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

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Introduction

The TLR5-agonist flagellin (FLG) is the key protein component of bacterial flagella expressed by a number of organisms including Pseudomonas aeruginosa. The pro-inflammatory activity of bacterial FLG has been well documented in leukocytes although the effects on airway epithelial function are less clear.

The acute addition of FLG to murine trachea ex-vivo has been demonstrated to inhibit Na^+ transport (Kunzelmann et al. FASEB J 2006, 20(3):545-6) although effects on the human tissue have not been examined. 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. These conditions 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 potently attenuates epithelial resistance

HBEC donors (4 CF*, 3 non-CF) were seeded onto 12 well Transwell inserts and cultured submerged for the first 7 days followed by 15 days at air-liquid interface (Atherton et al. AJP LCMP 2003, 285(3):L730-9). Cells were treated with FLG (1.6 – 1,000 ng/mL; basolateral media) for the 14 days at air-liquid interface. Trans-epithelial resistance was monitored during culture using an EVOM voltmeter.

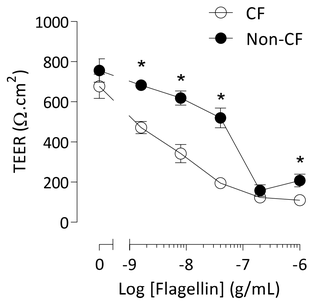

Figure 1

Mean data (± SEM) illustrating the effect of flagellin on TEER in both CF (mean of 4 donors) and non-CF derived HBEC (mean of 3 donors). TEER was measured on day 15 of culture at air-liquid interface. * indicates a significant difference between TEER at the specified concentration of flagellin (multiple t-tests with Holm-Sidak correction; P<0.005).

FLG-treatment reduced TEER of HBEC at concentrations as low as 1.6 ng/mL. CF-derived HBEC appeared to be more sensitive to the effects of flagellin albeit we have only examined a small number of donor codes (4x CF and 3x non-CF).

*generously provided by Dr Scott Randell, UNC. Donors were: F508del/R553X, F508del/G542X (2x donors), F508del/R709X

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Flagellin increases goblet cell marker expression

HBEC were seeded onto 24 well Transwell-HTS inserts and cultured submerged for the first 7 days followed by 14 days at air-liquid interface. Cells were treated with FLG (12 – 1,000 ng/mL; basolateral media) for the 14 days at air-liquid interface. HBEC were fixed in 4% formaldehyde and then stained with antibodies specific for the gel-forming mucins MUC5AC and MUC5B and acetylated α-tubulin, a marker of ciliated cells together with fluorescent secondary antibodies. Using a motorised stage and protocol written to take 4 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 induced a concentration-dependent increases in staining for both MUC5AC and MUC5B in both CF and non-CF donors. Sample data from one of the CF donors (KK016G) are shown in Figure 2. Staining for acetylated α-tubulin was also increased with FLG treatment although at higher concentrations, much of this staining appeared to cytoplasmic rather than associated with cilia. Sample images are shown in Figure 3.

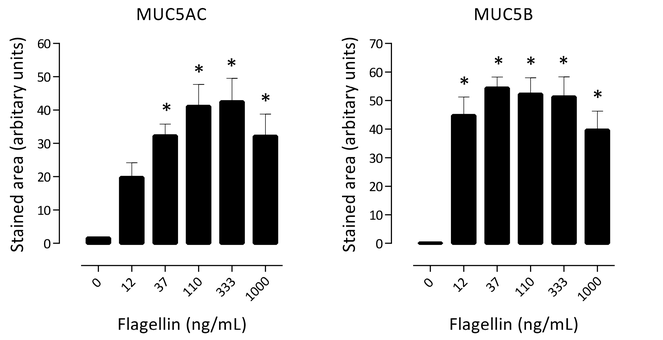

Figure 2

Flagellin treatment induced a concentration-dependent increase in the expression of both MUC5AC and MUC5B. Mean (± SEM) staining data from CF donor KK016G are shown. N=4 inserts per group. A one-way ANOVA with post-hoc Dunnett's test was used to test for significant differences from the untreated control group. * indicates P<0.05.

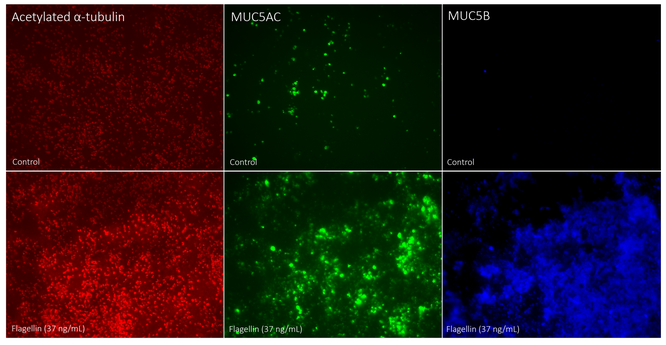

Figure 3

Sample images illustrating the effects of Flagellin (37 ng/mL) treatment on the cellular phenotype of CF-HBEC donor KK016G. Flagellin treatment increased staining for cells positive for both MUC5AC and MUC5B. Staining for the ciliated cell marker acetylated α-tubulin was also increased although much of the positive material appeared to be cytoplasmic rather than associated with the cilia.

Flagellin enhances anion secretion

HBEC (1x CF and 1x non-CF donor) were cultured on Snapwell inserts and treated with FLG (12 & 37 ng/mL; basolateral media) as per the imaging studies (during 15 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.

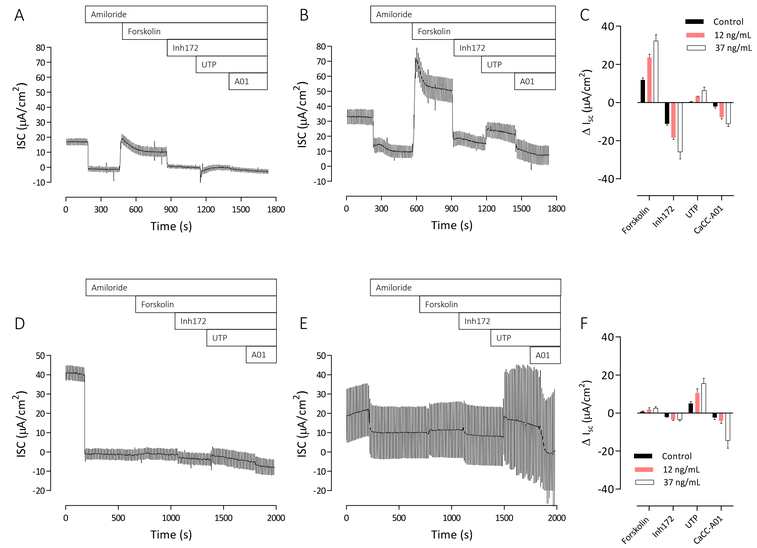

Figure 4

Sample short-circuit current traces illustrating the effects of 14 days of flagellin treatment (37 ng/mL) on the ion transport phenotype of non-CF (A & B) and CF-derived HBEC (D & E). Mean data (± SEM; n=8) are shown for both the non-CF (C) and CF HBEC (F). Vertical deflections represent the current response to a ±2 mV pulse every 30 sec.

Summary & conclusions

Flagellin can potently influence the structure and function of HBEC when exposed to cells during the 14 days of differentiation at air-liquid interface. The resulting phenotype is an epithelium with a reduced barrier function (attenuated TEER), an increased expression of goblet cell markers and an enhanced ability to secrete anions. In non-CF HBEC, both CFTR and TMEM16A-mediated anion secretory responses are enhanced, whereas in CF-derived cells, only the increase in TMEM16A function is apparent.

The ability of the epithelium to increase its anion secretory capacity in response to flagellin exposure 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.