ORIGINAL ARTICLE

Mucin Production and Hydration Responses to Mucopurulent Materials in Normal versus Cystic Fibrosis Airway Epithelia

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Abstract

Rationale: Cystic fibrosis (CF) airways disease produces a mucoobstructive lung phenotype characterized by airways mucus plugging, epithelial mucous cell metaplasia/hyperplasia, chronic infection, and inflammation. Simultaneous biochemical and functional *in vivo* studies of mucin synthesis and secretion from CF airways are not available. *In vitro* translational models may quantitate differential CF versus normal mucin and fluid secretory responses to infectious/inflammatory stimuli.

Objectives: We tested the hypothesis that CF airways exhibit defective epithelial fluid, but not mucin, secretory responses to bacterial/inflammatory host products.

Methods: Well-differentiated primary human bronchial epithelial cultures were exposed to supernatant from mucopurulent material (SMM) from human CF airways as a test of bacterial/inflammatory host product stimulus. Human bronchial epithelia (HBE) with normal CF transmembrane conductance regulator function were compared with ΔF508/ΔF508 CF HBE.

Measurements and Main Results: Acute (up to 60 min) SMM exposure promoted mucin secretion, but mucins were degraded by the proteolytic enzymes present in SMM. Chronic SMM exposure induced upregulation of mucin synthesis and storage and generated absolute increases in basal and stimulated mucin release in normal and CF cultures. These responses were similar in normal and CF cultures. In contrast, SMM produced a coordinated CF transmembrane conductance regulator–mediated Cl^- secretory response in normal HBE, but not in CF HBE. The absence of the fluid secretory response in CF produced quantitatively more dehydrated mucus.

Conclusions: Our study reveals the interplay between regulation of mucin and fluid secretion rates in inflamed versus noninflamed conditions and why a hyperconcentrated mucus is produced in CF airways.

Keywords: cystic fibrosis; airway mucins; airway hydration; airway inflammation

Cystic fibrosis (CF) is a mucoobstructive airways disease characterized by chronic mucus accumulation, infection, and inflammation. CF is believed to exhibit an element of failed mucus transport that contributes to disease pathogenesis. Mucins are the major components of airway mucus that generate the biophysical properties that support mucus transport (1, 2). Secretory cells in mammalian airways produce two polymeric mucins, MUC5AC and MUC5B, large glycoproteins that comprise the polymer scaffold of the mucus gel (3).

In healthy subjects, and in those with mucoobstructive disease, acute insults promote rapid secretion of mucins onto airway surfaces via exocytosis of mucin granules (4). Persistent exposure to airway insults induces increased synthesis of airway mucins associated with an increased number of mucin-producing cells (5). Although mucin secretion is essential for clearance in normal airways (6), it may be maladaptive in airways of subjects with CF (7–9). In these subjects, bacterial exoproducts and inflammatory mediators promote mucous cell hyperplasia/metaplasia and upregulate

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At a Glance Commentary

Scientific Knowledge on the Subject: Simultaneous biochemical and functional *in vivo* studies of mucin synthesis and secretion from cystic fibrosis (CF) airways are not available. *In vitro* translational models quantitating differential CF versus normal mucin and fluid secretory responses to infectious/inflammatory stimuli have not been developed.

What This Study Adds to the Field: The integrated mucin and fluid secretory responses to the intralumenal mucopurulent materials that characterize the mucoobstructive state of CF airways were investigated in primary cultures of normal and CF human bronchial epithelia. Normal airways mounted an adapted response that included increased MUC5AC and MUC5B secretion and robust CF transmembrane conductance regulator–mediated fluid secretion, to produce a dilute mucus to "flush" toxicants from airway surfaces. CF airways exhibited similar increases in mucin secretion but failed to secrete fluid, producing an inappropriately hyperconcentrated mucus that contributes to airway obstruction.

mucin synthesis and secretion (5, 10). In airways expressing normal CF transmembrane conductance regulator (CFTR) function, these insults coordinately promote increased CFTR-mediated fluid secretion to hydrate mucus and promote mucus-dependent clearance. The lack of functional CFTR in CF leads to airway surface dehydration, producing mucus hyperconcentration and mucus stasis/adhesion (10).

The present study investigated the integrated mucin and fluid secretory responses to the intralumenal mucopurulent materials that characterize the chronic mucoobstructive state of CF airways. Primary cultures of well-differentiated normal (non-CF) human bronchial epithelia (HBE) versus CF HBE were exposed to a supernatant of mucopurulent material (SMM) from human CF airways (11, 12). SMM contains the soluble infectious and inflammatory components present in the lumens of CF airways associated with chronic infection (13, 14). The acute versus chronic effects of SMM on MUC5AC and MUC5B transcription, translation, secretion, and proteolysis were compared in normal versus CF cultures. The impact of CFTR function on ion and fluid transport responses to SMM was also investigated in normal versus CF HBE to characterize the integrated effects of hydration on the properties of mucus (i.e., percent solids), which governs mucus transport rates.

Methods

For further details on the applied Methods, *see* the online supplement.

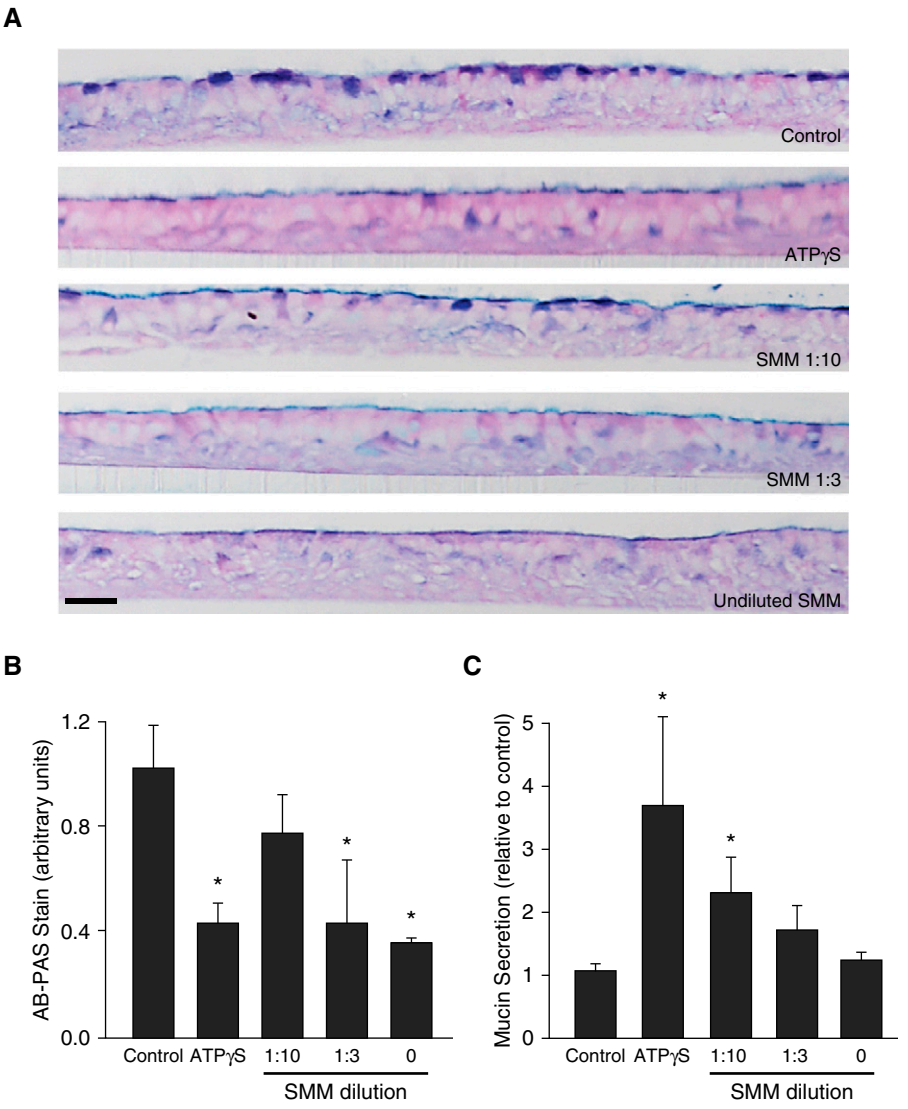

Figure 1. Acute supernatant of mucopurulent material (SMM) exposure induces mucin release and promotes degradation of secreted mucins. After washing away accumulated mucins, non–cystic fibrosis (normal) human bronchial epithelial cultures were mucosally exposed to various doses of SMM for 1 hour. Secreted mucins were collected from the epithelial surface and the cultures were fixed. (*A*) Alcian blue/periodic acid–Schiff (AB/PAS) staining of human bronchial epithelial cultures exposed to phosphate-buffered saline (control), 100-μM ATPγS, or various doses of SMM. ATPγS was used as a positive control for mucin release. (*B*) Quantification of the dose-dependent effect of acute SMM-promoted mucin exocytosis. (*C*) Secreted mucin levels (measured by ELISA) from the cultures depicted in *A*. Data represent mean ± SD; $n = 4$–5. *$P < 0.05$, ATPγS-treated or SMM-exposed versus control cultures. Scale bar = 20 μm. ATPγS = adenosine 5′-O-(3-thio)triphosphate.

Human Bronchial Epithelial Culture and Mucopurulent Materials from CF Airways

Normal human bronchial epithelial cells were isolated from excised non-CF lungs rejected for transplant by local and distant organ procurement agencies; HBE from ΔF508/ΔF508 CF lungs were obtained at the time of a transplant. Non-CF lungs originated from patients with no history of inflammatory or infectious disease known to affect the respiratory airways. Human bronchial epithelial cells and mucopurulent materials from the airways of excised human CF lungs were obtained in accordance with institutional review board–approved protocols and provided by the Tissue Procurement and Cell Culture Core from the Cystic Fibrosis Center at the University of North Carolina at Chapel Hill as previously described (11, 12). Well-differentiated primary non-CF and CF human bronchial epithelial cultures were studied as previously reported (11, 12). SMM was obtained as previously detailed (11) and pooled from 14 CF lungs infected with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The SMM demographics are shown in Table E1 in the online supplement.

Mass Spectrometry of SMM

SMM samples were subjected to mass spectrometry–based proteomics analysis as previously described (15).

Treatment of HBE with SMM

Before the experiments, the apically accumulated mucus was removed with phosphate-buffered saline (PBS). Cultures were mucosally exposed to 30 μl PBS or SMM at different dilutions over various times. Apical mucus was collected as previously reported (16). In some studies, cultures were immediately fixed for histology.

Histology and Quantification of Mucin Stores

Cultures were fixed in 10% formalin and embedded in paraffin. Sections were stained with alcian blue/periodic acid–Schiff (AB/PAS) reagent and examined by conventional light microscopy. The intracellular AB/PAS-positive area (an index of intracellular mucin stores) was quantitated as previously reported (17), using the software ImageJ (http://rsb.info.nih.gov/ij/).

Mucin Antibodies

Immature (nonglycosylated protein) mucin antibodies used a mouse MUC5AC antibody (MAB2011, clone CLH2) and a rabbit MUC5B antibody (5BVNTR-1) generated for this study recognizing the sequence SSPGTATALPALRSTATTPTATS. Mature (globular protein) mucin antibodies used *1*) a rabbit polyclonal that recognizes all vertebrate polymeric mucins (18), *2*) a MUC5AC rabbit polyclonal (MAN5AC) that recognizes the C-terminal peptide sequence RNQDQQGPFKMC (19), and *3*) a MUC5B mouse antibody (EU-MUC5Ba) that recognizes the peptide sequence RNREQVGKFKMC (20).

Purified Mucins

MUC5AC and MUC5B were isolated from HT-29 cells and human saliva, respectively (21). Mucins purified from sputum from patients with CF were used to generate standard curves for the ELISA assay (17). Concentrations of purified mucins were determined by their refractive index (16).

Analysis of Mucin Gene Expression by qRT-PCR

These analyses were performed as previously described (22, 23).

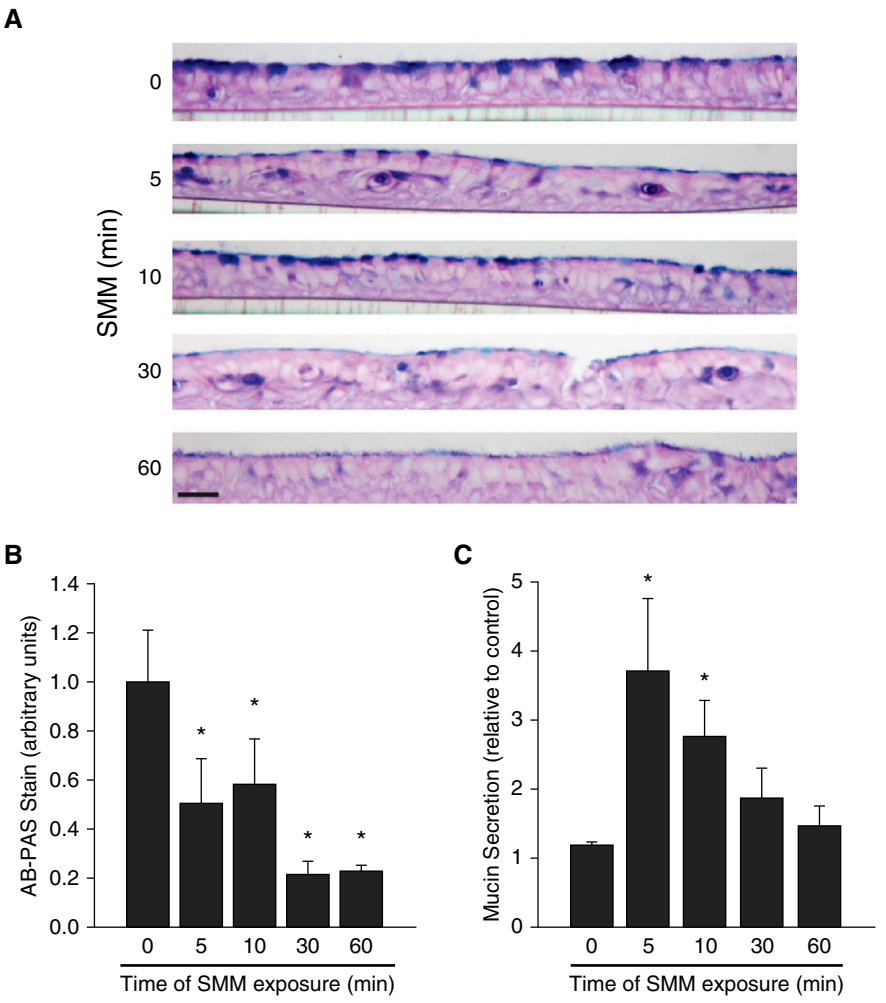

Figure 2. Time course for acute supernatant of mucopurulent material (SMM)-induced mucin release and degradation of secreted mucins. (*A*) Alcian blue/periodic acid–Schiff (AB/PAS) staining of non–cystic fibrosis (normal) human bronchial epithelial cultures mucosally exposed to undiluted SMM for various amounts of time up to 60 minutes. (*B*) Quantification of the time-dependent effect of acute SMM-promoted mucin exocytosis. (*C*) Secreted mucin levels (measured by ELISA) from the cultures depicted in *A*. Apical secretions were collected in buffer containing protease and elastase inhibitors. Cultures were fixed immediately after sample collection. Depletion of mucin stores occurs as early as 5 minutes and is almost complete by 60 minutes. Approximately 60% of secreted mucins are degraded in 1 hour, as compared with secreted mucins after 5-minute SMM exposure. Data represent mean ± SD. $n = 5$. *$P < 0.05$, SMM-exposed versus control cultures. Scale bar = 20 μm.

Assessment of Mucin Protein

Extracted human bronchial epithelial samples were dialyzed against 6-M urea buffer, and their mucins were resolved by electrophoresis in 1% agarose gels, and analyzed by Western blotting (3).

Mucin Secretion

Apical secretions were collected after each treatment (19), and mucin quantities measured by ELISA (3, 16). For inhibition of mucin proteolysis, a cocktail of protease inhibitors with broad spectrum for serine, cysteine, and metalloproteases and a specific elastase inhibitor were added to the samples immediately after collection. To evaluate total accumulation of secreted mucins during 72-hour SMM exposure, the mucosa was lavaged five times with PBS to collect accumulated mucins. Cultures were subsequently exposed to 100-μM adenosine 5′-O-(3-thio)triphosphate (ATPγS) to release mucins from intracellular stores, which were collected in 400 μl PBS. Total mucins were measured by ELISA using the previously mentioned mucin antibodies.

Bioelectric Studies

These were performed in HBE mounted in Ussing chambers as previously reported (24).

Airway Surface Liquid Height

A total of 20 μl PBS or SMM containing 2 mg/ml rhodamine-dextran was added to prewashed cultures at the start of the experiment and airway surface liquid (ASL) height measured with XZ confocal microscopy as previously detailed (25).

Assessment of Mucus Percent Solids

The percentage solids of mucus from normal and CF human bronchial epithelial cultures was measured as previously reported (8).

Statistical Analysis

All experiments were performed on cultures established from at least three different donors ($n \geq 3$ independent biologic replicates). Data are expressed as mean values ± SD, and analyzed by ANOVA, using the GraphPad InStat software. Statistical significance was defined as P less than 0.05.

Results

Acute Effect of SMM on Mucin Secretion and Degradation in Normal (Non-CF) HBE

We first tested whether acute exposure of normal HBE to SMM promoted mucin secretion. Cultures were exposed to different dilutions of SMM for 1 hour, followed by collection of lumenal secretions (containing residual SMM) for assessment of mucin concentrations. As a positive control for mucin secretion, cultures were exposed to ATPγS, a purinergic agonist that induces maximal mucin exocytosis (3, 4, 17). Cultures were fixed and stained with AB/PAS to visualize their intracellular mucin content.

Exposure of normal human bronchial epithelial cultures to undiluted SMM produced a maximal discharge of mucin stores, based on the comparability of AB/PAS-positive intracellular content to that following a maximal concentration of ATPγS (Figure 1A). Dilution of SMM by a factor of 3 elicited approximately the same mucin discharge effect as the undiluted material (Figure 1A). In contrast, a 10-fold dilution of SMM exhibited a decreased

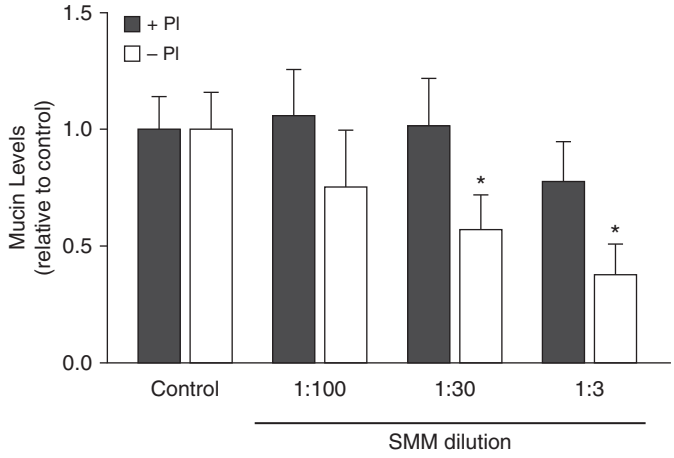

Figure 3. Protease inhibition prevents mucin degradation by supernatant of mucopurulent material (SMM). Mucins harvested from non–cystic fibrosis (normal) human bronchial epithelia were incubated with different doses of SMM in presence or absence of protease inhibitors (PI) for 30 minutes. Samples were evaluated for MUC5B, because it is the major mucin produced and secreted by human bronchial epithelial cultures. Data represent mean ± SD. $n = 3$. *$P < 0.05$, SMM-exposed versus control cultures not incubated with PI.

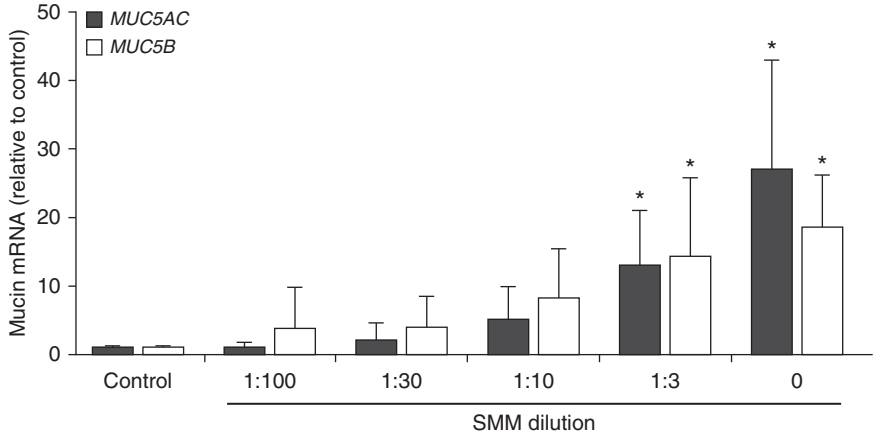

Figure 4. Dose–response for chronic supernatant of mucopurulent material (SMM) exposure on mucin gene expression. Non–cystic fibrosis (normal) human bronchial epithelial cultures were washed and mucosally exposed to various doses of SMM for 48 hours. Cultures were washed again and extracted in RNeasy guanidine-based buffer, and MUC5AC and MUC5B mRNA levels were determined by qRT-PCR. Data represent mean ± SD. $n = 6$. *$P < 0.05$, SMM-exposed versus control cultures.

effect on mucin exocytosis, as evidenced by increased residual mucin storage in mucous cells (Figure 1A). The quantification of the dose-dependent effect of acute SMM-promoted mucin exocytosis is depicted in Figure 1B.

We next assessed the quantity of mucins secreted following 1-hour exposure to various dilutions of SMM measured by ELISA (16) using a subunit antibody that detects all mature polymeric mucins tested so far (canine, bovine, mouse, rat; unpublished data from L.H.A.). In contrast with the histology data depicted in Figures 1A and 1B, the quantity of secreted mucins was inversely correlated with both SMM concentration and intracellular stores (Figure 1C). Because secreted mucins were in contact with SMM for 1 hour, we speculated that proteases present in SMM caused proteolytic degradation of antigenic epitopes of secreted mucins in a time-dependent manner. To address this possibility, a time course for full-strength SMM-induced mucin secretion was performed, mucosal samples collected, and cultures fixed for histologic analysis. Mucin release occurred within 5 minutes of undiluted SMM exposure, and the mucin stores were fully discharged after a 30-minute exposure (Figures 2A and 2B). Peak mucin secretion was observed at 5 minutes, following which a progressive reduction in ELISA-measureable mucin was detected (Figure 2C). These data suggest that SMM induces a time-dependent proteolytic loss of antigenic epitopes of secreted mucins.

To further investigate the proteolytic degradation of secreted mucins by SMM, secreted mucins from normal human bronchial epithelial cultures were exposed to SMM for 30 minutes in the presence or absence of protease inhibitors. Samples were selectively assayed for MUC5B because it is the major mucin in human bronchial epithelial cultures (3). SMM induced MUC5B degradation in a dose-dependent manner, and this effect was blunted by the addition of protease inhibitors (Figure 3). These findings agree with previous observations that proteolytic degradation of mucin antigenic epitopes can be induced by the elastase and other proteases that are components of CF sputum (23, 26).

Because the acute stimulatory effect of SMM on mucin secretion could be caused, at least in part, by neutrophil elastase (27, 28), we subjected SMM to proteomic analysis for identification of proteases. Table E2 depicts the proteases, and protease inhibitors, present in SMM. Several neutrophil-derived proteases, including neutrophil elastase, cathepsin G, and the neutrophil metalloproteases collagenase (metalloproteinase 8) and gelatinase (metalloproteinase 9; MMP-9), were identified in SMM. These proteases are present in ASL and BAL fluid from patients with CF (29, 30). Protease inhibitors (e.g., leukocyte elastase inhibitor, plasma protease C1 inhibitor, metalloproteinase inhibitors 1 and 2 and alpha-1 antitrypsin) were also identified in SMM (*see* Table E2). These findings are consistent with the notion that proteases present in SMM contributed to both mucin secretion and proteolysis. SMM also contains nucleotides (14) and a wide assortment of T-helper cell type 1 cytokines (*see* Figure E1), which may also contribute to mucin secretion in inflamed airways.

Chronic Effect of SMM on Mucin Synthesis and Secretion

Chronic SMM-induced mucin production was studied at 48 and 72 hours because preliminary studies indicated that

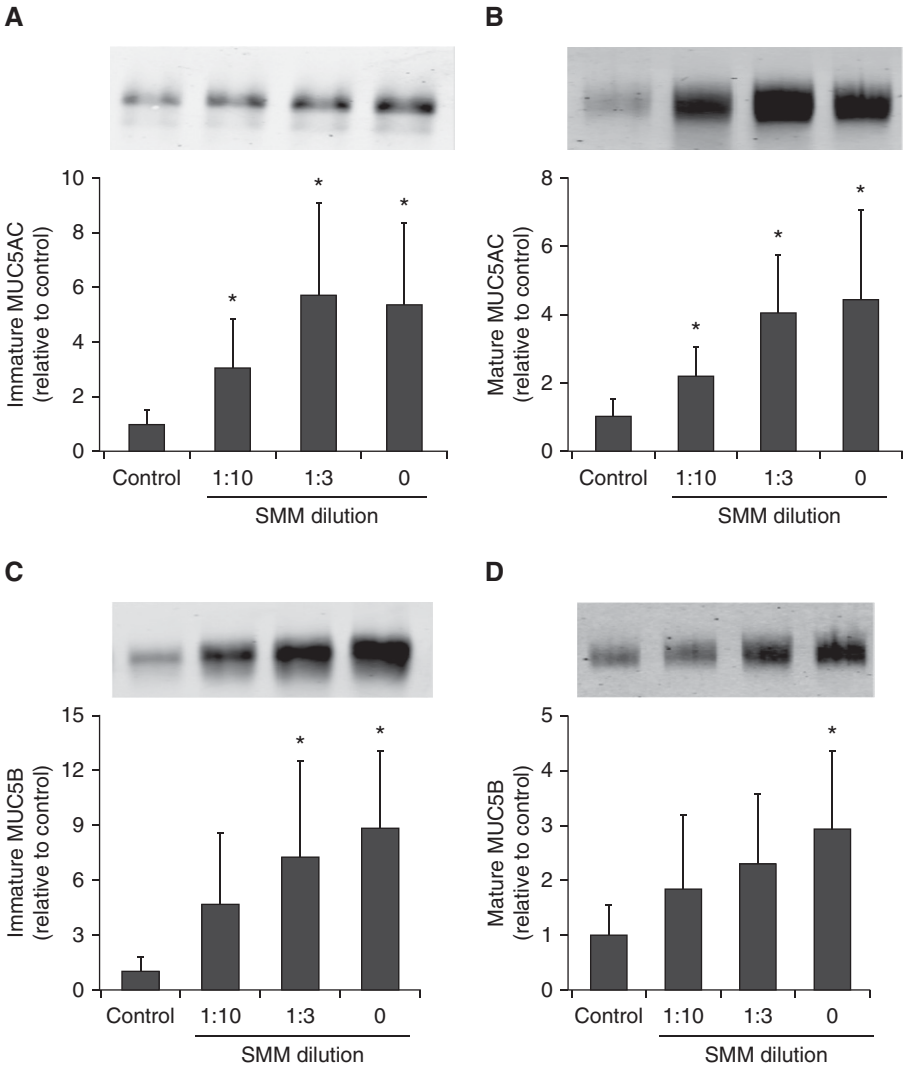

Figure 5. Dose–response for chronic supernatant of mucopurulent material (SMM) exposure on MUC5AC and MUC5B protein synthesis. Non–cystic fibrosis (normal) human bronchial epithelial cultures were washed and mucosally exposed to different dilutions of SMM for 48 hours. Cultures were extracted in RNeasy guanidine-based buffer, and aliquots electrophoresed in agarose. Immunoblots were probed with antibodies to the immature and mature forms of MUC5AC (*A* and *B*) and MUC5B (*C* and *D*). Data represent mean ± SD. $n = 5–6$. $^*P < 0.05$, SMM-exposed versus control cultures.

SMM-induced mucous cell metaplasia was evident at 48 hours and maximal at 72 hours (data not shown). SMM promoted large increases in MUC5AC and MUC5B mRNA levels at 48 hours in a dose-dependent manner (Figure 4). Whole cell extracts from 48-hour SMM-treated cultures revealed that SMM exposure for 48 hours stimulated production of both the immature and mature forms of MUC5AC and MUC5B, an effect dependent on SMM dilution (Figure 5).

The upregulation of the mRNA and protein production levels of MUC5AC and MUC5B observed at 48 hours with undiluted SMM exposure was maintained at 72 hours, but a few differences were noted (Figure 6). First, the relative levels of MUC5AC mRNA waned substantially at 72 hours versus 48 hours with undiluted SMM exposure (Figure 4), producing a relatively greater increase in MUC5B versus MUC5AC mRNA at 72 hours (Figure 6). Second, whereas there were no significant changes in the relative levels of immature MUC5AC and MUC5B protein between 48- and 72-hour SMM exposure (Figures 5 and 6), the levels of both mature MUC5AC and MUC5B protein increased after 72-hour SMM exposure (compare Figure 6 with Figure 5). The upregulation of the level of mature MUC5B was greater than mature MUC5AC, perhaps reflecting the fact that human bronchial epithelial cultures typically express more MUC5B than MUC5AC (3).

We next compared the mucin secretory responses of normal versus CF HBE with chronic (72 h) SMM exposure to mimic the chronic exposure that occurs *in vivo* (Figure 7). Unlike our previous report (3), MUC5AC, but not MUC5B, secretion rates were greater under basal conditions in CF than normal cultures. We speculate that this difference may reflect variable epigenetic regulation of MUC5AC expression that can be manifest in CF cultures (31).

With respect to responses to an inflammatory challenge, 72-hour SMM increased secretion of MUC5AC and MUC5B equally in both non-CF and CF cultures (Figure 7). Indeed, chronic exposure to SMM produced an equivalent upregulation of mucin biosynthesis, mucin secretion, and lumenal accumulation of mucins in both normal and CF HBE. We speculate that, unlike the acute SMM exposure, the proteolytic capacity of SMM was overwhelmed by the SMM-induced mucin overproduction/secretion in both non-CF and CF cultures.

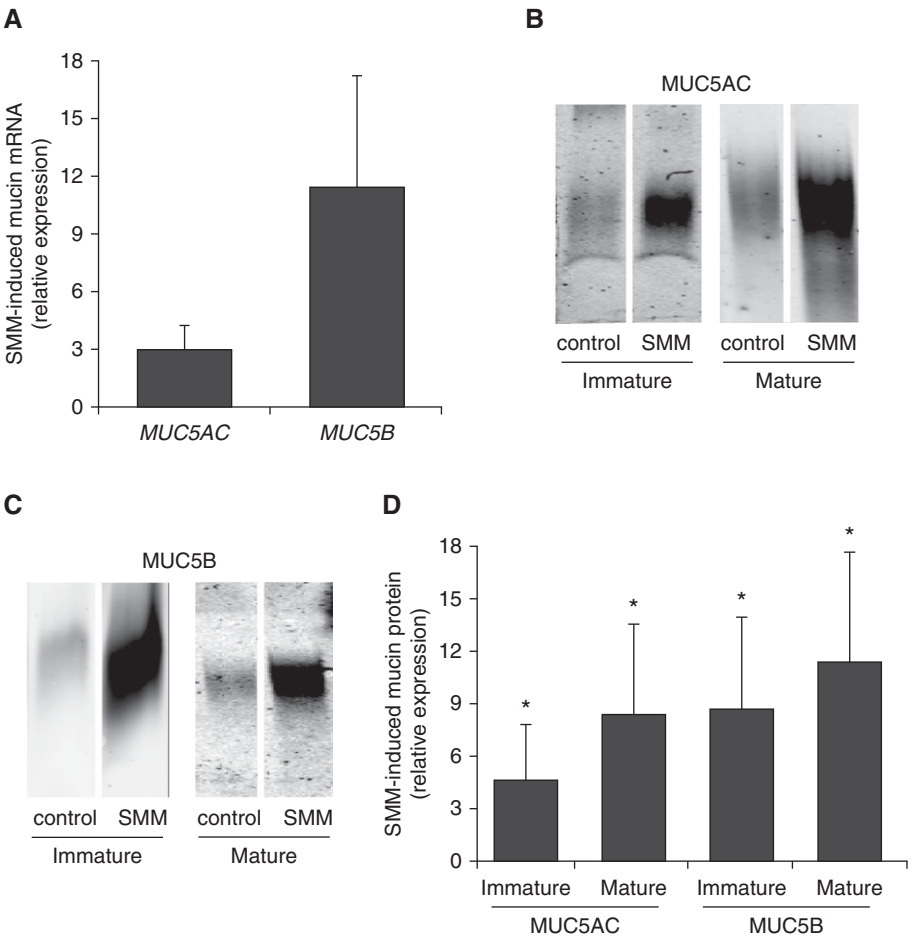

Figure 6. Effect of chronic (72 h) supernatant of mucopurulent material (SMM) treatment on MUC5AC and MUC5B mRNA and protein levels. Non–cystic fibrosis (normal) human bronchial epithelial cultures were washed and mucosally exposed to undiluted SMM for 72 hours. Cultures were extracted in RNeasy guanidine-based buffer. Aliquots were either used for determination of MUC5AC and MUC5B mRNA levels by qRT-PCR (*A*) or electrophoresed in agarose for evaluation of the immature and mature forms of MUC5AC (*B*) and MUC5B (*C*). (*D*) Compiled data expressed as fold change of SMM/phosphate-buffered saline from the experiments illustrated in *B* and *C*. Data represent mean ± SD. $n = 6$–8. $^*P < 0.05$, SMM-exposed versus control (phosphate-buffered saline) cultures.

Characterization of Ion Transport Responses to SMM

Bioelectric studies were conducted in normal and CF HBE lumenally exposed to PBS or SMM for 72 hours. In the absence of SMM, the amiloride-sensitive current was higher in CF versus non-CF cultures (Figures 8A and 8B). Notably, SMM exposure decreased the amiloride-sensitive current in normal cultures, whereas it did not change the amiloride-sensitive current in CF cultures (Figures 8A and 8B). Forskolin-induced Cl^- secretory currents were present in PBS-exposed normal HBE, whereas they were absent in CF HBE (Figures 8A and 8B). Importantly, the forskolin-induced current in normal cultures was doubled by the SMM treatment (Figure 8A). In contrast, there was no induction of forskolin stimulated Cl^- secretory channels in CF HBE exposed to SMM, consistent with the lack of functional CFTR activity in the CF cultures (Figure 8B). Moreover, SMM potentiated uridine-5′-triphosphate–induced currents, which reflect a calcium-mediated response, in both normal and CF HBE (Figures 8A and 8B), in agreement with previous studies indicating that SMM-induced

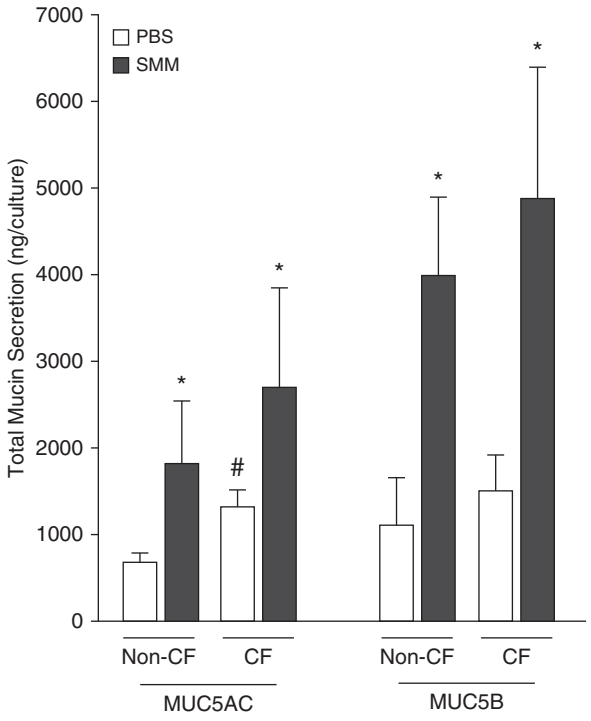

Figure 7. Chronic (72 h) supernatant of mucopurulent material (SMM) treatment of non–cystic fibrosis (CF) and CF human bronchial epithelial cultures promotes increased mucin secretion. Accumulated secreted mucins and ATPγS (100 μM)-induced release of intracellular stored mucins from non-CF (normal) and CF human bronchial epithelial cultures exposed to phosphate-buffered saline (PBS) (control) or undiluted SMM for 72 hours. The apically accumulated mucins were assayed by ELISA, using mucin-specific antibodies, as detailed in METHODS. Data represent mean ± SD. $n = 5$. $^{*}P < 0.05$, SMM-exposed non-CF or CF versus control (PBS-treated) cultures. $^{\#}P < 0.05$, CF versus non-CF cultures exposed to PBS. ATPγS = adenosine 5′-O-(3-thio)triphosphate.

inflammation expands the endoplasmic reticulum calcium stores, resulting in an upregulation of calcium-mediated responses (11, 12). The compiled data depicting differences in amiloride-sensitive, and forskolin- and uridine-5′-triphosphate–induced currents in normal and CF HBE are shown in Figure 8C.

The effect of SMM to increase forskolin-induced currents in non-CF cultures was additionally addressed in time course and dose–response studies for SMM. Forty-eight-hour SMM exposure promoted maximal increases in forskolin-induced currents, and this effect was dose dependent (*see* Figure E2).

ASL Volume Responses to SMM

To directly address the impact of SMM regulation of ion transport on airway hydration, ASL height was measured in normal and CF HBE exposed to SMM for 72 hours. As compared with PBS administration, SMM robustly increased ASL height in normal cultures but had no effect on ASL height in CF cultures (Figure 9A). Mucosal addition of $CFTR_{Inh\text{-}172}$ to normal HBE blocked the SMM-induced increase in ASL height, consistent with a CFTR-dependent fluid secretory mechanism (Figure 9B). Taken together, these findings indicate that in normal airway epithelia with functional CFTR, the upregulation of mucin production/secretion resulting from infection/inflammation is accompanied by a robust CFTR-mediated airway hydration.

Mucus Hydration Responses to SMM

We next evaluated the impact of 72-hour SMM on the percent of secreted mucus solids in normal and CF cultures. Exposure of normal HBE to SMM decreased the percent solids of secreted mucus (Figure 10). In contrast, SMM treatment of CF cultures increased the percentage of mucus solids (Figure 10). These data suggest that SMM increases airway hydration in normal HBE, whereas this effect is absent in CF HBE because of the functional loss of CFTR.

Discussion

CF airways are chronically exposed *in vivo* to an intralumenal mucopurulent material containing host and bacterial products. Mucous cell metaplasia/hyperplasia is a hallmark of CF airway epithelia in response to the infectious and inflammatory airway milieu. However, the effects of an intralumenal infectious/inflammatory milieu on mucus cell hyperplasia, balance between mucin secretion and proteolysis, and coordinate fluid transport in normal versus CF airway epithelia have not been well characterized.

The relative rates of mucin versus liquid secretion determine the hydration status of airway mucus, denominated by the percent solids content. Importantly, mucus clearance rates and disease severity scale inversely to mucus hydration (26, 32, 33). The present study used human bronchial epithelial cultures to evaluate the effects of CF airway lumenal contents (SMM) as a probe of the inflammatory stress chemically presented to CF airway surfaces. The effects of SMM on mucin and liquid secretory responses of normal versus ΔF508/ΔF508 CF cultures and the consequent effects on mucus hydration were compared.

Acute exposure of normal HBE to SMM promoted mucin secretion in a dose- and time-dependent manner (Figures 1 and 2). We speculated that the acute effect of SMM on mucin secretion was likely caused, in part, by proteases present in SMM, and a proteomics analysis identified multiple proteases in SMM (*see* Table E2). A component of CF sputum shown to stimulate mucin release from airway epithelia, neutrophil elastase (28, 34), was identified in SMM. Cathepsin G, also reported to promote mucin secretion (35), was also detected in SMM. SMM also contains MMP-9 (*see* Table E2). MMP-9 activity is increased during CF pulmonary exacerbations (29), and it cleaves alpha-1 antitrypsin (36), the major antiprotease of the lower respiratory tract that inactivates neutrophil elastase (37). Therefore, MMP-9 may increase the neutrophil elastase activity in SMM and contribute to increased neutrophil elastase–triggered mucin release into the airways.

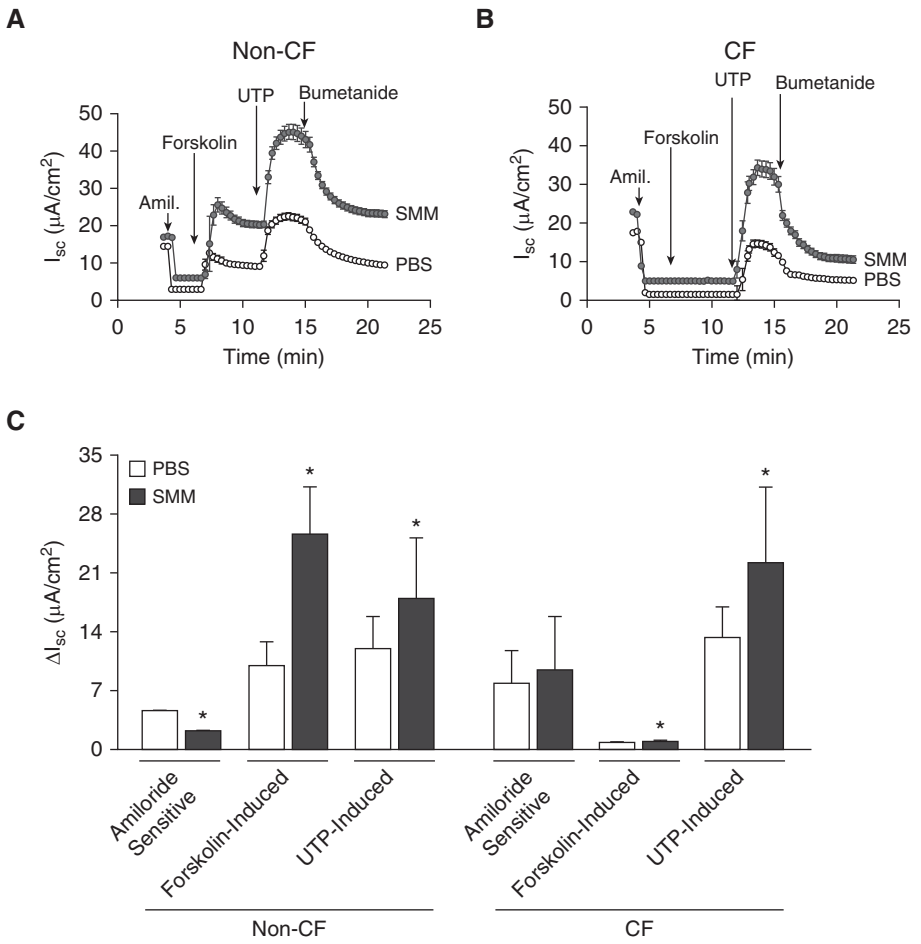

Figure 8. Effect of supernatant of mucopurulent material (SMM) on the bioelectric properties of non–cystic fibrosis (CF) and CF human bronchial epithelia. Bioelectric measurements were conducted in non-CF (normal) and CF human bronchial epithelial cultures, as described in METHODS. (*A* and *B*) Effect of sequential addition of amiloride (Amil.) (300 μM; apical), forskolin (10 μM; apical), UTP (100 μM; apical), and bumetanide (100 μM; basolateral) on short circuit currents in non-CF (*A*) and CF (*B*) cultures exposed to phosphate-buffered saline (PBS) or SMM for 72 hours. (*C*) Compiled data from *A* and *B* for amiloride-sensitive and forskolin- and UTP-induced currents. Data represent mean ± SD. $n = 8$–12. $^*P < 0.05$, SMM-exposed versus control (PBS-exposed) cultures. I_{SC} = short circuit current; UTP = uridine-5′-triphosphate.

The acute effects of SMM on mucin secretion likely were also mediated by intracellular Ca^{2+} mobilization resulting from activation of purinoceptors in HBE by nucleotides (11, 38), which are contained in SMM (14). The concentration of ATP in SMM is approximately 1 μM, similar to the ATP levels observed in CF sputum (14). Notably, mucosal exposure of CF HBE to 1-μM ATP elicits intracellular Ca^{2+} signals in the 400-nM range (39), which promotes mucin exocytosis. Other constituents in SMM, such as ILs, acting via activation of receptor-dependent intracellular Ca^{2+} mobilization, and tumor necrosis factor (TNF)-α may also be implicated in calcium-mediated mucin exocytosis (40–42).

Acute SMM exposure also led to proteolysis of secreted mucins in a dilution- and time-dependent manner (Figures 1 and 2). This effect was also mediated by proteases present in SMM (*see* Table E2), based on the observation that protease inhibitors protected secreted mucins from SMM-induced proteolysis (Figure 3). These data are in agreement with previous reports that serine proteases mediate mucin epitope proteolysis (43).

Prolonged SMM exposure had large stimulatory effects on the transcription, translation, and secretion of MUC5AC and MUC5B (Figures 4–7). Several components in SMM are candidates to upregulate mucin synthesis. For example, long-term exposure to neutrophil elastase induces mucous hyperplasia, resulting in a larger population of mucin-secreting cells (44). In addition to proteases, SMM contains bacterial exoproducts and T-helper cell type 1 cytokines previously reported in CF secretions, including IL-1β and TNF-α (*see* Figure E1). IL-1β, TNF-α, and LPS increase the expression of mucins via transcriptional and post-transcriptional mechanisms, providing mechanisms for increased mucin production on a per cell basis (45, 46). The increased mucin gene transcription was also associated with increased levels of mucin secretion, with secretion of MUC5B chronically exceeding secretion of MUC5AC (Figure 7). This finding is consistent with the increased basal mucin secretion caused by increased secretory cell numbers noted in previous studies (17).

Our data indicate that CF HBE exposed to SMM exhibit similar mucin secretory responses as normal HBE exposed to SMM, whereas fluid secretory responses differed between normal and CF HBE exposed to SMM. Three observations supported this conclusion. First, forskolin-induced currents (an index of CFTR-mediated Cl^- secretion) were upregulated by SMM in normal, but not in CF, HBE (Figure 8). Second, SMM increased ASL height in normal, but not in CF, cultures (Figure 9). Third, the percent of mucus solids was decreased in normal HBE following SMM exposure, whereas the concentration of mucus increased after SMM exposure in CF cultures (Figure 10).

These data are pertinent to clinical data from CF subjects. A key distinction between sputum from normal versus CF individuals is the relative difference in airway dehydration that may reflect the presence versus absence of functional CFTR in normal versus CF subjects, respectively. Mucus concentrations have been reported as approximately 2% solids in normal subjects and greater than or equal to 7% solids in CF subjects (47). Importantly, mucus percent solids correlate directly with loss of mucociliary clearance *in vitro* and *in vivo*, and with FEV_1 in subjects with chronic bronchitis (8). It has been proposed that the

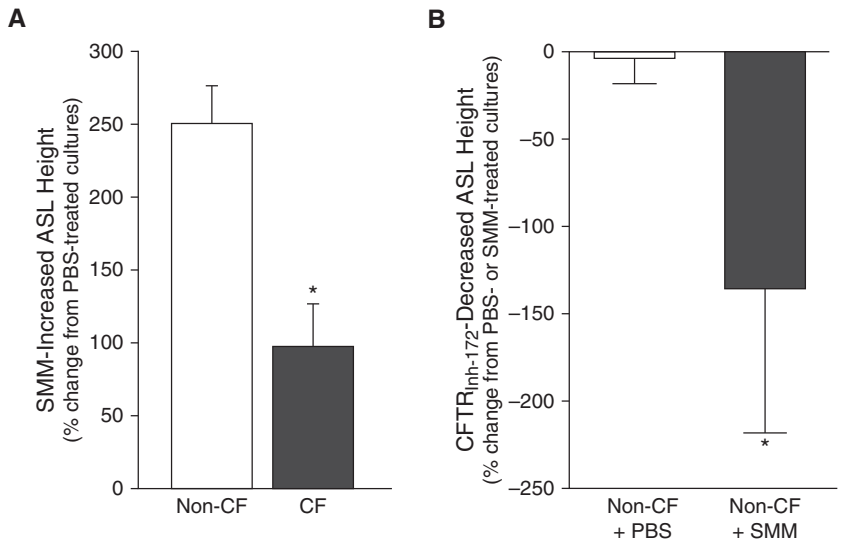

Figure 9. Effect of supernatant of mucopurulent material (SMM) on airway surface liquid (ASL) height in non–cystic fibrosis (CF) versus CF human bronchial epithelial cultures. (*A*) Non-CF (normal) and CF human bronchial epithelial cultures were exposed to phosphate-buffered saline (PBS) or SMM for 72 hours. ASL height was evaluated as described in METHODS, and is shown as the percentage increase after treatment with SMM over PBS for both groups. Data represent mean ± SD. $n = 5$–6. $^*P < 0.05$, CF versus non-CF cultures. (*B*) Non-CF human bronchial epithelial cultures were exposed to SMM for 48 hours and ASL height was evaluated by XZ confocal microscopy. $CFTR_{Inh\text{-}172}$ was added apically as a dry powder in perfluorocarbon and ASL height was remeasured. ASL height is expressed as percentage inhibition of ASL height by $CFTR_{Inh\text{-}172}$ relative to ASL height from PBS- or SMM-treated non-CF human bronchial epithelia. Data represent mean ± SD. $n = 4$. $^*P < 0.05$, SMM + $CFTR_{Inh\text{-}172}$ versus SMM. CFTR = CF transmembrane conductance regulator.

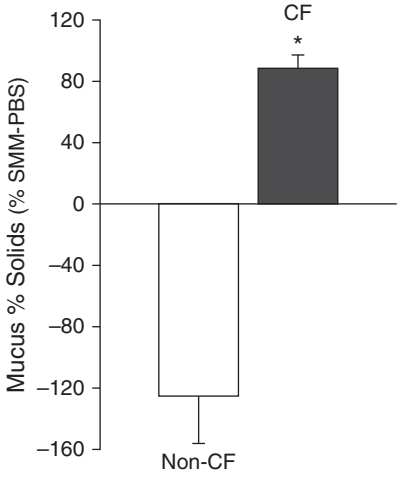

Figure 10. Effect of supernatant of mucopurulent material (SMM) on mucus percent solids in non–cystic fibrosis (CF) versus CF human bronchial epithelial cultures. Non-CF (normal) and CF cultures were exposed to phosphate-buffered saline or SMM for 72 hours. Mucus percent solids was evaluated as described in METHODS. Data represent mean ± SD. $n = 8$ for all groups. $^*P < 0.05$, CF versus non-CF cultures. PBS = phosphate-buffered saline.

link between the percent solids content and reduced mucociliary clearance is the mucus osmotic pressure (8). The greater than or equal to 7% mucus solids concentrations in CF generate osmotic pressures predicted to produce severe compression of the periciliary liquid layer, with resultant mucus adhesion and stasis (47). Our data suggest that normal airways can produce a more copious but "thin" (transportable) mucus to capture and flush secretory toxic materials from the lung, whereas CF airways fail to generate this adaptive response.

Our findings might be also relevant to non-CF bronchiectasis (NCFB), a milder obstructive disease than CF, as evidenced most simply by the large difference in life span between NCFB and CF subjects. We speculate that the relative concentrations of mucus may contribute to the greater severity of CF versus NCFB lung disease (26). Such a difference in CF versus NCFB mucus concentrations may reflect the different mucin versus liquid secretory responses to the mix of bacterial and host defense materials in the airway lumens of NCFB versus CF subjects. Indeed, NCFB is associated with less hyperconcentrated mucus than CF (48, 49), with mucus concentrations reported as approximately 3–4% solids in NCFB subjects (47). Osmotic pressures in the NCFB 3–4% range are predicted to modestly compress cilia, slow mucus clearance, and likely produce disease (8, 47). Studies using primary human bronchial epithelial cultures and SMM from NCFB subjects are necessary to address the integrated mucin and fluid secretory responses to the intralumenal mucopurulent materials to test for quantitative differences between NCFB and CF airways.

In summary, exposure of human bronchial epithelial cultures to the bacterial and host defense products in SMM produced acute mucin release, whereas longer term SMM exposure induced upregulation of mucin synthesis, storage, and release. These mucin responses seemed similar in normal and CF cultures. In contrast, SMM produced a coordinated CFTR-mediated Cl^- secretory and ASL volume responses in normal, but not in CF, HBE. The absence of the fluid secretory response in CF produced a more dehydrated (concentrated) mucus that is predicted to exacerbate mucus adhesion and accumulation, rather than exhibit the normal hydrating/flushing response as an adaptive host response. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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