Role of Endoplasmic Reticulum Stress in Cystic Fibrosis–Related Airway Inflammatory Responses

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Chronic airway infection and inflammation are hallmarks of cystic fibrosis (CF) pulmonary disease. The altered airway environment resulting from infection and inflammation can affect the innate defense of the airway epithelia. Luminal bacterial and inflammatory stimuli trigger an adaptation in human airway epithelia, characterized by a hyperinflammatory response to inflammatory mediators, which is mediated by an expansion of the endoplasmic reticulum (ER) and its Ca^{2+} stores. Recent studies demonstrated that a form of ER stress, the unfolded protein response (UPR), is activated in airway epithelia by bacterial infection–induced airway inflammation. UPR-dependent signaling is responsible for the ER Ca^{2+} store expansion–mediated amplification of airway inflammatory responses. These studies highlight the functional importance of the UPR in airway inflammation and suggest that targeting the UPR may be a therapeutic strategy for airway diseases typified by chronic inflammation. This article reviews the contribution of airway epithelia to airway inflammatory responses, discusses how expansion of the ER Ca^{2+} stores in inflamed airway epithelia contributes to airway inflammation, describes the functional role of the UPR in these processes, and discusses how UPR activation might be relevant for CF airways inflammatory disease.

Keywords: airway inflammation; airway epithelia; cystic fibrosis; endoplasmic reticulum calcium stores; unfolded protein response

Chronic airway infection and inflammation are hallmarks of cystic fibrosis (CF) pulmonary disease. The altered airway environment resulting from infection and inflammation can affect the airways epithelial innate defense. In accord with this notion, we have described an adaptive response in human bronchial epithelia (HBE) triggered by luminal bacterial and inflammatory stimuli. This HBE adaptation reflected a hyperinflammatory response to inflammatory mediators (e.g., increased uridine triphosphate [UTP] or bradykinin [BK]–induced IL-8 secretion, which was mediated by an expansion of the endoplasmic reticulum [ER] and its Ca^{2+} stores) (1, 2). Our previous studies suggested that a form of ER stress, the unfolded protein response (UPR), is activated in airway epithelia by bacterial infection–induced airway inflammation and mediates airway inflammatory responses such as secretion of inflammatory mediators.

In this article, we review the contribution of airway epithelia to airway inflammatory processes, discuss how expansion of the ER Ca^{2+} stores in inflamed airway epithelia contributes to airway inflammation, and describe the functional importance of the UPR in these processes. In addition, we discuss recent findings suggesting that activation of the UPR is functionally

important for the pathophysiology of CF airways inflammatory disease. Some of the results of these studies have been previously reported in the form of abstracts (3, 4).

THE CONTRIBUTION OF AIRWAY EPITHELIA TO AIRWAY INFLAMMATION IN CHRONICALLY INFECTED AND INFLAMED AIRWAYS

CF is a multiorgan disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). In airway epithelia of patients with CF, the absence of CFTR-mediated Cl^- secretion coupled with increased Na^+ absorption (5) results in dehydration of the periciliary layer (6), decreased mucus clearance, and accumulation of thickened mucus in airway lumens. As a consequence of these alterations, CF airways are plagued by persistent infection (7–10) and inflammation (9, 11–13). CF airways disease is associated with increased cytokine concentrations of IL-1, IL-6, IL-8, and tumor necrosis factor (TNF)- α , whose syntheses are dependent on activation of the transcription factor nuclear factor (NF)- κB (14–16), and neutrophil infiltration (17). The CF airway epithelium plays a central role in the airway inflammatory response, as suggested by studies showing persistent activation of NF- κB (18), elevated production of IL-6 and IL-8, and decreased secretion of antiinflammatory factors by CF airway epithelia (18–21). These observations suggested that alterations in CF airway epithelial cytokine production contribute to the inflammatory response of CF airways.

THE ROLE OF INTRACELLULAR Ca^{2+} MOBILIZATION IN AIRWAY EPITHELIAL SECRETION OF INFLAMMATORY MEDIATORS

The role of intracellular Ca^{2+} (Ca^{2+}_i) signaling in inflammatory responses is well established (22). Inflammatory and infectious stimuli can activate phospholipase C (23, 24) and increase Ca^{2+}_i through two pathways: (1) inositol 1,4,5-trisphosphate–induced Ca^{2+} release from the ER, which is the major Ca^{2+} storing, buffering, and signaling compartment within cells, and (2) Ca^{2+} entry mediated by plasma membrane channels (25–27). Ca^{2+}_i mobilization resulting from heterotrimeric G protein–coupled receptor activation by inflammatory mediators or infectious agents is functionally relevant to inflammatory responses in several systems, including airway epithelia (22).

Activation of G protein–coupled receptors results in Ca^{2+}_i mobilization associated with activation of NF- κB and secretion of inflammatory mediators (28–31). In addition, infectious agents, or their products, activate NF- κB in a Ca^{2+}_i mobilization–dependent way (32–34). The proinflammatory mediator BK triggers Ca^{2+}_i mobilization (35) and induces IL-8 secretion in non-CF and CF human airway epithelia (2, 36). Likewise, activation of airway epithelial purinoceptors by UTP results in increased IL-8 secretion (2). Furthermore, the CF pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* promote IL-8 secretion by a Ca^{2+}_i mobilization–dependent mechanism in airway epithelial cells (37). The delay between Ca^{2+}_i

(Received in original form January 28, 2010; accepted in final form August 17, 2010)

Supported by grants RIBEIR00Z0, RIBEIR00G0, and RIBEIR07G0 from the Cystic Fibrosis Foundation (C.M.P.R.) and grant HL34322 from the National Institutes of Health (R.C.B.).

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Proc Am Thorac Soc Vol 7. pp 387–394, 2010

DOI: 10.1513/pats.201001-017AW

Internet address: www.atsjournals.org

mobilization and the onset of cytokine secretion reflects the kinetics of activation of Ca^{2+} -dependent transcription (38) and protein translation. After rises in Ca^{2+} , NF- κ B is freed from I κ B inhibition and translocates to the nucleus, where it can reside for tens of minutes, resulting in persistent transcriptional activation (38), despite the relaxation of Ca^{2+} levels toward baseline levels, as occurs in human airway epithelia (1, 2).

Ca^{2+} signals induced by airway epithelial activation by inflammatory or bacterial factors can affect airway inflammatory responses by affecting mucin secretion. For example, purinoceptor activation with ATP, released in part by inflammatory cells (39), triggers mucin secretion in airway goblet cells (40–42).

THE RELATIONSHIP AMONG AIRWAY INFECTION AND INFLAMMATION, ER/ Ca^{2+} STORE EXPANSION, AND HYPERINFLAMMATORY AIRWAY EPITHELIAL RESPONSES

Although the two phases of Ca^{2+} mobilization (i.e., ER Ca^{2+} release and Ca^{2+} influx) induced by inflammatory and infectious stimuli can act in concert to modulate NF- κ B, the quantity of ER-releasable Ca^{2+} is a major factor controlling the magnitude of Ca^{2+} signals and, thus, NF- κ B activation. For instance, presenilin mutations in Alzheimer's disease have been associated with increases in ER size, Ca^{2+} stores, and cytokine production (43, 44). The role of ER-sequestered Ca^{2+} in inflammatory responses is further supported by studies illustrating a link between infection and ER expansion. For example, viral infection promotes ER proliferation associated with the massive production of viral proteins (45–47), and bacterial infection increases ER size (48).

These data led to the hypothesis that airway epithelia exposed to infectious and inflammatory stimuli exhibited increased ER mass and ER Ca^{2+} storage, with resulting larger ER-derived Ca^{2+} signals triggered by external stimuli mediating increased Ca^{2+} -dependent transcriptional activity of inflammatory mediators. Indeed, it has been shown that apical purinoceptor (P2Y₂) or BK receptor activation induced greater Ca^{2+} signals in short-term (6–11 d old) primary cultures of CF versus normal HBE (1), which resulted from an increased density and Ca^{2+} storage capacity of the apical ER (1). The ER expansion was similar in native airway epithelia from patients with CF and patients with primary ciliary dyskinesia who had chronic airway infection and inflammation (1), was independent of ER retention of mutated Δ F508 CFTR (1), reverted *in vitro* with time in the absence of infection and inflammation (1), and was induced in normal HBE by luminal exposure to supernatant from mucopurulent material (SMM) from human CF airways infected with *P. aeruginosa* and *Staphylococcus aureus* (SMM contains infectious and inflammatory factors derived from bacteria, inflammatory cells and inflamed epithelia [1, 2]).

Short-term primary cultures of Δ F508 CF HBE exhibited hyperinflammation (e.g., an amplified mucosal BK-dependent IL-8 secretion), which was lost in long-term (30–40 d-old) cultures, suggesting that this phenotype can revert to normal and be dissociated from the Δ F508 CFTR mutation (2). A CF-like hyperinflammatory response could be induced in long-term cultures of normal HBE by luminal exposure to SMM (2). Thus, the increased BK-induced IL-8 secretion in short-term primary cultures of CF HBE was associated with larger ER Ca^{2+} stores and increased BK-triggered, ER-derived Ca^{2+} signals, and the loss of the hyperinflammatory phenotype in long-term CF HBE correlated with the reversal of ER Ca^{2+} stores and ER-dependent Ca^{2+} signals to normal levels (1, 2). Together, these published observations led to the conclusion that the up-

regulation of the ER Ca^{2+} stores in inflamed airway epithelia reflected an epithelial adaptation to chronic airway infection and inflammation (1, 2).

RELEVANCE OF AIRWAY INFLAMMATION-INDUCED ACTIVATION OF THE UPR IN AIRWAY EPITHELIA

Previous studies have demonstrated that luminal infection increases total intracellular protein synthesis in HBE, reflecting the increased epithelial synthesis of inflammatory mediators (2). The up-regulation of protein synthesis in infected and inflamed airway epithelia affects ER function because the increased load of new, unfolded inflammatory mediators and defensive factors on the protein folding machinery alters ER homeostasis and triggers an ER stress response, the UPR (49–55). In eukaryotic cells, three different mechanisms have evolved to deal with the accumulation of unfolded proteins in the ER: (1) transcriptional induction of genes whose functions are associated with increasing the ER volume and capacity for protein folding, (2) translational attenuation to decrease the ER protein folding load, and (3) degradation of misfolded proteins. Thus, the UPR plays a key role in the folding, processing, export, and degradation of proteins originating from the ER, and it can be regarded as an adaptive, cell survival mechanism. However, if one or all of these mechanisms cannot relieve cell stress resulting from accumulation of unfolded proteins in the ER, the cell will undergo apoptosis or necrosis (50, 51, 53).

Eukaryotic cells exhibit three major UPR-sensing pathways, namely inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and PERK-like ER kinase/pancreatic eIF2 α kinase (PERK), whose activation during ER stress result in downstream activation of different signaling pathways. An additional review of UPR signaling is provided in Ron and Walter (56). Changes in ER homeostasis driven by increases in cellular protein synthetic rates, such as those experienced during airway epithelial inflammation, are transduced in the ER by sensors that detect the higher levels of unfolded, nascent proteins. These ER stress sensors activate signaling pathways responsible for the expression of several genes associated with the UPR. In mammalian cells, the requirement for increased folding of proteins is detected by two transmembrane ER stress sensors: IRE1 (57, 58), which exists in two isoforms, α and β , and ATF6 (50, 53, 59). Recent studies have shown that IRE1 signaling plays a key role in gut and airway inflammatory responses (60, 61). UPR activation leads to IRE1 dimerization and activation of its C-terminal endoRNase activity (51, 53). The IRE1 endoRNase activity splices the leucine zipper transcription factor X-box binding protein-1 (XBP-1) mRNA via removal of a 26-nucleotide intron, resulting in a frameshift of the XBP-1 mRNA transcript (62, 63). The spliced XBP-1 mRNA is translated into a transcription factor that up-regulates genes encoding ER chaperones (52, 62–64). Albeit mediated by a different mechanism of activation as compared with that from IRE1, activation of ATF6 also leads to the up-regulation of ER chaperones that facilitate the folding requirements of newly synthesized proteins (56).

The spliced XBP-1 has also been directly implicated in expansion of the ER compartment and the secretory pathway (65–69). The elaborated ER compartment in specialized secretory cells exemplifies this responsiveness. Studies with B lymphocytes and plasma cell differentiation have established the link between increased protein synthesis, activation of ER stress, and ER size. Differentiated plasma cells are programmed to secrete large quantities of immunoglobulins, a function accomplished by the acquisition of a highly developed ER (70). Such studies revealed a relationship among increased Ig synthesis,

activation of the UPR, and ER expansion (65, 71). The high rate of Ig secretion by plasma cells requires ER expansion (70), a process dependent on XBP-1 mRNA splicing (65). These findings agree with earlier data suggesting a role for the UPR in the coordination of the synthesis of phospholipids and new membranes and the up-regulation of a wide spectrum of genes of the secretory pathway (72, 73). Previous studies have demonstrated that the spliced XBP-1 promotes phospholipid biosynthesis, which is necessary for ER expansion (66–68). After UPR activation, new membranes are produced, and the resulting increased ER volume serves to dilute unfolded proteins and prepare the compartment to receive newly synthesized folding components, thereby restoring ER homeostasis (73). In addition to its role in ER expansion, the spliced XBP-1 triggers increases in protein synthesis, ribosomal number, lysosomal content, Golgi compartment, mitochondrial mass and function, and cell size (66). The function of spliced XBP-1 in coordinating structural and functional features characteristic of secretory cells is relevant for inflammatory responses of airway epithelia.

Besides IRE1 and ATF6, PERK serves as an additional sensor of ER stress (50, 53, 59). PERK has a luminal domain similar to that of IRE1 and a cytoplasmic domain similar to that of other eIF2 α kinases. UPR activation stimulates PERK's kinase activity, resulting in PERK-mediated phosphorylation of eIF2 α and attenuation of general protein synthesis (59). However, phosphorylation of eIF2 α is also associated with selective translation and expression of the activating transcription factor 4 (ATF4) (74). Of importance for airway inflammatory responses, ATF4 can induce the transcription of genes associated with the improvement of cellular metabolism and survival (e.g., amino acid import, glutathione biosynthesis, and resistance to oxidative stress) (75).

The following sections focus on IRE1-dependent XBP-1 signaling and PERK-mediated activation of the ATF4 pathway because recent data have uncovered key functional roles of these branches of the UPR in airway inflammatory responses in models relevant to CF airways disease.

AIRWAY INFLAMMATION INDUCES XBP-1 mRNA SPLICING IN CF HUMAN AIRWAYS AND IN INFLAMED MURINE AIRWAYS *IN VIVO*

The role of XBP-1 mRNA splicing in coordinating structural and functional features characteristic of secretory cells, including those in the intestinal tract and the airways, can be summarized as follows.

A XBP-1 conditional knockout mouse was mated to a Villin-*Cre* transgenic mouse to delete XBP-1 in the intestinal epithelial cells (60). The resulting mouse lacking XBP-1 specifically in intestinal epithelia exhibited spontaneous enteritis, an approximately 95% decrease in the number of Paneth cells, and a decrease in the granule protein lysozyme. In addition, the intestinal epithelia XBP-1^{-/-} mouse exhibited a decreased goblet cell staining by periodic acid Schiff and a decreased number of goblet cells per villus (60). Electron micrographs demonstrated that deletion of XBP-1 in intestinal epithelia resulted in a contracted ER and rudimentary electron-dense granules in Paneth cells and, similarly, a contracted ER and smaller cytoplasmic mucin granules in goblet cells (60). These studies demonstrated the importance of XBP-1 in secretory processes associated with intestinal inflammation, such as secretion of defense proteins (e.g., lysozyme) and mucins.

Earlier studies have suggested that the XBP-1 pathway is functionally relevant in CF airways inflammatory disease by demonstrating that exposure of primary HBE cultures to SMM promotes XBP-1 mRNA splicing (2). In addition, chronically

infected and inflamed native CF airway epithelia exhibit an increased protein expression of the UPR markers calreticulin and protein disulfide isomerase, which are gene targets of XBP-1 (2). A follow-up study provided evidence that mRNA splicing of XBP-1 occurs in airway inflammation *in vivo*, based on the following observations. (1) Native infected/inflamed CF human bronchial airway epithelia exhibit increased levels of spliced XBP-1 as compared with noninfected/inflamed normal human bronchial epithelia (Figure 1). (2) ER stress-activated indicator (ERAI) mice (76) exhibit evidence of ER stress-dependent XBP-1 mRNA splicing after intrapulmonary bacterial challenge (61). ERAI mice are a transgenic line of mice where UPR activation-dependent XBP-1 mRNA splicing couples to expression of Venus (a variant of GFP) fluorescence (76). The ER stress indicator was constructed by fusing XBP-1 and Venus. Upon UPR activation, the spliced indicator mRNA is translated into an XBP-1–Venus fusion protein, whose expression can be detected by its fluorescence. Hence, there is no Venus expression in the absence of XBP-1 mRNA splicing, but, in the presence of XBP-1 splicing, Venus expression occurs, and its fluorescence is an index of spliced XBP-1. ERAI mice have been successfully used to monitor physiological and pathological ER stress *in vivo* (76).

To test whether airway infection promoted XBP-1 mRNA splicing *in vivo*, ERAI mouse airways were exposed to 40 μ l PBS or PBS containing 1×10^6 colony-forming units of *P. aeruginosa* strain PAK (77). Although this is a model of acute pneumonia (77), it also induces airway inflammation 24 hours after infection. XBP-1 mRNA splicing, as reflected by increased Venus fluorescence, was increased 24 hours after *P. aeruginosa* infection in lining epithelia from inflamed airways (Figures 2C and 2D). In contrast, XBP-1 splicing/Venus fluorescence was low in noninflamed airway sites in PBS- and *P. aeruginosa*-challenged airways (Figures 2A, 2B, 2E, and 2F). Quantification of Venus fluorescence intensity, expressed per surface airway epithelial area, strengthened the notion that XBP-1 mRNA splicing was increased in airway epithelia from inflamed airways (Figure 2G). These findings demonstrated that luminal *P. aeruginosa* infection-induced airway inflammation activates the UPR and promotes XBP-1 mRNA splicing in airway epithelia *in vivo*.

Figure 1. Native cystic fibrosis (CF) human bronchial epithelia exhibit increased activation of X-box binding protein-1 (XBP-1). (A) Southern blot illustrating that XBP-1 mRNA splicing is increased in native infected/inflamed CF human bronchial epithelia as compared with noninfected/inflamed normal native human bronchial epithelia. Data are representative of three tissue codes from normal and CF epithelia. (B) Compilation of the XBP-1 mRNA splicing data expressed as a percentage of XBP-1 mRNA splicing from normal epithelia. Reproduced with permission from Ref. 61.

Figure 2. Airway inflammation induced by *Pseudomonas aeruginosa* infection promotes X-box binding protein-1 mRNA splicing (activation) in murine airway epithelia *in vivo*. (A, C, and E) Differential interference contrast and (B, D, and F) Venus expression in airway epithelia from noninflamed, PBS-challenged (A and B), inflamed, *Pseudomonas aeruginosa* (*P.a.*)-challenged (C and D), and noninflamed, *P.a.*-challenged (E and F) endoplasmic reticulum stress-activated indicator mice airways. Arrows point to airway epithelia. Bar, 20 μ m. (G) Compiled

data for Venus fluorescence intensity, expressed per surface epithelial area, from all groups. * $P < 0.05$, *P.a.*-challenged and inflamed versus PBS-challenged, noninflamed. Reproduced with permission from Ref. 61.

ACTIVATION OF XBP-1 BY AIRWAY INFLAMMATION TRIGGERS THE ER Ca^{2+} STORE EXPANSION IN AIRWAY EPITHELIA

Data linking inflammation with airway epithelial ER Ca^{2+} store expansion *in vivo* were recently provided by findings from wild-type mouse airways exposed to *P. aeruginosa* strain PAK (77). Histological analyses of *P. aeruginosa*-challenged lungs at 24 hours revealed areas that were clearly inflamed and areas that showed no evidence of inflammation (61). As visualized by calreticulin (ER Ca^{2+} store marker) staining, ER Ca^{2+} store expansion was only observed in epithelia from airways that were inflamed 24 hours after inoculation with *P. aeruginosa* (61). These data illustrated that epithelial ER expansion and UPR activation coupled to XBP-1 mRNA splicing are a feature of inflamed airways.

We have recently investigated the role of XBP-1 in human airway epithelial inflammatory processes mediated by ER Ca^{2+} store expansion by overexpressing the spliced XBP-1 or a dominant negative XBP-1 (DN-XBP-1) in human bronchial airway epithelia (61). Overexpression of spliced XBP-1 promoted morphological and functional expansion of the ER and its Ca^{2+} stores in the absence of extracellular stimulus (61). In contrast, the ER Ca^{2+} store expansion resulting from exposure to mucosal infectious and inflammatory factors from CF airways was blunted in epithelia expressing the DN-XBP-1 (61). These data demonstrate that activation of XBP-1 is a key mechanism responsible for the ER/ Ca^{2+} store expansion in inflamed human airway epithelia. In addition, these studies indicate that activation of XBP-1-dependent ER/ Ca^{2+} store expansion is a mechanism responsible for the amplified inflammatory mediator secretion we have previously reported in inflamed human bronchial epithelia (2, 61).

ACTIVATION OF XBP-1 BY AIRWAY INFLAMMATION MEDIATES INCREASED AIRWAY EPITHELIAL CYTOKINE SECRETORY RESPONSES

We have considered that activation of XBP-1 mRNA splicing during airway inflammation may mediate ER Ca^{2+} store expansion-dependent hyperinflammation. Because a hyperinflammatory response was, in part, typified by an increased mucosal BK-dependent IL-8 secretion in airway epithelia (2), we investigated IL-8 secretory responses induced by mucosal BK in cultures of 16HBE14o⁻-expressing control, spliced XBP-1, or DN-XBP-1 vectors. In control vector-expressing cultures,

mucosal BK induced a modest increase in IL-8 secretion in comparison with vehicle-treated cultures (Figure 3). In contrast, cultures expressing the spliced XBP-1 exhibited a higher IL-8 secretory response, as compared with control vector expressing cultures in the absence of BK, and potentiation of BK-induced IL-8 secretion (Figure 3). Finally, expression of DN-XBP-1 inhibited mucosal BK-induced IL-8 secretion (Figure 3). These data illustrate that constitutive activation of spliced XBP-1 induces a hyperinflammatory response (e.g., increased IL-8 secretion in airway epithelia).

CFTR MUTATIONS ARE NOT ASSOCIATED WITH ACTIVATION OF XBP-1-DEPENDENT SIGNALING

Freshly isolated CF airway epithelia exhibit increased levels of XBP-1 mRNA splicing (Figure 1). However, our previous work (2) and the findings from other investigators suggest that CFTR mutations are not linked to the higher levels of spliced XBP-1 found in inflamed CF airway epithelia. Although CF human bronchial epithelia homozygous for the Δ F508 CFTR mutation exhibit an increased IL-8 secretion in short-term primary

Figure 3. X-box binding protein-1 (XBP-1) promotes IL-8 secretion and plays a functional role in cytokine secretion in inflamed airway epithelia. Serosal IL-8 levels from polarized 16HBE14o⁻ cultures expressing control, spliced XBP-1, or dominant negative XBP-1 (DN-XBP-1) vectors resulting from 8-hour exposure to vehicle or 5 μ M mucosal bradykinin. * $P < 0.05$ XBP-1s versus control vector. Reproduced with permission from Ref. 61.

cultures, their higher IL-8 secretion is lost in long-term cultures (2). Moreover, exposure of long-term CF cultures to SMM reproduced the increased IL-8 secretion found in short-term CF cultures, and this response was coupled with the ability of SMM to induce XBP-1 mRNA splicing (2).

Follow-up studies have also suggested a dissociation of CFTR mutations from XBP-1 mRNA splicing. For instance, no differences in XBP-1 mRNA splicing were found in passage 1 normal versus CF airway epithelial cultures (78). The endogenous levels of CFTR expression in primary cultures of airway epithelia are low, as observed in airway epithelia *in vivo*. Dissociation of the $\Delta F508$ CFTR mutation from IRE1/XBP-1 signaling and airway epithelial inflammatory responses has been further documented in a study showing no significant differences in basal and *P. aeruginosa* (or flagellin)-induced IL-8 secretion, intracellular Ca^{2+} mobilization, and IRE1 activity in CF15 cells overexpressing wild-type or $\Delta F508$ CFTR (79). In contrast, high-level expression of recombinant $\Delta F508$ CFTR in Calu-3 cells led to increased XBP-1 mRNA splicing (80). We speculate that the absence versus presence of activation of the IRE1/XBP-1 pathway in the latter two studies may have resulted from cell-specific differences or differences in $\Delta F508$ CFTR expression levels induced in cell lines (i.e., whereas lower levels of expression of mutated CFTR do not trigger ER stress, high levels of expression of $\Delta F508$ CFTR induce XBP-1 mRNA splicing). The relevance of *in vitro*-promoted exaggerated expression of $\Delta F508$ CFTR-triggered activation of XBP-1 signaling in airway epithelial inflammatory responses remains to be established. Nevertheless, the findings from primary cultures of normal and CF epithelia expressing endogenous CFTR mutations suggest that airway epithelial inflammation, rather than mutated CFTR, is responsible for activation of ER stress mediated by XBP-1 mRNA splicing.

SIGNIFICANCE OF INFECTION-TRIGGERED AIRWAY EPITHELIAL UPR ACTIVATION FOR CF AIRWAY INFLAMMATION *IN VIVO*

A model linking airway epithelial inflammation-induced ER stress-dependent XBP-1 mRNA splicing—and the consequent

XBP-1-dependent increased airway epithelial secretion of inflammatory mediators—is presented in Figure 4. Under normal conditions, these XBP-1-mediated functions are beneficial for the host by amplifying the inflammatory response to clear the airways of infection. However, we speculate that XBP-1-dependent functions can mediate adaptive and maladaptive responses during chronic airway inflammation. For example, expansion of ER Ca^{2+} stores can provide a compensatory and protective function for infected airways via an increased Ca^{2+} -dependent mucociliary clearance (1). The higher Ca^{2+} signals resulting from ER Ca^{2+} store expansion may be particularly beneficial to patients with CF, who depend on Ca^{2+} -activated Cl^- channels (CaCC) to compensate for the lack of CFTR-dependent Cl^- secretion (81). Because of the increased expression of ER Ca^{2+} stores at the apical domain in CF airway epithelia (1), luminal infectious and inflammatory stimuli generate greater Ca^{2+} signals in close proximity to CaCC, allowing the airways to transiently restore defective mucus clearance.

However, UPR-induced ER Ca^{2+} store expansion also amplifies the airway epithelial inflammatory response, which may adversely affect the homeostasis of CF airways. After intraluminal infection-triggered ER Ca^{2+} store expansion, the airway epithelium is primed to secrete higher levels of inflammatory factors (e.g., IL-8) in response to the larger Ca^{2+} signals triggered by Ca^{2+} -mobilizing stimuli such as BK or ATP/UTP (1). The amplified cytokine response resulting from persistent bacterial infection in CF may be maladaptive because inflammatory cells cannot rid the lumen of bacteria that are protected in the thickened mucus environment characteristic of CF airways (82). Thus, rather than eradicating the bacterial infection, the chronic, but ineffective, UPR-mediated hyperinflammatory response of CF airways may have harmful consequences by promoting paradoxical airway wall destruction due to proteolytic enzyme release.

It is plausible to speculate that the resulting activation of the PERK/eIF2 α /ATF4 UPR pathway should also be functionally relevant for the pathophysiology of CF airways inflammatory disease. The increased synthesis of inflammatory mediators in CF airways likely causes profound consequences for the metabolic and oxidative status of inflamed airway epithelia. Protein

Figure 4. Model for airway epithelial inflammation-induced activation of X-box binding protein-1 (XBP-1) and activating transcription factor (ATF)4 and the functional consequences of XBP-1- and ATF4-mediated responses for airway inflammation. Airway epithelial inflammation triggers an unfolded protein response mediated by activation of IRE1 and PERK due to an increased flux of newly synthesized unfolded inflammatory factors into the endoplasmic reticulum (ER). Activation of inositol-requiring enzyme 1 (IRE1)-dependent XBP-1 mRNA splicing and pancreatic eIF2 α kinase (PERK)-dependent eIF2 α phosphorylation-induced ATF4 translation results in, respectively, ER/ Ca^{2+} store expansion-mediated hyperinflammation and protection against the amino acid loss and oxidative stress consequent to airway epithelial inflammation.

secretion can constitute an irreversible loss of amino acids into the extracellular environment and produce net loss of equivalents from the cell. The foldase PDI catalyzes oxidative protein folding in the ER, resulting in the transfer of electrons from the nascent polypeptide chain to the oxidoreductase ERO1 (75). ERO1 is then oxidized by molecular oxygen, which functions as the terminal electron acceptor. Therefore, the greater the secretory burden, the greater the loss of amino acids and reducing equivalents. Previous studies have revealed that the PERK/ATF4 UPR pathway plays a key role in protecting cells against amino acid deprivation and oxidative stress (75, 83). In cells devoid of PERK, activation of the UPR results in accumulation of reactive oxygen species, and ATF4 and PERK knockout cells require amino acid and cysteine supplementation, most likely to replenish amino acids lost during secretion and increase glutathione levels, respectively (75). The importance of ATF4 in regulation of amino acid metabolism and oxidative stress responses was not only revealed by these studies (PERK^{-/-} cells cannot activate eIF2 α -dependent translational up-regulation of ATF4, and ATF4^{-/-} cells lack ATF4 protein) but was also underscored in microarrays with ATF4^{-/-} cells, illustrating that ATF4 induces the transcription of genes involved in amino acid import, glutathione biosynthesis, and resistance to oxidative stress (75).

ATF4-induced amino acid transport likely plays a protective role in inflamed CF airway epithelia because SMM-exposed HBE exhibit an augmented metabolic response (e.g., increased protein synthesis [2] and lactate production) (C.M.P. Ribeiro, unpublished observation). Moreover, the antioxidant functions of ATF4 may also be relevant for infected or inflamed CF airways because oxidative stress is a hallmark of CF airways disease (84, 85). Glutathione levels in the airway surface liquid from patients with CF are reduced (86), which may result, at least in part, from the lack of CFTR-mediated glutathione transport (reviewed in [86]). However, infected/inflamed CF airway epithelia may also contribute for the oxidative stress of CF airways through increased generation of reactive oxygen species, independently of mutated CFTR. Indeed, primary cultures of human bronchial airway epithelia exposed to SMM exhibit a higher expression of oxidative stress genes, including protective genes that are targets of ATF4 (87). A model depicting the functional role of ATF4 signaling in airway epithelial inflammatory responses is shown in Figure 4.

FUTURE DIRECTIONS

Because the knowledge generated by the studies suggesting a functional role for XBP-1 in airway inflammatory responses was limited to primarily *in vitro* studies of selected cell types, *in vivo* models that allow the study of UPR in mammalian airways and, specifically, secretory cell types (e.g., mucin-secreting cells) are necessary. Glimcher and colleagues have generated a LoxP-flanked XBP-1 mouse, which allows for conditional knockdown of XBP-1, as demonstrated by the specific knockout of XBP-1 in intestinal epithelia (60). The generation of the airway epithelia XBP-1^{-/-} mouse, using the LoxP-flanked XBP-1 mouse, will advance the understanding of cellular pathways relevant to chronic airway inflammation.

Previous studies have linked XBP-1 with a genetic risk for human inflammatory bowel disease by showing that several single-nucleotide polymorphisms within the XBP-1 gene locus conferred risk for Crohn's disease and ulcerative colitis (60). Similar studies are necessary to address whether XBP-1 is linked with genetic risks for human airway inflammatory diseases.

An unresolved area involves the relative roles of IRE1 α and IRE1 β in UPR-mediated signaling in airway inflammatory re-

sponses. A central question is whether IRE1 β has a similar activation scheme to IRE1 α . Structural studies with the core region of the ER luminal domain of IRE1 α suggest that dimerization of this domain, resulting from IRE1 α activation during ER stress, creates a groove reminiscent of the peptide-binding domains of histocompatibility complexes (88). Additional studies have shown that oligomerization is central to IRE1 α function and is an intrinsic attribute of its cytosolic domains (89). Studies that address whether IRE1 β exhibits a similar sequence of activation during airway inflammation and whether it has a distinct functional role versus IRE1 α in the airways will expand the understanding of airway secretory mechanisms.

An additional role for the UPR in CF airway inflammatory responses has been suggested by initial studies investigating the PERK/eIF2 α /ATF4 pathway, indicating that it plays a key role in innate immune responses of human airway epithelia by inducing ATF4-dependent expression of genes involved in protection against amino acid loss and oxidative stress (87) and NF- κ B-mediated transcription of inflammatory mediators (3, 4). Future studies addressing the role of the PERK/eIF2 α /ATF4 pathway in airway inflammatory responses may result in novel therapies for CF airway inflammation associated with oxidative stress. A previous study has shown that the eIF2 α UPR pathway can be modulated by salubrinal, a selective inhibitor of eIF2 α dephosphorylation that does not affect ATF6- or IRE1-dependent signaling and promotes cell protection (90). This raises the possibility that a therapeutic strategy for conferring protection against airway epithelial inflammation may be based on small molecules, like salubrinal, to up-regulate the ATF4 pathway.

The functional consequences of UPR activation for inflammatory responses relevant to CF airways disease deserve further investigation. Studies with mice exhibiting airway epithelial deletion of UPR pathways will provide insight into the relevance of ER stress responses in the pathogenesis of CF. Future studies are required to establish how the UPR is triggered in CF; whether activation of the IRE1-dependent XBP-1, ATF6, or PERK signaling pathways plays a favorable or a negative role in CF pathophysiology; and whether manipulation of these pathways can be therapeutically beneficial for patients with CF.

Author Disclosure: C.M.P.R. received grant support from Cempla Pharmaceuticals (\$50,001–\$100,000). R.C.B. was a consultant for Gilead Sciences (\$1,001–\$5,000), Parion Sciences, Inspire Pharmaceuticals (\$10,001–\$50,000), Pulmatrix (\$5,001–\$10,000). He is on the Board of Parion Sciences (\$10,001–\$50,000) and owns patents through Inspire Pharmaceuticals and Parion Sciences. He owns stocks or options of Parion Sciences and Inspire Pharmaceuticals (more than \$100,001).

Acknowledgments: The authors thank Lisa Brown for editorial assistance during the preparation of this manuscript.

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