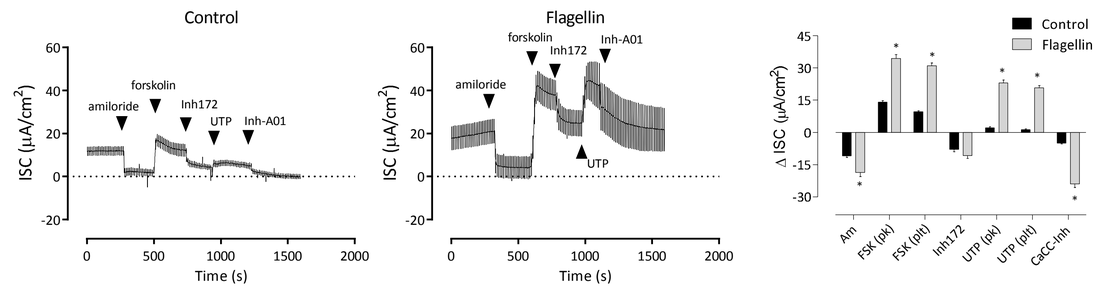

Figure 2.

Sample raw data traces (above) illustrating the ion transport phenotype of control and FLG treated HBEC. Vertical deflections reflect the current response to an imposed 2 mV pulse. Mean data ± SEM (left; n=6 inserts per group) from the ion transport studies. * indicates a significant difference from the matched control group (P<0.05). Am = amiloride, FSK = forskolin, (pk) = peak response, (plt) = steady state plateau response, Inh172 = CFTR-Inh172 (10 µM). Inh-A01 = CaCC-A01

Flagellin treatment resulted in a reduced baseline resistance from 839 ± 30 Ω.cm^2 to 361 ± 18 Ω.cm^2 (P<0.05; note larger vertical deflections in FLG-treated raw data traces; Figure 2). In addition, the baseline and amiloride-sensitive ISC was significantly increased in the FLG treated group. The ISC response to forskolin (CFTR-mediated) was also significantly increased as was the response to UTP. Of note, the FSK-stimulated ISC was only partially inhibited by Inh172 at 10 µM. In a follow-up study (Figure 3), a higher concentration of Inh172 (30 µM) effectively blocked the CFTR-dependent ISC.

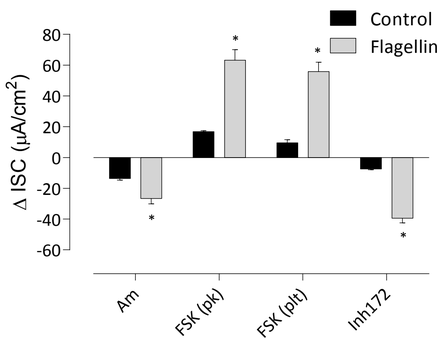

Figure 3.

Mean data ± SEM (n=6 inserts per group) from a repeat ion transport study using Inh172 at a higher concentration (30 µM). * indicates a significant difference from the matched control group (P<0.05). Am = amiloride, FSK = forskolin, (pk) = peak response, (plt) = steady state plateau response, Inh172 = CFTR-Inh172 (30 µM).

The effects of flagellin treatment were next examined using primary CF-HBEC (F508del/R553X). Flagellin treatment reduced the baseline resistance from 750 ± 37 Ω.cm^2 to 75 ± 4 Ω.cm^2 (P<0.05). The induction of such a significant drop in resistance made the subsequent interpretation of ion transport data challenging and of questionable value (Figure 4).

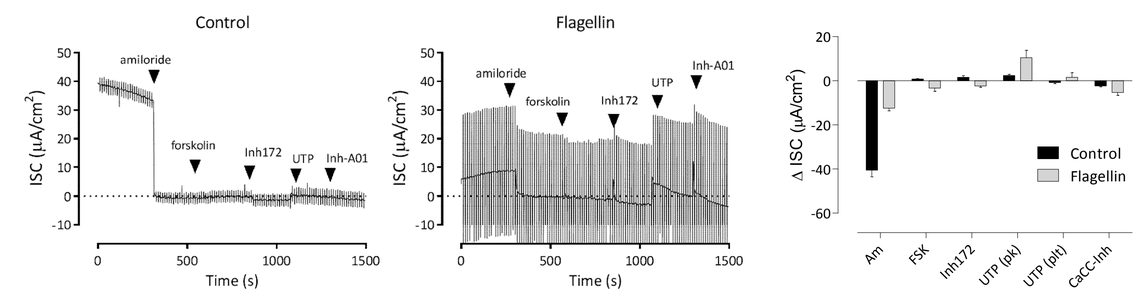

Figure 4.

Sample raw data traces (above) illustrating the ion transport phenotype of control and FLG treated CF-HBEC. Vertical deflections reflect the current response to an imposed 2 mV pulse. Mean data ± SEM (left; n=11 inserts per group) from the ion transport studies. Am = amiloride, FSK = forskolin, (pk) = peak response, Inh172 = CFTR-Inh172 (10 µM). Inh-A01 = CaCC-A01

Conclusions

Exposure of non-CF HBEC to flagellin during the 14 days of differentiation at air-liquid interface can modify both the cellular composition of the epithelium as well as the ion transport phenotype and thus the regulation of mucosal hydration.

The ability of the epithelium to simultaneously increase its anion secretory capacity will be predicted to provide additional fluid to hydrate the expanded mucus gel although whether this is sufficient to prevent plugging, especially in the CF airway, is presently unknown.

Additional studies using multiple CF-HBEC donor cells will be required to understand the effects of FLG in the disease epithelium.

1. Kunzelmann et al., (2006) FASEB J 20(3):545-6
2. Atherton et al., (2003) AJP LCMP 285(3):L730-9
3. Generously provided by Dr Scott Randell, University of North Carolina

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