Cystic Fibrosis Airway Epithelial Ca\(^{2+}\) Signaling

THE MECHANISM FOR THE LARGER AGONIST-MEDIATED Ca\(^{2+}\), SIGNALS IN HUMAN CYSTIC FIBROSIS AIRWAY EPITHELIA

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In cystic fibrosis (CF) airways, abnormal epithelial ion transport likely initiates mucus stasis, resulting in persistent airway infections and chronic inflammation. Mucus clearance is regulated, in part, by activation of apical membrane receptors coupled to intracellular calcium (Ca\(^{2+}\)) mobilization. We have shown that Ca\(^{2+}\)i signals resulting from apical purinoceptor (P2Y2-R) activation are increased in CF compared with normal human airway epithelia. The present study addressed the mechanism for the larger apical P2Y2-R-dependent Ca\(^{2+}\)i signals in CF human airway epithelia. We show that the increased Ca\(^{2+}\)i mobilization in CF was not specific to P2Y2-Rs because it was mimicked by apical bradykinin receptor activation, and it did not result from a greater number of P2Y2-R or a more efficient coupling between P2Y2-Rs and phospholipase C-generated inositol 1,4,5-trisphosphate. Rather, the larger apical P2Y2-R activation-promoted Ca\(^{2+}\)i signals in CF epithelia resulted from an increased density and Ca\(^{2+}\) storage capacity of apically confined endoplasmic reticulum (ER) Ca\(^{2+}\) stores. To address whether the ER up-regulation resulted from ER retention of misfolded F508 CFTR or was an acquired response to chronic luminal airway infection/inflammation, three approaches were used. First, ER density was studied in normal and CF sweat duct human epithelia expressing high levels of F508 CFTR, and it was found to be the same in normal and CF epithelia. Second, apical ER density was morphometrically analyzed in airway epithelia from normal subjects, F508 homozygous CF patients, and a disease control, primary ciliary dyskinesia; it was found to be greater in both CF and primary ciliary dyskinesia. Third, apical ER density and P2Y2-R activation-mobilized Ca\(^{2+}\)i, which were investigated in airway epithelia in a long term culture in the absence of luminal infection, were similar in normal and CF epithelia. To directly test whether luminal infection/inflammation triggers an up-regulation of the apically confined ER Ca\(^{2+}\) stores, normal airway epithelia were chronically exposed to supernatant from mucopurulent material from CF airways. Supernatant treatment expanded the apically confined ER, resulting in larger apical P2Y2-R activation-dependent Ca\(^{2+}\)i responses, which reproduced the increased Ca\(^{2+}\)i signals observed in CF epithelia. In conclusion, the mechanism for the larger Ca\(^{2+}\)i signals elicited by apical P2Y2-R activation in CF airway epithelia is an expansion of the apical ER Ca\(^{2+}\) stores triggered by chronic luminal airway infection/inflammation. Greater ER-derived Ca\(^{2+}\)i signals may provide a compensatory mechanism to restore, at least acutely, mucus clearance in CF airways.

Airway epithelia constitute the major interface between inspired air and the airway wall. These epithelia are highly polarized and exhibit a series of integrated functions that provide mechanical cleansing, i.e. mucus clearance, as a primary mode of lung defense. The individual airway epithelial functional components that mediate mucus clearance, including ion transport, mucin secretion, and ciliary beat frequency (1), are regulated in part by intracellular calcium (Ca\(^{2+}\)) (2, 5). Airway epithelial Ca\(^{2+}\)i mobilization can be elicited byselective autocrine and/or paracrine activation of apical or basolateral membrane heterotrimeric G protein-coupled receptors (GPCRs)1 linked to phospholipase C (PLC) stimulation, which generates inositol 1,4,5-trisphosphate (IP3) and induces Ca\(^{2+}\)i release from endoplasmic reticulum (ER) stores (3, 4). 5’ nucleotides (ATP/UTP), released by airway epithelial cells and sensed by apical membrane purinoceptors (P2Y2-Rs) (5), may be the dominant autocrine regulators of Ca\(^{2+}\)i mobilization in airway epithelia (3).

In airway epithelia of patients with cystic fibrosis (CF), the functional absence of the cystic fibrosis transmembrane conductance regulator (CFTR) results in a diminished periciliary liquid layer depth (6) and a reduction in mucus clearance. Likewise, abnormal mucus clearance is found in patients with primary ciliary dyskinesia (PCD), a syndrome that results from defective airway epithelial ciliary proteins linked to a decreased ciliary activity (7). In both diseases, persistent airways infection and inflammation are the predominant clinical phenotypic characteristics consequent to the impairment of mucus clearance (8).

There are clues that airway epithelia may regulate the Ca\(^{2+}\)i

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1 The abbreviations used are: GPCR, G protein-coupled receptor; PLC, phospholipase C; IP3, inositol 1,4,5-trisphosphate; IPiR, IPi receptor; ER, endoplasmic reticulum; P2Y2-R, purinoceptor; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; PCD, primary ciliary dyskinesia; SMM, supernatant from mucopurulent material from CF airways; PBS, phosphate-buffered saline; RPA, ribonuclease protection assay; DIOiC3(3), dihexaoxacarbocyanine; CaCC, Ca2+-activated Cl- channel; Ins, inositol.

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signaling pathway in response to airways disease. For example, in vivo studies established that the nasal transepithelial electrical potential difference responses to agents that activate P2Y2-Rs and promote Ca\(^{2+}\), mobilization (e.g., ATP/UTP) are greater in CF patients than in normal subjects (9, 10). In vitro studies also detected a larger apical Ca\(^{2+}\)-dependent Cl\(^-\) conductance in CF airway epithelia (9, 11–13). In addition, we recently reported that apical P2Y2-R activation promotes greater Ca\(^{2+}\), mobilization in CF compared with normal human nasal epithelia (4). However, the underlying mechanism(s) responsible for the regulation of the increased Ca\(^{2+}\) responsiveness of CF airway epithelia and its functional consequences are unknown.

Therefore, in the present study we investigated the mechanism for the augmented apical GPCR-derived Ca\(^{2+}\), signals in CF airway epithelia by probing the P2Y2-R/Ca\(^{2+}\), signaling system in functional, biochemical, and cell biological studies in normal and CF human airway epithelia. Further, we investigated whether the raised Ca\(^{2+}\), signals in CF are a manifestation of the molecular pathogenesis of mutant CFTR (\(\Delta F508\) CFTR) or are an acquired host response to chronic infection/inflammation in vivo. We compared ER density in non-infected human sweat duct epithelia from normal and \(\Delta F508\) CF individuals and in human airway epithelia from normal, \(\Delta F508\) CF, and PCD patients with chronic airway infection and inflammation. In addition, ER density and apical P2Y2-R activation-induced Ca\(^{2+}\), mobilization were studied in short term and long term cultures of normal and CF bronchial epithelia. Finally, to directly address whether chronic airway infection/inflammation, independently of defective CFTR, up-regulated apically confined ER Ca\(^{2+}\) stores, ER density and UTP-mobilized ER Ca\(^{2+}\) were investigated in normal airway epithelia chronically exposed to supernatant from CF airways mucopurulent material (SMM).

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Freshly Excised Tissue**—Tissues and cells were obtained under the auspices of protocols approved by the Institutional Committee on the Protection of the Rights of Experimental Subjects. Nasal scrapes obtained from normal, ages 25–46, or PCD, ages 31–47, individuals were used for in vitro studies. Whole tissue from CF lungs infected with *Staphylococcus aureus* and regions of interest were designated in the apical domains with the MetaMorph software. The same acquisition parameters (e.g., laser power, contrast, brightness, and pinhole value) were employed for each channel to acquire the images from normal and CF cultures or native tissue in experiments performed on the same day. The fluorescence intensity values from the regions of interest were averaged from paired monolayers or 30–40 days later (long term well differentiated). Normal and CF cultures were apically washed with sterile PBS, and the serosal media were replenished every 2–4 days.

**Nasal scrapes examined from normal** (n = 4, ages 33–35, \(\Delta F508\) homozygous CF (n = 4, ages 25–46), or PCD (n = 3, ages 34–57) individuals and skin biopsies from normal (n = 4, ages 28–47) and \(\Delta F508\) homozygous CF (n = 5, ages 31–47) individuals were used for ER density studies.

Ca\(^{2+}\), **Studies**—6–11- or 30–40-day-old cultures were loaded with fura-2/AM, and Ca\(^{2+}\), measurements were taken with a fluorometer (SpectraMax, Molecular Devices). Calreticulin and IP3R were studied in normal and CF cultures treated with PBS or supernatant from CF airways mucopurulent material (SMM, see “Studies with Infectious/Inflammatory Material from CF Airways”). Immunostaining of Calreticulin and IP3 receptors (IP3-Rs) and DIOCs3\((3)\) Staining—The immunostaining was carried with Alexa Fluor 594-labeled IP3-Rs in cultures and depaразinfied native bronchial epithelial or skin biopsy sections was performed according to a modification of our previous method (17). The calreticulin and IP3-R staining, samples were incubated with a rabbit anti-calreticulin antibody (1:100 dilution, Affinity Bioreagents) and a mouse anti-IP3-R antibody that recognizes all IP3-R isoforms (1:100 dilution, Calbiochem) for 40 min at 37 °C. This incubation was followed by three PBS washes and a 30-min incubation at 25 °C with a fluorescein-labeled goat anti-rabbit antibody (1:20 dilution for calreticulin, Kirkegaard & Perry Laboratories) and with a Texas Red-labeled goat anti-mouse antibody (1:200 dilution for IP3-Rs, Jackson Immunoresearch Laboratories). As controls, the primary antibodies were omitted or substituted with rabbit and mouse y-globulins (Jackson Immunoresearch Laboratories). For antibody-independent ER staining, and rhodamine-labeled calreticulin antibody were labeled with 30 μg/mL dihexaaxocarbocyanine dye DIOC6(3) by incubation with 250 ng/mL DIOC6(3) for 1 min at room temperature in PBS washed by three washes with PBS as we have reported previously (17). The Ca\(^{2+}\), store markers and the DIOCs(3) signals were studied by laser confocal microscopy (Leica, model TCS 4D, PL APO 63x/1.20 mm water lens) in the XZ or XY scanning mode.

To quantify the fluorescence intensity of labeled calreticulin and IP3-Rs and DIOCs(3), multiple scans were obtained from each sample, and regions of interest were designated in the apical domains with the MetaMorph software. The same acquisition parameters (e.g., laser power, contrast, brightness, and pinhole value) were employed for each channel to acquire the images from normal and CF cultures or native tissue in experiments performed on the same day. Two fields were chosen for each sample, and the fluorescence intensity values from the regions of interest were averaged from paired monolayers or 30–40 days later (long term well differentiated). Normal and CF cultures were apically washed with sterile PBS, and the serosal media were replenished every 2–4 days.

**Electron Microscopy**—Nasal scrapes from normal, \(\Delta F508\) homozygous CF, and PCD individuals were fixed in 2% glutaraldehyde plus 2% paraformaldehyde plus 0.25% tannic acid, post-fixed in 1% Os\(_2\)O\(_4\), and processed for electron microscopy (19). Apical ER morphometric scoring was performed double blind. Only ciliated cells were analyzed, and repairing cells with migrating basal bodies (procentrioles) and micrographs with tangential cuts were excluded. The apical domain was defined as the region 1–3.4 μm (to exclude basal bodies) from the apical plasma membrane. The criteria for identifying ER were: parallel membranes in elongated or oval strands, containing an amorphous center without cristae (to exclude mitochondria), or electron-dense particles (to exclude rough ER, because it is increased in repairing cells), and not stacked (to exclude Golgi). Data are expressed as number of ER strands/μm\(^2\).

**Studies with Infectious/Inflammatory Material from CF Airways**—Mucopurulent material (SMM, see “Studies with Infectious/Inflammatory Material from CF Airways”). Calreticulin Western Blot Analysis—Monolayers of normal and CF bronchial epithelial cells were harvested with remodeling to exclude Golgi. Data are expressed as number of ER strands/μm\(^2\).
Fig. 1. The larger apical UTP-mobilized \( \text{Ca}^{2+} \) in CF airway epithelia is not agonist specific. A and B, representative \( \text{Ca}^{2+} \) tracings depicting the effect of apical UTP-mobilized \( \text{Ca}^{2+} \), in short-term primary culture monolayers of normal and CF human bronchial epithelia, respectively. C, summary \( \Delta \text{Ca}^{2+} \), values (peak-baseline \( \text{Ca}^{2+} \)) from UTP-stimulated cultures. D and E, representative \( \text{Ca}^{2+} \) tracings depicting the effect of apical bradykinin (BK)-mobilized \( \text{Ca}^{2+} \), in short-term primary culture monolayers of normal and CF human bronchial epithelia, respectively. F, compiled \( \Delta \text{Ca}^{2+} \), values (peak-baseline \( \text{Ca}^{2+} \)) from BK-stimulated cultures. Data are expressed as mean \pm \text{S.E.} \); \( n = 3 \) for BK studies in both groups. *, \( p < 0.05 \).
ER Ca\(^{2+}\) that could be mobilized in response to IP\(_3\) generation resulting from GPCR activation. To measure the Ca\(^{2+}\) storage capacity of the apical ER, a protocol was developed based on findings that ER Ca\(^{2+}\) stores are functionally confined to the plasma membrane domain ipsilateral to P2Y\(_2\)-R activation (21). 6–11-Day-old normal and CF monolayers, bathed in bilateral nominally Ca\(^{2+}\)-free buffer, were exposed to 100 \(\mu\)M basolateral UTP to deplete P2Y\(_2\)-R-sensitive basolateral Ca\(^{2+}\) stores. Basolateral UTP increased Ca\(^{2+}\) to the same extent in both cultures (Fig. 3A), suggesting that basolateral ER Ca\(^{2+}\) store capacity was the same in normal and CF epithelia. Apical ER Ca\(^{2+}\) store capacity was then measured with perfusion with 0.6 mM bilateral La\(^{3+}\) to block plasma membrane Ca\(^{2+}\)-ATPases and Ca\(^{2+}\) influx channels (22) followed by 1 \(\mu\)M apical thapsigargin (an ER Ca\(^{2+}\)-ATPase inhibitor) to release Ca\(^{2+}\) from apical ER stores (23). Ca\(^{2+}\) rose and was sustained because of inhibition of the plasma membrane and ER Ca\(^{2+}\)-ATPases by La\(^{3+}\) and thapsigargin, respectively (Fig. 3A). The greater rise in Ca\(^{2+}\) in CF (Fig. 3A) indicated that the quantity of Ca\(^{2+}\) sequestered in the apical ER was functionally greater in CF compared with normal bronchial epithelial cultures. Fig. 3B illustrates the summary \(\Delta\text{Ca}^{2+}\) data from these experiments. These results led us to speculate that the density of apical ER Ca\(^{2+}\) stores is increased in short term 6–11-day-old cultures of CF airway epithelia.

Apical ER Ca\(^{2+}\) Stores Are Morphologically Expanded in Short Term CF Airway Epithelial Cultures and Native CF Airway Epithelia—We next measured the expression of two ER Ca\(^{2+}\) store markers, IP\(_3\)Rs and calreticulin (an intraluminal ER protein involved in Ca\(^{2+}\) sequestration), in 6–11-day-old monolayers of normal and CF bronchial epithelia by confocal immunofluorescence microscopy (Fig. 4). The relative cellular distribution of these Ca\(^{2+}\) store markers was similar in normal and CF epithelia (i.e. they localized predominantly toward the apical pole and the fluorescent signals from calreticulin and IP\(_3\)Rrs were increased in CF cultures (Fig. 4, A and B)). No immunostaining of Ca\(^{2+}\) store markers was detected when the primary antibodies were omitted or nonspecific IgGs were used (data not shown). These data suggest an increased expression of calreticulin and IP\(_3\)Rs in CF cultures; however, because the IP\(_3\)-R antibody used recognizes all IP\(_3\)-R isoforms, it is possible that the change in IP\(_3\)-R signal may reflect a switch in the predominance of one isoform versus others rather than a change in the total amount of all IP\(_3\)Rs in CF. The increase in CF calreticulin immunofluorescence was confirmed by calreticulin immunoblotting from whole cell lysates of early stage culture monolayers of normal and CF bronchial epithelia (Fig. 4C).

The apical ER volume of normal and CF epithelia was also studied by an antibody-independent method, utilizing DIO\(_{10,01}\) fluorescence to stain the ER (17). Fig. 4D illustrates that DIO\(_{10,01}\) staining was greater in native CF compared with normal bronchial airway epithelia. The mean apical fluorescence intensity from the CF group exceeded that of the normal group by a factor proportional to that observed with antibody-dependent methods (Fig. 4E).

To test whether the topography and expression levels of the ER Ca\(^{2+}\) stores found in CF versus normal short term primary culture monolayers mimicked those in vivo, confocal immunofluorescence microscopy studies of IP\(_3\)Rs and calreticulin were performed in native normal and CF bronchial epithelia (Fig. 4, F and G). Similar to our findings in short term primary culture monolayers (Fig. 4, A and B), ER Ca\(^{2+}\) stores were distributed toward the apical domain of native epithelia, and their expression was increased in CF (Fig. 4, F and G). Collectively, these data, coupled with the direct measurement of ER density by electron microscopy (Fig. 5), strongly suggested that the functional increase in apical ER Ca\(^{2+}\) storage detected in CF epithelia (Fig. 3) was because of an increase in the ER volume in the apical domain.

Is the ER Expansion in CF a Result of Misfolded ΔF508 CFTR or an Acquired Response to Chronic Airway Infection?—Two approaches were used to address this question. First, we tested the hypothesis that the ER up-regulation could be linked to abnormal CFTR folding in the absence of infection/inflammation. We elected to study sweat ducts because they are a source of non-infected CF epithelium and they exhibit relatively high levels of CFTR expression (24). Fig. 5, A and B, depict the immunostaining of calreticulin in native sweat ducts from normal and ΔF508 homozygous CF individuals, respectively. Quantification of calreticulin immunofluorescence revealed that its expression was the same in normal and CF sweat duct epithelia (Fig. 5C), suggesting that the ER expansion observed in CF airway epithelia was not a consequence of ER retention of ΔF508 CFTR.

Second, we evaluated whether the ER expansion in CF was acquired by studying airway epithelia from patients with an unrelated disease characterized by chronic airways infection, e.g. PCD (8). We quantified morphometrically the ER density in freshly isolated nasal scrapes from normal (Fig. 5D), ΔF508 homozygous CF (Fig. 5E), and PCD (Fig. 5F) individuals. ER density, as depicted by red arrows, was increased in both CF and PCD compared with normal epithelium (Fig. 5G). Apical UTP-mobilized Ca\(^{2+}\), was also increased 2-fold in short term primary culture monolayers of PCD compared with normal bronchial epithelia (data not shown). These data suggest that
the apical ER volume is expanded in CF in response to luminal airway infection and inflammation.

The ER Expansion Observed in Short Term CF Cultures Reveals to Normal in CF Epithelia Cultured for Long Term in the Absence of Luminal Infection—To address directly the possible relationship between luminal infection/inflammation and ER expansion in CF airway epithelia, normal and ΔF508 homozygous CF bronchial epithelia were cultured for long term for 30–40 days, and ER density was investigated by calreticulin immunofluorescence as described in the studies depicted in Fig. 4A. Fig. 6, A and B, depicts the calreticulin expression in a normal culture and a ΔF508 homozygous CF culture, respectively, illustrating that apical calreticulin expression was no longer up-regulated in the long term CF compared with the normal culture. Fig. 6C shows the summary data for apical calreticulin staining from long term normal and CF cultures. These findings demonstrate that the ER expansion observed in short term CF cultures is lost upon prolonged culturing under sterile conditions. We next investigated whether the morphological reversal of ER expression to normal correlated with a functional reversal of agonist-sensitive Ca$^{2+}$ stores to normal in long term ΔF508 CF cultures. Fig. 6, D and E, illustrates that activation of apical P2Y$_R$-Rs with mucosal ATP (100 μM) elicited a similar Ca$^{2+}$ mobilization in long term normal and CF cultures, respectively. Fig. 6F depicts the summary ∆Ca$^{2+}$, data (peak-baseline Ca$^{2+}$, values) from these studies. These data demonstrate that the characteristic phenotype of short term 6–11-day-old ΔF508 CF cultures (e.g. apical ER expansion associated with increased agonist-sensitive ER Ca$^{2+}$ stores) reverts to normal after long term culturing in the absence of luminal infection and is independent of mutated CFTR.

Can the CF Phenotype, e.g. Increased Density of Apically Confined ER Ca$^{2+}$ Stores Coupled to Larger Apical P2Y$_R$-R Activation-dependent Ca$^{2+}$ Signals, Be Transferred to Normal Airway Epithelia by Prolonged Exposure to Mucopurulent Material from CF Airways?—The above findings suggest that the expanded apical ER phenotype does not depend on ER retention of ΔF508 CFTR but results from an epithelial adaptation to the in situ CF airway infectious/inflammatory milieu, and it is lost in vitro with time in the absence of airway infection. Based on these findings, we hypothesized that the ER expansion and the consequent up-regulation of functional Ca$^{2+}$ stores could be induced in normal airway epithelia exposed to an infectious/inflammatory luminal environment. To test this notion, the luminal surfaces of 30–40-day-old normal bronchial epithelia were exposed for up to 48 h to either PBS or SMM harvested from CF airways, and the following parameters were measured. First, the apical ER compartment, visualized by calreticulin immunofluorescence, was assessed in PBS- versus SMM-treated cultures. Fig. 7A illustrates that apical ER density was increased in SMM- compared with PBS-treated cultures in a time-dependent manner. The compiled data from these studies are shown in Fig. 7B. SMM-treated short term cultures of normal epithelia also expressed an increased ER density (not shown). Second, we tested whether the increased apical ER expression in SMM-treated cultures resulted in larger Ca$^{2+}$, signals in response to apical P2Y$_R$-R activation with 100 μM mucosal UTP. UTP-mobilized Ca$^{2+}$, was increased in cultures pretreated with SMM compared with PBS (Fig. 7C), mimicking the larger mucosal UTP-dependent Ca$^{2+}$, signals found in short term primary cultures of CF bronchial airway epithelia (Fig. 1, A–C). These findings demonstrate that chronic luminal exposure of normal cultures to SMM reproduces the increased ER density and apical GPCR activation-dependent Ca$^{2+}$, signals observed in short term cultures of CF airway epithelia.

**DISCUSSION**

We have hypothesized previously that an increased Ca$^{2+}$, signal coupled to Cl$^-$ secretion would provide a compensatory mechanism to offset the absent cAMP-mediated Cl$^-$ transport in CF (3). Perhaps the strongest evidence for the role of Ca$^{2+}$-, mediated Cl$^-$ secretion in protecting against CF lung disease came from CF knock-out mice, which express a large endogenous Ca$^{2+}$,-activated Cl$^-$ conductance in the airway epithelia and are devoid of airway disease (13, 25). CF patients do exhibit larger Ca$^{2+}$,-dependent responses triggered by luminal purinoceptor agonists (9, 10), but the mechanism(s) of this response in human airways has remained unclear.

In this study, we have elucidated the mechanism for the increased apical GPCR activation-dependent Ca$^{2+}$, signals in CF human airway epithelia (4) (Fig. 1) by showing that it resulted from the expansion of the apical ER Ca$^{2+}$, store compartment (Figs. 3–5 and 7) rather than from a greater number of P2Y$_R$-Rs or coupling efficiency between P2Y$_R$-R activation and PLC activity (Fig. 2). Although it is plausible that the higher Ca$^{2+}$,-dependent Cl$^-$ secretion in human CF airway epithelia may in part be a consequence of an increased Ca$^{2+}$,-activated Cl$^-$ channel (CaCC) number, our data demonstrating higher Ca$^{2+}$, mobilization in CF compared with normal human nasal (4) or bronchial (Fig. 1) epithelia suggest that higher Ca$^{2+}$, levels at the vicinity of the apical membrane CaCC likely mediate this response.

A number of observations led to the conclusion that the enlarged ER in CF reflected an acquired epithelial response to luminal airway infection/inflammation rather than an ER stress response to misfolded ΔF508 CFTR (26). First, non-infected native sweat duct epithelia from ΔF508 homozygous
CF patients exhibited normal ER size (Fig. 5). Second, the apical ER density was similarly increased in native CF and PCD airway epithelia (Fig. 5). Third, the expanded ER phenotype observed in short term (6–11-day-old, Fig. 4) primary cultures of F508 CF airway epithelia reverted to normal in long term (30–40-day-old, Fig. 6) F508 CF cultures. Fourth, chronic in vitro exposure of normal airway epithelia to bacterial and inflammatory factors (such as SMM) harvested from CF airways increased the ER size and Ca\(^{2+}\) storage (Fig. 7), mimicking the phenotype of short term CF cultures. These data suggest that the elaborated apical ER phenotype observed in short term primary cultures of CF airway epithelia, which mimics that of native CF airway epithelia, 1) reflects a “memory” of the in situ infectious environment of CF airways, 2) reverts in vitro in the absence of luminal airway infection, and 3) is independent of the intrinsic ΔF508 CFTR defect.

Regarding host defense mechanisms that clear airway surfaces, the raised Ca\(^{2+}\) release due to ER expansion, especially confined to the apical domain (4), (27), may provide an adaptive response for both the normal and the CF airways. The higher Ca\(^{2+}\) mobilization may be particularly useful to CF patients, who depend solely on CaCC to compensate for the absent cAMP-mediated Cl\(^{-}\) secretion in CF (3). Thus, the approximate doubling of Ca\(^{2+}\) mobilization in CF is predicted to double the magnitude of Cl\(^{-}\) secretion (4). This relatively larger component of Cl\(^{-}\) secretion may allow CF airways to transiently restore defective mucus clearance. In this scenario, cough (shear stress)-induced release of ATP/UTP (5) into airway surface liquid would produce larger Ca\(^{2+}\) signals via apical P2Y\(_2\)-R activation and would more effectively activate CaCC.

**Fig. 4.** Apical ER Ca\(^{2+}\) stores are morphologically expanded in CF cultures and native CF tissue. A, XZ confocal scans from short term primary culture monolayers of normal and CF bronchial airway epithelia immunostained for calreticulin or IP\(_{3}\)Rs. Bar, 10 μm. B, percent of apical fluorescence intensity from calreticulin and IP\(_{3}\)Rs (normalized to fluorescence intensity values from normal cultures; n = 5). C, Western blot for calreticulin from whole cell lysates (50 μg of protein/lane) from short term primary culture monolayers of normal and CF bronchial airway epithelia (representative from three normal and three CF cultures). D, XY confocal scans from normal and CF sections of native bronchial epithelia stained with DIOC\(_{6}(3)\). E, percent of apical DIOC\(_{6}(3)\) fluorescence intensity (normalized to fluorescence intensity values from normal sections; n = 5). F, XY confocal scans from normal and CF sections of native bronchial epithelia immunostained for calreticulin and IP\(_{3}\)Rs, Bar, 10 μm. G, percent of apical fluorescence intensity from calreticulin and IP\(_{3}\)Rs (normalized to fluorescence intensity values from normal sections; n = 5 for calreticulin, and n = 3 for IP\(_{3}\)Rs in normal and CF). Data are expressed as mean ± S.E.; *, p < 0.05, CF versus normal epithelia.
**FIG. 5.** ER density is similar in native ΔF508 homozygous CF versus normal sweat duct epithelia but greater in airway epithelia from native CF and PCD infected airways. XY confocal scans of the ER (as depicted by calreticulin staining) in normal (A) and ΔF508 homozygous CF (B) native sweat duct epithelia are shown. Bar, 10 μm. C, compiled ER density data from the apical epithelial domain of normal and ΔF508 homozygous CF sweat ducts (n = 4 and 5 individuals for normal and CF, respectively). Micrographs of the apical region of normal (D), ΔF508 homozygous CF (E), and PCD (F) nasal ciliated epithelial cells are shown. Red and black arrows depict the ER and the intercellular spaces, respectively (see panels D, E, and F). G, compiled ER density data from the apical epithelial domain of the three groups (n = 3–4 individuals). Data are expressed as mean ± S.E.; *, p < 0.05, CF or PCD versus normal epithelia.

**FIG. 6.** Apical ER density and ER Ca^{2+} storage is the same in normal and CF airway epithelia cultured for long term in the absence of luminal infection. ER density, depicted by calreticulin staining, in 30–40-day-old long term cultures of normal (A) and CF (B) bronchial airway epithelia. The apical surface corresponds to the upper area in figures. Bar, 10 μm. C, compiled data for calreticulin fluorescence from the apical domain of normal and CF cultures. D and E, representative Ca^{2+}, tracings depicting the effect of (100 μM) apical ATP-mobilized Ca^{2+}, in 30–40-day-old long term cultures of normal and CF human bronchial epithelia, respectively. F, compiled ΔCa^{2+}, values (peak-baseline Ca^{2+},) from ATP-stimulated cultures. Data are expressed as mean ± S.E. (n = 3–5).
and ciliary beat to transiently restore mucus clearance (28).

Conversely, this adaptive airway epithelial response to chronic luminal infection/inflammation involving the up-regulation of apical ER Ca$^{2+}$ stores can have an adverse effect, because the increased agonist-induced Ca$^{2+}$ mobilization in CF airway epithelia may also play a role in Ca$^{2+}$-dependent airway inflammatory responses (29). A role for increased ER Ca$^{2+}$ stores has been raised in the pathogenesis of neurological diseases. For example, ER expansion has been described in a model for Gaucher disease, where neuronal cells expressed an increase in ER and ryanodine receptors and a greater ER-dependent Ca$^{2+}$ release in response to glutamate or caffeine stimulation (30). Thus, the increased glutamate-dependent Ca$^{2+}$ signal may be linked with the neuronal toxicity and cell death characteristic of Gaucher disease. In contrast, most cases of Alzheimer disease are caused by presenilin mutations, and it is thought that ER protein (presenilin) retention (31) leads to increases in ER size and Ca$^{2+}$ storage capacity in this disease (32–34).

In conclusion, our findings add to the understanding of the mechanisms and roles of Ca$^{2+}$-, dependent signal transduction in airway epithelia by elucidating that 1) ER Ca$^{2+}$ stores can have an adverse effect, because the increased agonist-induced Ca$^{2+}$ mobilization in CF airway epithelia may also play a role in Ca$^{2+}$-dependent airway inflammatory responses (29). A role for increased ER Ca$^{2+}$ stores has been raised in the pathogenesis of neurological diseases. For example, ER expansion has been described in a model for Gaucher disease, where neuronal cells expressed an increase in ER and ryanodine receptors and a greater ER-dependent Ca$^{2+}$ release in response to glutamate or caffeine stimulation (30). Thus, the increased glutamate-dependent Ca$^{2+}$ signal may be linked with the neuronal toxicity and cell death characteristic of Gaucher disease. In contrast, most cases of Alzheimer disease are caused by presenilin mutations, and it is thought that ER protein (presenilin) retention (31) leads to increases in ER size and Ca$^{2+}$ storage capacity in this disease (32–34).

In conclusion, our findings add to the understanding of the mechanisms and roles of Ca$^{2+}$-, dependent signal transduction in airway epithelia by elucidating that 1) ER Ca$^{2+}$ stores can have an adverse effect, because the increased agonist-induced Ca$^{2+}$ mobilization in CF airway epithelia may also play a role in Ca$^{2+}$-dependent airway inflammatory responses (29). A role for increased ER Ca$^{2+}$ stores has been raised in the pathogenesis of neurological diseases. For example, ER expansion has been described in a model for Gaucher disease, where neuronal cells expressed an increase in ER and ryanodine receptors and a greater ER-dependent Ca$^{2+}$ release in response to glutamate or caffeine stimulation (30). Thus, the increased glutamate-dependent Ca$^{2+}$ signal may be linked with the neuronal toxicity and cell death characteristic of Gaucher disease. In contrast, most cases of Alzheimer disease are caused by presenilin mutations, and it is thought that ER protein (presenilin) retention (31) leads to increases in ER size and Ca$^{2+}$ storage capacity in this disease (32–34).

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