Regulation of Antiprotease and Antimicrobial Protein Secretion by Airway Submucosal Gland Serous Cells*

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Airway submucosal gland serous cells express the cystic fibrosis transmembrane conductance regulator (CFTR) and secrete antimicrobial, anti-inflammatory, and antioxidant molecules. In cystic fibrosis, diminished gland secretion may impair innate airway host defenses. We used Calu-3 cells as a serous cell model to study the types of proteins released, the pathways that release them, and the possible involvement of CFTR activity in protein release. Many proteins were secreted constitutively into the apical fluid and showed increased release to agonists. We identified some of them by high pressure liquid chromatography-mass spectrometry and reverse transcriptase PCR, including lysozyme, siderocalin (the protein NGAL), which inhibits bacterial growth by binding iron-containing siderophores, HSC-71, which is thought to have anti-inflammatory properties, and the serine protease inhibitors α-1-antitrypsin and α-1-antichymotrypsin, which may function as antimicrobials as well as play a potential role in diminishing the activation of epithelial Na⁺ channels by serine proteases. We used an enzyme-linked immunosorbent assay to quantify lysozyme secretion by Calu-3 cells in response to various agonists and inhibitors. Forskolin increased the lysozyme secretion rate (Jlyz) from 32 to 77 ng/hr/cm² (n = 36, p < 0.005). Thapsigargin increased Jlyz from 40 to 63 ng/hr/cm² (n = 16, p < 0.005), and forskolin plus thapsigargin further increased the forskolin-stimulated Jlyz by 48% (n = 9, p < 0.05). 1-Ethyl-benzimidazolinone and carbachol were less effective. Glibenclamide inhibited basal and stimulated Jlyz, but clotrimazole was without effect. CFTRinh₁₁₇₂ caused a small (15%) but significant inhibition of forskolin-stimulated Jlyz without affecting basal Jlyz. Thus, Calu-3 cells secrete diverse proteins that in aggregate would be expected to suppress microbial growth, protect the airways from damage, and limit the activation of epithelial Na⁺ channels via serine proteases.

The main function of the airways is to conduct air to and from the respiratory bronchioles and alveoli for gas exchange. Airborne particles, including pathogens, are adsorbed by the mucus layer lining the airways, where they are inactivated by innate mucosal defense system and removed via mucociliary and cough clearance. In cystic fibrosis (CF), this process is defective, allowing bacteria and fungi to grow within static airway mucus. That leads to a process of inflammation that eventually occludes and destroys the airways. Most of the mucus lining the upper airways is produced by submucosal glands, and gland secretion is defective in CF (1). Glands are complex structures that comprise a ciliated duct connected to a non-ciliated collecting duct into which multiple tubules empty. Each tubule is lined by mucous cells in its proximal part and serous cells at the distal ends. Serous cells are reported to secrete a rich mixture of proteins that have antimicrobial, antiprotease, antioxidant and anti-inflammatory functions, although there is to date relatively little direct evidence of such secretion. Within submucosal glands, serous cells are the main site for cystic fibrosis transmembrane conductance regulator (CFTR) expression.

Studies of submucosal gland secretion performed in animals, healthy human subjects, and humans with cystic fibrosis have led to a model in which cholinergic stimulation, which elevates [Ca²⁺], leads to secretion from both mucous and serous cells, whereas stimulation with vasoactive intestinal peptide (VIP), which elevates [cAMP], stimulates only serous cells. In cystic fibrosis, secretion to VIP or forskolin is completely lost (1), whereas the response to cholinergic stimulation is altered, which leads to thicker mucus (2, 3). Joo et al. interpreted their results with a model of gland secretion in which the lack of functional CFTR causes the electrolyte-driven fluid secretion from serous cells to be deleted, leaving protein secretion intact (1). However, because no mucus is observed leaving CF gland ducts after stimulation with VIP, these results imply that the proteins are trapped within the glands. An alternative view is that CFTR mediates the secretion of mucins and serous proteins and that its loss will cause CF mucus to have a diminished complement of defense molecules (4).

The inaccessibility of serous cells makes them difficult to study directly. Therefore, the Calu-3 cell line, a serous cell model, has been used as a surrogate to study fluid and protein secretion (5). Previous studies have shown that Calu-3 cells are capable of releasing proteins such as glycoproteins/mucins and lysozyme in response to cAMP agonists (4, 6–8). In this study, we harvested uncontaminated mucus secreted by porcine glands and collected apical fluid from Calu-3 cells, analyzing bands via silver-stained SDS-PAGE gels. We then identified five proteins not previously known to be secreted by either glands or Calu-3 cells and reviewed evidence that they inhibit serine proteases and have antimicrobial functions. Be-
cause airway surface ENaCs are known to be activated by serine proteases, the secretion of serine protease inhibitors by gland serous cells could play a role in diminishing Na⁺-driven fluid absorption via ENaCs. We also demonstrate that proteins are released in response to elevations of either [Ca^{2+}i] or [cAMP], with the latter pathway being more effective in Calu-3 cells. Our attempts to implicate CFTR in protein release were not conclusive; the most effective and specific CFTR inhibitor we used, CFTRinh172 (3), produced only a slight (but significant) decrease in forskolin-stimulated lysozyme release.

**MATERIALS AND METHODS**

**Tissues and Mucus Collection**—Porcine tracheas were harvested <1 h postmortem from adult Yorkshire pigs that had been killed after acute experiments unrelated to the present studies. Tissue was maintained until use in ice-cold Krebs-Ringer bicarbonate buffer bubbled with 95% O₂ and 5% CO₂. The Krebs-Ringer bicarbonate composition was 115 mM NaCl, 2.4 mM KH₂PO₄, 0.4 mM KH₂PO₄, 25 mM NaHCO₃, 1.2 mM MgCl₂, 1.2 mM CaCl₂, and 10 mM glucose (pH 7.4), and osmolality was adjusted to ~290 mosm. To minimize endogenously generated prostaglandins, 1 μM indomethacin was present in the bath throughout the experiment. The experimental setup for the agonist-stimulated mucus secretion was as described previously (9–11). Secretable mucus bubbles were collected by aspiration and stored at −20 °C until use.

**Calu-3 Cell Culture and Apical Fluid Collection**—Calu-3 cells were grown in a T₆₀ tissue culture flask containing a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 nutrient mixture supplemented with 15% fetal bovine serum (Sigma), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamate at 37 °C in a humidified atmosphere containing 5% CO₂. Cells were passaged once a week with 0.25% trypsin. After 3 weeks of an initial submucosal gland mucus from porcine trachea was evaluated with its processed form, NGAL, and the heat shock cognate protein precursor lipocalin-2 and its processed form, siderocalin (12). A representative LC-MS peptide mixture was analyzed using LC-MS. The acquired mass data from glands and Calu-3 cells, and some of these were digested by trypsin. Peptide mixtures were analyzed using LC-MS. The acquired mass spectra from trypsin-digested products were compared with the known trypsin digest protein sequence database using Mascot software (Matrix Science). An ELISA for human lysozyme was initially carried out with a kit from Biomedical Technologies Inc. We then developed an in-house ELISA for human lysozyme. Briefly, rabbit anti-lysozyme antibody (Zymed Laboratories Inc.) was coated onto 96-well ELISA plates at 5 μg/ml using 50 mM carbonate-bicarbonate buffer (Sigma). Coating was done by overnight incubation at 4 °C. After washing, the plates were incubated with a 1:50 000 dilution of rabbit anti-sheep IgG (H + L) horseradish peroxidase conjugate (Sigma) for 1 h. The plate was washed 3–5 times with PBS-Tween and incubated with the substrate, 3,3',5,5'-tetramethylbenzidine (Zymed Laboratories Inc.) for 20 min in the dark. Absorbance at 450 nm was recorded using an automated ELISA microplate reader (Molecular Devices).

**RESULTS AND DISCUSSION**

**Identification of Secreted Proteins**—Uncontaminated submucosal gland mucus from porcine tracheas was evaluated with the silver staining of Tris-Tricine SDS gradient gels (Fig. 1). Under the conditions used, at least 40 bands, from ~5 to 200 kDa, could be resolved. No differences were found between forskolin- or carbachol-induced mucus in either the number or intensities of the bands, indicating that these two methods of stimulation have equivalent effects on the protein secretion by submucosal glands (Fig. 1a).

The same methods were used to evaluate apical secretions collected from unstimulated (basal) and forskolin-stimulated Calu-3 cells. As shown in Fig. 1b, no silver-stained bands were found from the lane of apical washings from Calu-3 cells taken right before the 2 h incubation (WO lane). The absence of proteins in the preincubation wash fluid proves that washing did not release a burst of secretion or dislodge detectable levels of cells. Forskolin stimulation increased the quantity of protein secreted, but there was no evidence for additional bands or an alteration in the relative band intensities of stimulated versus basally secreted proteins, suggesting that no proteins were secreted either purely constitutively or purely in response to stimulation. Our cells typically have some basal level of a short circuit current (Isc) and fluid secretion, and the question of whether this represents a low grade of stimulation by unknown factors has not been resolved. Although carbachol was a potent stimulus for gland protein secretion (Fig. 1a), it was less effective in stimulating protein secretion from Calu-3 cells (gel data not shown; see ELISA results).

A comparison of forskolin-stimulated gland and Calu-3 secretions indicated that our collection methods (see “Materials and Methods”) yield less total protein from Calu-3 cells versus glands. Nevertheless, although not immediately apparent in Fig. 1, many bands of comparable molecular weight and comparable relative intensities were observed in the silver-stained SDS gels from glands and Calu-3 cells, and some of these were selected for further analysis using LC-MS.

Six proteins secreted by Calu-3 cells were identified using LC-MS, namely lysozyme, α-1-antichymotrypsin, α-1-antitrypsin, and the heat shock cognate protein HSC71. Because the 198-amino acid NGAL precursor lipocalin-2 and its processed form, NGAL, and the heat shock cognate protein HSC71. Because the 198-amino acid NGAL precursor lipocalin-2 (~22.8 kDa) and the 178-amino acid NGAL (~20.7 kDa) are not clearly distinguished in terms of function, we discuss them together as siderocalin (12). A representative LC-MS
analysis for α-1 antitrypsin is shown in Fig. 1c. Analysis for two bands was inconclusive.

Reverse transcriptase PCR analysis of Calu-3 lysates was used to confirm the presence of transcripts for these five proteins (counting NGAL and lipocalin-2 as one protein) using primers designed on reported sequences of each protein. We detected clear evidence for the expression of all of them in Calu-3 lysates (data not shown). The complementary results from reverse transcriptase PCR and LC-MS provide evidence that these proteins were correctly identified and are indeed secreted into the apical media by Calu-3 cells. We consider it significant that antimicrobial, anti-inflammatory, or anti-proteolytic actions have been reported for each of these proteins (see below).

Lysozyme was the first natural antimicrobial to be discovered and is abundant and well studied. It is secreted by human submucosal glands and Calu-3 cells (7) and was used in this study as an archetype to quantify the effects of agonists and submucosal glands and Calu-3 cells (7) and was used in this study. Secretory lysozyme is secreted by human placental collagen. Bottom section, silver-stained protein bands from control wash out (WO) of the apical surface with a minimal medium, a 2-h incubation with a vehicle (Bs, basal), or a 2-h exposure to 10 μM forskolin (Fsk). Molecular weight markers are on the left. c, identification of secreted proteins from Calu-3 by LC-MS. Results for α-1-antitrypsin are shown as representative. Predicted mature 394-amino acid residues plus an N-terminal 24-amino acid signal peptide (underlined) (30) of α-1-antitrypsin are shown here. A total of 11 tryptic fragments (boxed, boldface) matched the α-1-antitrypsin ~47 kDa protein.

The presence of α-1-antitrypsin and α-1-antichymotrypsin is of particular interest, because the activity of the ENaC is activated, at least in part, by serine proteases (14). It is well documented that serine protease inhibitors protect the respiratory tract from proteolytic damage (15). Loss of functional CFTR in airways increases Na⁺-driven fluid absorption by surface epithelial cells because of the increased activity of ENaC. Extracellular serine proteases such as trypsin and chymotrypsin can increase ENaC activity, whereas a serine protease inhibitor, aprotinin, decreases ENaC activity (16). We hypothesize that a balance between serine proteases, (such as trypsin, channel-activating serine proteases, neutrophil elastase, and cathepsin G) and serine protease inhibitors (such as α-1-antichymotrypsin and α-1-antitrypsin) may be important for maintaining optimal properties of airway mucus. This hypothesis provides an additional mechanism to link CFTR and ENaC; in CF, the diminished surface availability of gland-derived serine protease inhibitors might further enhance fluid absorption and further dehydrate airway mucus.

HSC 71 is also called lipopolysaccharide-associated protein 1 or heat shock 70 kDa protein 10. In general, heat shock proteins are regarded as intracellular chaperones that aid the folding and assembly of oligomeric proteins and as intracellular proteases that mediate ubiquitin-dependent degradation of certain proteins via proteasomes. However, heat shock proteins, including HSC 71, can be released into the extracellular milieu under physiological conditions within small membrane-bound vesicles termed exosomes (17) by a number of cell types, including epithelial cells (18). Heat shock proteins are found in the peripheral circulation of healthy people, where they are thought to function as anti-inflammatory molecules because of their immune responses to pathogenic microbes (19). Our results indicate that they may serve the same function in airway surface liquid. The occurrence of HSC71 in exosomes raises the possibility that its release may be entirely constitutive. How-
ever, the band identified as HSC71 was clearly darker in stimulated preparations, and we found no evidence of any bands that were unaffected by stimulation (data not shown).

Siderocalin (NGAL), originally identified as a protein stored in neutrophil granules, is a member of the lipocalin superfamily, so named because of the ability of its members to bind the lipophilic components of ligands. However, whereas NGAL binds lipids with only millimolar affinity, it was recently shown (in a remarkable study that included crystallographic evidence for binding) that NGAL binds bacterial catecholate-type ferric siderophores with subnanomolar affinity (12). As pointed out by Goetz et al. (12), the binding of siderophores will complement iron sequestering by lactoferrin to starve bacteria of iron and is potentially much more efficient because it targets iron already bound for bacterial use. They showed directly that NGAL is bacteriostatic and argue that the recognition machinery of NGAL should allow a large range of catecholate-type siderophores to be bound. Because siderophores and not lipids are the natural ligands of NGAL, the authors propose that NGAL be renamed siderocalin (12).

Quantification of Protein Secretion Using Lysozyme as a Marker Protein—As shown above, lysozyme is one of many proteins secreted into the apical fluid by unstimulated Calu-3 cells. Because relative band densities were maintained across different stimulation conditions, we used lysozyme as a representative protein for the quantification of apically secreted proteins. The lysozyme secretion rate in ng/hr/cm² was measured by ELISA in the presence or absence of an agonist or an antagonist. The confluent Calu-3 monolayers used in the ELISA experiments had a mean transepithelial resistance of 600 ± 16 Ω cm² and a mean transepithelial potential difference of 9.2 ± 0.2 mV (n = 205, apical negative). Basal and agonist treatments were done for 2-h periods in a highly humidified CO₂ incubator. Unstimulated Calu-3 cells secreted lysozyme basally at an average rate of 31.7 ± 2.3 ng/hr/cm² (range 8–91 ng/hr/cm², n = 51).

Forskolin Stimulated Lysozyme Secretion—Forskolin (10 μM), which directly activates adenylate cyclase to increase [cAMP], significantly increased the lysozyme secretion rate from 31.7 ± 3.0 to 77.3 ± 9.1 ng/hr/cm² (160.5 ± 28.8%, n = 36, p < 0.005; Fig. 2a). The percentage increase in lysozyme secretion is much larger than the percentage increases in Iₑₑₑ (20) or fluid secretion (21) induced by forskolin in Calu-3 cells. The rates for both unstimulated and forskolin-stimulated lysozyme secretion were relatively constant during the 2-h measurement periods (Fig. 2b). The constant rate of forskolin-stimulated lysozyme secretion is consistent with the rates of forskolin-stimulated gland mucus secretion, which are also constant over time (1, 10). We did not find any evidence that mechanical stimulation during the apical fluid collection process caused increased lysozyme secretion.

To determine whether a relationship existed between basal and forskolin-stimulated lysozyme secretion, the magnitudes of basal and forskolin-stimulated lysozyme secretion rates for the same monolayer were plotted. As shown Fig. 2c, the rates are positively correlated (r² = 0.4). This result eliminates the possibility that larger increases were observed in cells with low basal rates of secretion.

Carbachol- and Thapsigargin-stimulated Lysozyme Secretion—The cholinergic agonist carbachol is a potent secretagogue for mucus secretion from airway submucosal glands (Fig. 1a). Cholinergic agents cause a fast and transient burst of mucus secretion followed by sustained secretion at a rate that is much slower than that for the initial transient burst but is still usually 2–3 times greater than the rate of sustained secretion to forskolin or VIP (10). However, in Calu-3 cells lysozyme secretion was only modestly stimulated by carbachol (100 μM), increasing from 24.1 ± 4.1 to 31.6 ± 5.7 ng/hr/cm² (40.3 ± 24.5%, n = 11, p < 0.05; Fig. 3a). In Iₑₑₑ experiments with Calu-3 cells, the Iₑₑₑ response to carbachol is large but transient, with little or no sustained increase (5). The reason for the rapid adaptation of the Calu-3 Iₑₑₑ response to carbachol is not known, but if the Iₑₑₑ spike represents a spike in [Ca²⁺]), and if carbachol-stimulated lysozyme secretion is mediated by increases in [Ca²⁺], then any initial large spike in lysozyme secretion would be averaged over the 2-h sample period of our...
experiments. However, an alternative possibility is that the lysozyme secretion from Calu-3 cells is driven by some aspect of muscarinic stimulation other than an increase in \([\text{Ca}^{2+}]_i\).

To test this possibility further, we used 1-EBIO, a benzimidazolone compound that blocks Ca\(^{2+}\) uptake into the endoplasmic reticulum, resulting in increased \([\text{Ca}^{2+}]_i\). Thapsigargin (333 nM) increased lysozyme secretion rates from 40 ± 5 to 63 ± 8.6 ng/h/cm\(^2\) (67.3 ± 19.6%, \(n = 16\), \(p < 0.005\); Fig. 3c). Even with the sustained \([\text{Ca}^{2+}]_i\) increase produced by thapsigargin, the magnitude of lysozyme secretion over the 2-h period was much smaller in comparison to \(I_w\) or net fluid secretion increases of 200–300% (20, 21).

Thapsigargin in the presence of forskolin produces additive or synergistic effects on fluid secretion by Calu-3 cells (21). To see if protein secretion is also increased by combining cAMP and \(\text{Ca}^{2+}\) agonists, lysozyme secretion was measured for cells treated with 10 \(\mu\text{M}\) forskolin alone or with 10 \(\mu\text{M}\) forskolin plus 333 nM thapsigargin. As shown in Fig. 3d, thapsigargin plus forskolin was a more effective stimulus than either used alone, with thapsigargin causing a further increase of almost 50% over forskolin alone (from 61.1 ± 6.7 to 85.9 ± 11.1 ng/h/cm\(^2\), 47.9 ± 25.6%, \(n = 9\), \(p < 0.05\)).

1-EBIO Is a Weak Agonist for Lysozyme Secretion—The results with forskolin and thapsigargin point to a dissociation between protein and fluid secretion in Calu-3 cells. To investigate this possibility further, we used 1-EBIO, a benzimidazolone that was shown by Devor et al. to powerfully activate \(I_w\) in Calu-3 cells by coordinate action on both apical CFTR and basolateral \(\text{Ca}^{2+}\)-activated K\(^+\) channels, with both effects possibly being mediated by direct interactions with the respective channels (22). 1-EBIO also strongly stimulates fluid secretion by Calu-3 cells, increasing net fluid secretion by >170% (21). In contrast to its powerful effects on electrolyte-mediated fluid secretion, 1-EBIO (1 mM) stimulated only a small increase of lysozyme secretion, from 34.3 ± 6.7 to 44.4 ± 11.6 ng/h/cm\(^2\) (31.4 ± 11.9%, \(n = 10\), \(p < 0.05\); Fig. 3b).

**Summary of Agonist Effects**—These results lead to three conclusions. First, Calu-3 cells have both cAMP- and \(\text{Ca}^{2+}\)-mediated protein secretory pathways, similar to what has been shown for exocytosis by pancreatic \(\beta\) cells (23) and some epithelial cells (for a review, see Ref. 24). Second, the cAMP pathway appears to be more robust than that of \(\text{Ca}^{2+}\), at least under the conditions of our experiments, and, finally, the effects of the agonists on the rate of protein secretion do not correspond to their effects on \(I_w\) and net fluid secretion.

**Effects on Protein Secretion of Three Antagonists of Fluid and Electrolyte Secretion**—Although there is ample evidence that fluid and protein secretion can be independently regulated, coordinate action is the rule, raising the question of what common pathways participate in such linkage. Of most interest is the possibility that CFTR plays a critical role in the process such that protein secretion would be altered, at least in some cells, by the loss of functional CFTR (4).
process of protein secretion by Calu-3 cells, protein secretion by Calu-3 monolayers was assessed in the presence of various inhibitors (see “Materials and Methods” for details of these experiments). We tested three inhibitors known to affect ion transport: (i) glibenclamide, a nonspecific inhibitor of CFTR and other transporters; (ii) clotrimazole, a specific inhibitor of Ca<sup>2+</sup>-activated K<sup>+</sup> channels; and (iii) CFTRinh<sub>172</sub>, the most potent and specific inhibitor of CFTR presently available (25).

The rationale was that a comparison of the effects of these three inhibitors might support or refute the specific involvement of CFTR in protein secretion.

Glibenclamide (0.6 mM) inhibited basal and stimulated lysozyme secretion (Fig. 4a). Basal lysozyme secretion was reduced 25%, from 26.1 ± 3.4 to 20.2 ± 4.2 ng/h/cm<sup>2</sup> (n = 14, p < 0.05). Forskolin-stimulated secretion was inhibited 41%, from 74.0 ± 13.7 to 45.5 ± 10.4 (n = 19, p < 0.0005); and thapsigargin-stimulated secretion was inhibited 47%, from 57.5 ± 6.9 to 30.7 ± 4.5 ng/h/cm<sup>2</sup> (n = 6, p < 0.0005).

Clotrimazole (30 μM) was without a significant effect on basal or stimulated lysozyme secretion (Fig. 4b). Basal secretion went from 45.1 ± 13.9 to 49.6 ± 20.3 (n = 5, not significant), forskolin-stimulated secretion went from 79.3 ± 17.2 to 85.4 ± 21.6 ng/h/cm<sup>2</sup> (n = 5, not significant), and thapsigargin-stimulated secretion went from 57.9 ± 28.3 to 86.6 ± 26.2 ng/h/cm<sup>2</sup> (n = 5, not significant).

CFTR<sub>inh172</sub> (10 μM) did not inhibit basal lysozyme secretion (39.6 ± 4.0 to 42.7 ± 3.9 ng/h/cm<sup>2</sup>, n = 7), and was not tested with thapsigargin. It produced a small (15%), but significant decrease in forskolin-induced lysozyme secretion from 63.2 ± 4.7 to 53.3 ± 4.4 ng/h/cm<sup>2</sup> (n = 11, p = 0.008; Fig. 4c).

Summary of Inhibitor Effects—The mechanisms responsible for the various effects observed on protein secretion are unknown. At the levels used here, glibenclamide inhibits at least CFTR, ATP-sensitive K<sup>+</sup> channels, and carnitine palmitoyltransferase 1 (26). Although clotrimazole is a specific blocker of intermediate conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels, its K<sub>i</sub> for blocking those channels is in the nanomolar range, and other effects could operate at the doses used here. Nevertheless, none of the clotrimazole treatments reached significance, although they were consistently associated with increases in protein release, which, in the case of thapsigargin, was a 50% increase that approached significance. Thus, a powerful inhibition of ion transport does not necessarily translate into inhibition of protein secretion. Finally, the small but significant inhibitory effect of 10 μM CFTR<sub>inh172</sub> on forskolin-mediated lysozyme secretion might be mediated via CFTR, but CFTR<sub>inh172</sub> is a new compound with micromolar potency, and it is possible that it has other targets.

Protein Secretion by Calu-3 Cells in the Context of Submucosal Gland Mucus Secretion—In their model of submucosal gland secretion (1), Joo et al. hypothesized that serous cells secrete electrolyte-driven fluid secretion and protein secretion in response to both acetylcholine and VIP. In cystic fibrosis, they showed that electrolyte-driven fluid secretion is deleted, and hypothesized that protein secretion was intact. Because in their experiments no mucus escaped the CF glands in response to VIP, the inference is that any secreted proteins are trapped in the gland until partial secretion is produced via acetylcholine (1).

In the present work, Calu-3 cells were used as a surrogate for serous cells, whereas forskolin and thapsigargin were used to mimic the effects of VIP and acetylcholine. Although clearly imperfect as models, Calu-3 cells allow the study of electrolyte transport and protein secretion in a relatively homogeneous, serous like cell, whereas mucus secreted by glands is an admixture of multiple cell types.
To the extent that the cell model and pharmacological tools are valid, these results support a model of gland secretion in which serous cells provide a component of mucus that is rich in antimicrobial and anti-proteolytic compounds. Whereas the lack of CFTR may have only a modest effect on protein secretion from serous cells, the deletion of the HCO$_3^-$-rich fluid secretion from serous cells could cause secreted proteins to be entrapped in underhydrated, viscous mucus (27, 28). That result should have profoundly negative consequences for the bioavailability of these proteins within the airway surface mucus, where they are essential to slow the proliferation of inhaled pathogens. In addition, the lack of serine protease inhibitors will render the airways more vulnerable to damage, and the unchecked proteolytic activation of ENaC may further dry and thicken the already underhydrated airway secretions, further crippling mucociliary and cough clearance (29). Thus, we hypothesize that the chronic airway infections of cystic fibrosis arise as the consequence of static mucus with reduced antimicrobial capacity.

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