Acidic duodenal pH alters gene expression in the cystic fibrosis mouse pancreas

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Cystic fibrosis (CF) is an autosomal recessive disease that affects multiple organs, primarily through alterations of exocrine fluid and glycoprotein secretions (41). In the human disease, the pancreas is one of the major organs affected, and CF leads to widespread destruction of the exocrine portion (41). Importantly, many CF patients identified by newborn screening may begin life with significant pancreatic function (7, 27) but most CF patients eventually become pancreatic insufficient (22, 65).

CFTR in the gastrointestinal system is required for secretion of bicarbonate ion. CFTR is primarily a cAMP-regulated Cl− channel, and the secreted Cl− can be exchanged for HCO3− (13). Additionally, there is accumulating evidence for direct permeation of HCO3− through CFTR (11, 13). The human pancreas is very dependent on functional CFTR for bicarbonate ion secretion, which is believed to be essential for solubility of digestive enzymes secreted into the pancreatic duct system (25, 61). In fact, the strongest association of CFTR functional genotype to CF disease phenotype is found for the pancreas (22).

Individuals with one severe and one mild CFTR mutant allele are pancreatic sufficient, but they are at increased risk for acute pancreatitis that occurs with increasing frequency with age (14, 22). Pancreatitis is an inflammatory condition whose etiology is not fully understood. Early events in pancreatitis include damage to acinar cells that release cytokines and cause an inflammatory response that can become systemic and life-threatening (5). In CF, it is believed that secreted digestive enzymes precipitate in the poorly hydrated acidic environment of the proximal duct system (62). This may lead to enzyme activation that damages acinar cells, induces inflammation, and results in the eventual replacement of the exocrine cells with fibrotic tissue. Although the mechanism of damage in compound heterozygous individuals has not been demonstrated, partial CFTR function may predispose to sporadic enzyme aggregation in the proximal ducts leading to episodes of acute pancreatitis.

The CFTR null mouse model of CF has some unique features advantageous for elucidating aspects of CF pathogenesis, especially in the gastrointestinal system. Whereas the human pancreas depends on CFTR for bicarbonate ion secretion, the mouse pancreas normally expresses very low levels of CFTR (http://www.informatics.jax.org/searches/image.cgi?1136 and http://www.informatics.jax.org/searches/image.cgi?1137) and sufficient secretion can occur in the absence of CFTR in the mouse pancreatic ducts. This is likely due to expression of a Ca2+-regulated Cl− conductance in the mouse duct cells that allows Cl−/HCO3− secretion in the absence of CFTR (12, 31). As a consequence, the CFTR null mouse has only mild pathologies in the exocrine pancreas (16, 25, 58). On the other hand, the small intestine of the CF mouse is severely affected, and serves as a model for meconium ileus and distal intestinal obstruction syndrome (58), both common complications of human CF (43).

In contrast to human CF, the CF mouse is null for CFTR expression, whereas the most common CFTR mutation in humans is the ΔF508 allele. ΔF508 causes misfolding of CFTR and can induce endoplasmic reticulum (ER) stress responses, including NF-κB activation (39, 66). It was demonstrated by Weber et al. (66) that expression of ΔF508 CFTR in CHO cells induces NF-κB activation. A second mutant CFTR protein, G551D, which traffics correctly but does not have Cl− channel activity, did not induce NF-κB activation. Finally, transfection of wild-type CFTR or an empty plasmid did not activate NF-κB (66). The ER stress response is due to the presence of misfolded CFTR in the ER. This will not occur in the CFTR null mouse, because CFTR protein is absent altogether. Thus inflammation of human cells expressing ΔF508 CFTR may be a chronic inherent condition, and is not expected to occur in the CFTR null mouse. The observed pathologies in the CF mouse are most likely a result of the deficit in Cl− and HCO3− permeation of HCO3− by 10.220.32.247 on July 5, 2017 http://ajpgi.physiology.org/ Downloaded from
secretion (66). This difference is useful for studying the consequences of loss of CFTR on electrolyte transport without a confounding intrinsic inflammation. For these reasons, the CF mouse model allows exploration of more subtle and potentially important physiological interactions between the small intestine and the pancreas.

In the intestine, delivery of acidic chyme from the stomach activates signaling pathways that stimulate bicarbonate ion secretion. This neutralizes the acid and is essential for proper digestion and absorption. Bicarbonate ion is secreted from duodenal submucosal (Brunnner’s) glands, from the intestinal crypt epithelium, and from the ductal systems of the liver and pancreas. Dysfunction of intestinal pH regulation in CF patients may enhance signaling and have effects on exocrine pancreatic function.

We recently reported that the CF mouse duodenum is abnormally acidic (17), similar to human CF patients (3). There are increases in intestinal secretin and vasoactive intestinal peptide (VIP) gene expression, and increased pancreatic cAMP levels in CF mice. The acinar cells have dilations of the apical plasma membrane (16, 25) and reduced amylase stores (17), indicating increased zymogen exocytosis and decreased endocytosis (25). We proposed that the acidic duodenal pH in CF results in excessive signaling from the small intestine to the exocrine pancreas in an attempt to stimulate secretion of bicarbonate-rich pancreatic fluid to neutralize the acid in the duodenum (17). For the studies presented here, we hypothesized that acidic duodenal pH would affect the pancreas and could account for some of the CF-like changes to the tissue. To explore this question, Affymetrix GeneChip DNA microarrays were employed to look at expression levels of many genes. The hypothesis that the acidic lumen of the CF small intestine affects the exocrine pancreas was tested by using genetically modified and pharmacologically treated CF mice to normalize duodenal pH levels. Our data suggest that abnormal acidity in the CF duodenum has an important role in inducing inflammation of the pancreas and expression of stress-related genes, and that normalizing the duodenal pH can ameliorate these effects.

**MATERIAL AND METHODS**

**Animals.** Mice heterozygous for disruption of the CFTR gene (CFTR knockout mouse; cfr<sup>mut<sup>(NC)</sup></sup>) (58) congenic on the C57BL/6 background were bred to obtain mice with wild-type or disrupted CFTR alleles (17). Previous work has shown that CFTR<sup>(+/-)</sup> mice are phenotypically normal (58), and CFTR<sup>(+/-)</sup> mice were included in the control group for this gene. Comparison of gene expression in CFTR<sup>(+/-)</sup> to CFTR<sup>(+/-)</sup> mice showed no significant differences for the genes analyzed (data not shown). Gastrin null mice (26) were crossed with C57BL/6 mice for eight generations after which they were 95% C57BL/6 and 5% 129Sv on the basis of genome-wide allele typing analysis (Jackson Labs, Bar Harbor, ME; www.jax.org). To optimize obtaining the desired genotypes, CFTR<sup>(+/-)</sup>/gastrin<sup>(+/-)</sup> mice were bred with CFTR<sup>(+/-)</sup>/gastrin<sup>(-/)</sup> mice. Controls for the gastrin gene were gastrin<sup>(+/-)</sup> mice. It has been shown that gastrin heterozygous mice have normal plasma gastrin levels (26). Mice of both sexes were used for these experiments between 6 and 9 wk of age. Mice were genotyped by PCR analysis of tail-snip DNA as described (17, 26). To prevent intestinal obstruction that occurs in CF mice on solid chow, all mice including controls, were maintained from postnatal age of 10 days on Peptamen (Nestle, Deerfield, IL), a complete liquid diet (24). To pharmacologically block gastric acid production, 3-wk-old mice were treated with the proton pump inhibitor omeprazole for 3 wk by daily subcutaneous injection of 400 nmol/g body wt, in 0.1% methyl cellulose (68). All procedures were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

**In situ duodenal pH determination.** After overnight fasting with free access to water, mice were anesthetized with intraperitoneal injections of ketamine. The peritoneal cavity was opened, and the duodenal pH was measured by inserting a pH microprobe into a small slit through the duodenal wall as previously described (17). Mice were then killed for removal of the pancreas for analysis.

**Microarray analysis of gene expression.** Total RNA was prepared from freshly isolated pancreas by rapid homogenization in TRizol reagent and extraction according to the manufacturer’s instructions (Invitrogen; Carlsbad, CA). RNA was prepared from the pancreata of three 8-wk-old CFTR control mice [CFTR<sup>(+/+)</sup> gastrin<sup>(+/-)</sup>)] and three 8-wk-old CFTR homozygous null mice [CFTR<sup>(+/-)</sup> gastrin<sup>(+/-)</sup>]. RNA samples were submitted to the University of Kansas School of Medicine Microarray Core Facility for probing of the mouse U74Av2 GeneChip from Affymetrix that has 12,000 known genes and ~6,000 expressed sequence tags (Affymetrix; Santa Clara, CA). All processing and hybridization steps were done according to the manufacturer’s instructions (https://www.affymetrix.com/support/downloads/manuals/expression_s2_manual.pdf).

Data analysis was performed by using Affymetrix Microarray Suite 5.0 (MAS 5.0) software with the absolute expression algorithm and the default settings. The entire expression analysis dataset was exported to QuattroPro spreadsheet software (Corel; Ottawa, Ontario, Canada) and a t-test was performed to compare the three control and three CF samples. To conclude that a gene had increased expression in CF, three criteria were used: the gene must be called “Present” by MAS 5.0 software in all three CF samples; the t-test P value comparing CF to control must be < 0.05; and the average change must be greater than or equal to twofold. Similarly, to conclude that a gene had decreased expression in CF, the gene must be called Present in all three control samples and meet the other two criteria above. The complete datasets that include signal strength and Absent/Marginal/ Present calls from the MAS 5.0 software were deposited at the National Center for Biotechnology Information’s (NCBI) Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo; accession numbers GSM6764, GSM6777, GSM12128, GSM12180, GSM12181, GSM12182).

**Table 1. Primers for quantitative RT-PCR and product sizes**

<table>
<thead>
<tr>
<th>Gene (symbol)</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Product, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regenerating islet-derived γ (Reg3γ)</td>
<td>TGT CTT CCA TGA TCA AAA GC</td>
<td>AAT TCA TGA CAT CAG CAT TG</td>
<td>126</td>
</tr>
<tr>
<td>Regenerating islet-derived 3α (Reg3α)</td>
<td>AGG TGA AGA CTT CCA GAA GG</td>
<td>GGA GGA CAC AAA GAG CAG CT</td>
<td>196</td>
</tr>
<tr>
<td>Pancreatitis-associated protein (Pap)</td>
<td>CCA ACA GCC TGC TGC GTC AT</td>
<td>GCA TCA AAG CAG GTC TGT GG</td>
<td>182</td>
</tr>
<tr>
<td>Kininogen (Kng)</td>
<td>GAC TGA AAT GGC AAG GC</td>
<td>AAG TCA AAT GGC AAG AAG GC</td>
<td>212</td>
</tr>
<tr>
<td>Leucine-rich-α2 glycoprotein (Lrg)</td>
<td>GGA GCA GCT AGT GTC TCT TGT TG</td>
<td>AAG ATC AGG CAT TCC TCG AG</td>
<td>125</td>
</tr>
<tr>
<td>Interleukin 13 receptor-α (II13ra)</td>
<td>AGA AAA TTG CTC CAG AAA CT</td>
<td>TAT AGG TGA GGT TAT GCC AA</td>
<td>198</td>
</tr>
</tbody>
</table>

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Genes were functionally categorized by using online resources (www.Netaffx.com; Gene Ontology at www.geneontology.org; Mouse Genome Informatics at www.informatics.jax.org; the NCBI databases at www.ncbi.nlm.nih.gov), and the scientific literature.

Quantitative real-time RT-PCR. Quantitation of gene expression was performed with a LightCycler (Roche Biochemicals, Indianapolis, IN) using a one-step RT-PCR kit that uses the fluorescent double-stranded DNA-binding dye SYBRgreen I (Qiagen; Valencia, CA). The genes chosen were regenerating islet-derived3 (Reg3γ), leucine-rich α2-glycoprotein (Lrg), Reg3α, pancreatitis-associated protein (PAP), Muclin (also known as crp-ductin or dmbl), IL-13 receptor-α, and kininogen (Kng). Primers were designed to cross RNA splice junctions to minimize potential amplification from any contaminating genomic DNA. Control PCR reactions in which the RT enzyme was not added resulted in a signal of <0.1% of the complete RT-PCR reaction for all genes (data not shown). Primers used are listed in Table 1, and the primers for Muclin and GAPDH were as previously described (59). RT-PCR products from the LightCycler were reamplified with standard Taq (Promega, Madison, WI) and cloned by using the pDRIVE vector (Qiagen). All cloned cDNAs were sequenced, which verified the specificity of the PCR primers. To generate standard curves for the quantitative LightCycler reactions, plasmid DNA was linearized and used to prepare a dilution series covering 10 pg to 0.00001 pg (converted to copy number from the size of the plasmid + insert). Messenger RNA levels are expressed as copy number per copy of GAPDH mRNA, and GAPDH mRNA levels were not significantly different between CF and control groups on the microarrays.

Serum amylase determinations. Trunk blood was collected at death and used to determine serum amylase activities. Amylase enzyme activities were determined as described previously (17) and expressed per milliliter of serum.

Immunohistochemistry and morphometry. Pancreas tissue was immersion fixed in 4% paraformaldehyde and 5-μm paraffin sections were prepared. Sections were immunolabeled with rat anti-mouse neutrophil monoclonal antibody (cat. no. MCA771GA, Serotec; Raleigh, NC), and the Vectastain Elite ABC kit (Vector Labs; Burlingame, CA). The signal was developed by using VIP peroxidase substrate, and sections were counterstained with methyl green (Vector Labs). After staining for neutrophils, three to four samples from each group and at least 14 fields per sample were examined with a ×40 objective (~450 nucleated cells/field). Frozen sections (5 μm) were also prepared and labeled with tetramethylrhodamine isothiocyanate-phallolidin (Sigma, St. Louis, MO) to visualize F-actin under the apical plasma membrane of pancreatic acinar cells. To measure the acinar lumen diameters, fields were chosen that had the major axis of the lumen parallel to the plane of section. Pancreatic samples from three control, three CF, and two CF omeprazole-treated mice were imaged on a Nikon microscope using a ×100 oil immersion objective and a SPOT II camera (Diagnostic Instruments; Sterling Heights, MI). The digital images were analyzed by using Image J software (http://
rsh.info.nih.gov/ij/) to obtain the diameter at the widest point of the lumen for each image.

Statistical analysis. Data were compared by using an ANOVA with a post hoc Tukey’s test (Systat software; SPSS Science, Chicago, IL). The software was used to identify statistical outliers omitted from the final analysis. The number of samples for each determination is given in the figure legends. P values of <0.05 were considered as statistically significant.

RESULTS

Microarray analysis of pancreatic gene expression in CF mice. To get a global picture of the effects of CF on gene expression in the pancreas, the Affymetrix mouse U74Av2, GeneChip cDNA microarray was used to survey a large number of genes. At the time of its release, this array represented 12,000 unique probe sets of ~6,000 known genes and ~6,000 expressed sequence tags; since its release in 2001, many of the expressed sequence tags are now identified (www.affymetrix.com). Total pancreatic RNA samples from three control and three CF pancreas were used to probe microarrays. From the arrays, 25 genes were identified that were significantly upregulated 2-fold to 47-fold in the CF pancreas (Table 2). Many of the upregulated genes are considered pancreatic stress genes and are associated with inflammation of the pancreas, especially pancreatitis. These include Reg3γ and Reg3α, the PAP gene (30), and the recently identified pancreatitis-associated vacuolar membrane protein (Vmp1) gene (64). Other inflammation-associated genes upregulated in the CF pancreas include Lrg (51), complement component 4 (29), and Kng (10) (Table 2). Also upregulated were 3 protease inhibitors that are inflammatory markers: inter-α-trypsin inhibitor 4 (6, 28), Serpin A10 (28, 69), and Serpin A1α (37). The remainder of the upregulated genes are of unknown function or do not fall into an apparent pattern (Table 2).

From the microarrays, only four genes were found to be downregulated in the CF samples compared with controls, and the magnitude of change was modest for all four genes (Table 3). The functional significance of these genes is not apparent.

Genetic correction of the acidic duodenal pH in the CF mouse and confirmation of altered expression for selected genes using quantitative real-time RT-PCR. The duodenum of the CF mouse has an abnormally acidic pH compared with controls, and we reported evidence of increased signaling from the intestine to the exocrine pancreas (17). We proposed that increased signaling would result in overstimulation of the pancreas and may account for some of the CF-like pathologies observed in the CF mouse pancreas. To test this hypothesis, CF mice were crossed with gastrin-deficient mice that have a defect in gastric acid production (26) and therefore are expected to deliver less acid to the duodenum.

Consistent with previous work (17), the CF mice have a significantly more acidic duodenal pH compared with controls (Fig. 1). In the CFTR/gastrin double-null mice, the duodenal pH was ~50% less acidic than in the CF mice (Fig. 1). This pH value was intermediate between that of control and CF mice and was not statistically different from either one. Gastrin deficiency itself did not affect duodenal pH compared with control (Fig. 1).

From the genes found to be altered in the CF pancreas by microarray analysis, Reg3γ, Reg3α, PAP, Lrg, and Kng were chosen to verify expression changes using quantitative real-time RT-PCR (QRT-PCR). Also measured was expression of Mucln that did not show up on the arrays as changed but has previously been documented to have a modest twofold increase in the CF mouse pancreas (17) and may have a protective function as part of the epithelial innate defenses (38, 59). In addition, expression of IL13 receptor-α (IL13Ra) was measured. Expression of IL13Ra in CF was close to statistical significance on the arrays (P = 0.07) and is of interest because of its anti-inflammatory function (40).

Of these genes, all could be quantified in total pancreatic RNA samples except Kng, which was below the detection limit of QRT-PCR (2 copies per nanogram total RNA). Kng is known to be expressed in the normal liver (10) and was readily measured in liver total RNA using the primer pair listed in Table 1. The lack of detectable Kng expression in the pancreas

![Fig. 1. Correction of the acidic duodenal pH in cystic fibrosis (CF) mice by genetic means. CFTR(+/−) mice were crossed with gastrin(+/−) mice to obtain the indicated genotypes as described in MATERIALS AND METHODS. Duodenal pH was measured in anesthetized mice with a pH microprobe. Data values are means ± SE. The number of samples in each group (n) is given in parentheses below the bars. *Statistical P values: a, significant vs. control; b, not significant vs. control; c, significant vs. CF; d, not significant vs. CF; e, significant vs. gastrin KO; f, not significant vs. gastrin KO.](http://ajpgi.physiology.org/doi/abs/10.1152/ajpgi.00344.2004)
indicates that it is absent or expressed at only very low levels in both control and CF mouse pancreas. The array result indicating the presence of Kng mRNA in the pancreas is considered to be a false-positive and emphasizes the need to confirm array results by an independent technique such as RT-PCR.

All of the remaining genes tested by QRT-PCR had relatively abundant expression levels. The lowest level of expression in the control pancreas was for IL13Rα/H9251, which was expressed at ~0.06 copies per copy of GAPDH mRNA (Fig. 2E). Expression of the other genes in the control pancreas ranged from 0.75 copies per copy of GAPDH (Lrg) to 73 copies per copy of GAPDH (PAP). All of the genes were significantly upregulated in the CF pancreas and most were significantly decreased in the CFTR/gastrin double-null pancreas compared with CFTR null alone (Fig. 2). Reg3γ was upregulated 37-fold in the CF pancreas and was significantly reduced in the CFTR/gastrin double-null mouse with partial duodenal pH correction (Fig. 2A). Likewise, Lrg was upregulated 45-fold (Fig. 2B). Reg3α was upregulated 26-fold (Fig. 2C), PAP was upregulated 31-fold (Fig. 2D), and all were significantly reduced in the CFTR/gastrin double-null mouse. Muclin was upregulated 2.7-fold in the CF pancreas and was reduced in the CFTR/gastrin double-null mouse (Fig. 2E). The level of Muclin expression in the double-null mice was intermediate between control and CF and was not statistically different compared with either value (Fig. 2E).

Reg3γ expression in the double-null mice was also intermediate and not statistically different compared with either control or CF values (Fig. 2F). For comparison, gene expression levels were also measured in the gastrin null mice and were not significantly different compared with controls for any of the genes (Fig. 2, A–F).

Pharmacological correction duodenal pH and the effects on pancreatic gene expression in the CF mouse. The above experiments using the CFTR/gastrin double-null mouse

![Fig. 2](http://ajpgi.physiology.org/)
showed that partial correction of duodenal pH was accompanied by significant normalization of pancreatic gene expression. As an independent means of normalizing duodenal pH in the CF mouse, the proton pump inhibitor omeprazole was used (68). Omeprazole directly inactivates the parietal cell H+/K+-ATPase and effectively blocks gastric acid production (68). Omeprazole acts by a distinct mechanism compared with gastrin deficiency, which affects parietal cell maturation and responsiveness to secretory stimuli (54). Omeprazole-treated CF mice had duodenal pH values equivalent to the corresponding control value (Fig. 3).

In omeprazole-treated CF mice with complete correction of duodenal pH, pancreatic Reg3γ, Lrg, Muclin, and IL13Raα gene expression levels were significantly reduced compared with the untreated CF mice (Fig. 4, A, B, E, and F). Reg3α gene expression was reduced in omeprazole-treated CF mice compared with untreated CF mice, but the level of expression was still significantly elevated compared with control (Fig. 4C). PAP gene expression was also reduced in omeprazole-treated CF mice, and although the expression level was not significantly different than in untreated CF mice, it was also not significantly different than the control level (Fig. 4D).

Inhibition of the gastric proton pump causes hypergastrinemia (49). Hypergastrinemia is not expected to directly affect the pancreas (49), although it is conceivable there may be some indirect effects. To assess the possible effect of hypergastrinemia on pancreatic gene expression, QRT-PCR was performed using RNA from control mice treated with omeprazole and in CFTR/gastrin double-null mice treated with omeprazole. The latter mice cannot respond to inhibition of gastric acid secretion by elevating plasma gastrin, because they lack the functional gene. As shown in Fig. 4, A–F, control mice treated with omeprazole had no changes in expression in five of the six measured genes. The exception was Muclin whose expression was significantly downregulated by omeprazole treatment of control mice (Fig. 4E). The omeprazole-treated double-null mice also had the same levels of expression for five of the six genes studied compared with omeprazole-treated CF mice. Again, the exception was Muclin whose expression was significantly downregulated in omeprazole-treated double-null mice compared with omeprazole-treated CF mice. Nothing is known of transcriptional control of Muclin, so the effect of omeprazole is not currently understood. The majority of the genes investigated in omeprazole-treated mice are not responding to hypergastrinemia, and the effects are most likely due to decreased acid delivery to the intestines.

Measurement of pancreatitis parameters in control and CF mice. Several genes associated with pancreatitis were upregulated in the CF pancreas. This suggested that the tissue may be damaged in a way similar to pancreatitis. One of the indicators of pancreatitis is elevated digestive enzymes in the circulation (48). Therefore, serum amylase levels were measured in control and CF mice. Control mice had 11.9 ± 0.033 U amylase per milliliter of serum (mean ± SD, n = 8), and CF mice had 12.1 ± 0.062 U of amylase per milliliter of serum (n = 8), which was not a significant difference.

Another indicator of pancreatic inflammation associated with pancreatitis is an influx of neutrophils (32). The Lrg gene, which is strongly upregulated in the CF pancreas, has been suggested to be a marker of neutrophils (51). Therefore, pancreatic tissues were stained for neutrophils. Three to four samples were analyzed per group, and at least 14 fields were examined per sample. Neutrophils were rare in the control tissue and were observed only in blood vessels. In areas containing blood vessels, the number of neutrophils ranged from zero to three per field using a ×40 objective (1.6 ± 0.5 means ± SE; ~450 nucleated cells/field). A representative example of control pancreas is shown in Fig. 5A, where there is one neutrophil in a blood vessel. There were significantly more neutrophils in the CF pancreas compared with controls, and the numbers ranged from 3 to 24 per field containing blood vessels (7.3 ± 1.5; P < 0.05 vs. control). A representative example of CF tissue is shown in Fig. 5B, where the neutrophils are in small blood vessels. When CF mice were treated with omeprazole for 3 wk to normalize duodenal pH, the number of neutrophils decreased to control levels (1.6 ± 0.2), a representative example of which is shown in Fig. 5C.

Morphology of acinar lumen in CF pancreas without and with omeprazole treatment. It has previously been shown that the acinar lumen is frequently dilated in the CF mouse pancreas, indicating an imbalance between zymogen granule exocytosis and compensatory endocytosis (16, 25). To visualize the acinar lumen and whether correction of duodenal pH would affect the morphology of the pancreatic tissue, fluorescent phalloidin was used to stain the F-actin that underlies the apical plasma membrane of the acinar cell. As shown in a representative image in Fig. 6B, the acinar lumen was wider in the CF pancreas compared with control (Fig. 6A). In addition, the actin cytoskeleton underlying the apical membrane had an irregular appearance with protrusions along its length (Fig. 6B) compared with the narrower and straighter appearance in the control pancreas (Fig. 6A). The increased lumen diameter in CF was verified quantitatively and was significantly greater by ~25% on average compared with control (Fig. 6D). When CF mice were treated with omeprazole, the acinar lumen was straighter (Fig. 6C) and was significantly less wide than the untreated CF samples (Fig. 6D).
DISCUSSION

This study demonstrates that the CF mouse pancreas exhibits upregulation of genes associated with inflammation and pancreatic stress. Our previous work (17) provided evidence that the abnormally acidic duodenal pH in CF results in increased signaling from the small intestine to the pancreas to stimulate bicarbonate-rich fluid secretion. We hypothesized that excess stimulation would in turn induce expression of pancreatic stress genes. This was confirmed by analysis of gene expression in the CF mouse pancreas.

Pancreatic stress genes were upregulated in the CF pancreas and several of these genes are associated with pancreatitis. Pancreatitis is a condition that involves activation of zymogens within acinar cells and can lead to life-threatening systemic inflammation (4). Whereas some of the genes upregulated in the CF pancreas are associated with pancreatitis, the overall gene expression profile does not match that of experimental acute pancreatitis in rats. A recent and very thorough study of gene expression in experimental pancreatitis compared controls with two disease models (caerulein hyperstimulation and infusion of the pancreatic duct with taurocholate) and, importantly, included animals treated with high concentrations of the partial CCK agonist JMV-180 that does not induce pancreatitis (36). Among the genes upregulated in pancreatitis, several were also upregulated in the JMV-180-treated rats that did not develop pancreatitis. Notably, Reg III and PAP I were upregulated 30- to 40-fold 1 h after administration of JMV-180 (36). Thus a subset of these genes can be upregulated by a strong secretory stimulus that may be stressful but does not cause disease.

The CF mouse also lacked other indicators of pancreatitis. There was an increase in neutrophils in the CF pancreas, but this was modest compared with pancreatitis in which inflammatory cells can accumulate to be several percent of the total tissue cell number (32). Neutrophils in the CF tissue were usually within blood vessels or occasionally in the adjacent...
connective tissue. This suggests that there may be an increase in circulating neutrophils (neutrophilia) in the CF animals rather than actual infiltration into the pancreatic parenchyma. The observation that neutrophils were decreased by omeprazole treatment, which reduces inflammatory gene expression in the pancreas, suggests a role for pancreatic inflammation in this neutrophilia. This issue will require further work to clarify.

Pancreatic edema and elevated serum amylase are characteristic of pancreatitis and reflect the damage that occurs to the exocrine tissue (48). Neither of these was found in the CF mouse. The CF pancreas does not exhibit the dramatic changes associated with pancreatitis, but it does have some aspects of this condition, and it may be poised to develop pancreatitis on further insult. It has recently been shown that cAMP may have a role in pancreatitis, in that cAMP elevation sensitizes pancreatic acinar cells to zymogen activation by physiological concentrations of caerulein (42). The CF mouse pancreas has elevated cAMP levels (17) and it will be of interest to determine whether it is more susceptible to experimentally induced pancreatitis than normal controls.

Although the CF pancreas has signs of inflammation, there are also indications of anti-inflammatory responses. These include the upregulation of the IL13Ra-subunit, and the serine protease inhibitors Serpina1a, Serpina10, and Itih4. IL13 is an anti-inflammatory cytokine (18, 67), and upregulation of the IL13Ra-subunit is suggestive of an active downmodulation of inflammation in the CF pancreas. SerpinA1a inhibits neutrophil elastase (28), which can cause tissue damage and induce cytokine expression (46). SerpinA10 inhibits coagulation factor Xa (28), which is proinflammatory by signaling through the protease-activated receptors (53). Itih4 is an acute-phase protein that, like other serine protease inhibitors, is expected to downmodulate inflammation, although its physiological target protease and function are not currently known (52). These data indicate that the tissue is responding to the mild inflammation and with the stress imposed on it.

To test the hypothesis that the underlying cause of changes in the CF mouse pancreas is the acidic duodenum, two different approaches were used to correct duodenal pH. The expression of pancreatic stress/inflammatory genes was significantly normalized in both groups with corrected pH. Normalization of duodenal pH by omeprazole treatment also significantly reduced the degree of acinar lumen dilation and the number of neutrophils in the CF pancreas.

The group of genes most strongly upregulated in the CF pancreas are members of the Reg/PAP family. Although the functions of the Reg/PAP proteins are not completely understood, they are upregulated under stressful conditions and in several instances, foster cell survival. In the normal pancreas, the Reg genes are expressed at low levels in acinar cells and their expression can be dramatically upregulated when the pancreas is damaged as in pancreatitis (30). These peptides have also been found to be elevated in the serum of CF patients, indicating an upregulation in human CF disease (8, 33), but the tissue source was not identified.

The Reg genes were named regenerating islet-derived genes because of their growth effects on pancreatic beta cells (63). Moreover, they have been shown to have growth/survival effects on other cell types. For example, Regs were shown to be an autocrine signal in motorneurons during development (50) and a neurotrophic factor induced after nerve injury (2).
both cases, the Regs enhanced nerve cell survival. In regenerating small intestine after experimental partial resection, expression of these genes is upregulated (21). And, in inflammatory bowel disease, several Reg genes are upregulated and RegI was shown to be antiapoptotic (19). The theme emerging is that these peptides are involved in cell survival under various conditions. In the pancreas, these proteins may be protective under stressful conditions, such as pancreatitis or in the CF pancreas that is overstimulated by signaling from the small intestine or experimentally by JMV-180 (36).

It was noted that in the CFTR/gastrin double-null mice, expression of three of the six genes examined was still significantly elevated over control levels. And in the omeprazole-treated CF mice, two of the six genes were still significantly elevated over control levels. There are several possibilities for the incomplete normalization of gene expression levels. In the case of the CFTR/gastrin double-null mice it could be due to incomplete correction of duodenal pH. For the omeprazole-treated mice, it could be that the course of treatment was too short to totally reverse the pancreatic inflammation, which had time from birth of the animals to develop. A possibility common to both groups is that there are other effects of CF on the mouse pancreas in addition to the acidic duodenal pH. An obvious possibility is that the residual inflammation is due to the apparent malnourished state of these mice (17), which was not ameliorated in either experimental group (not shown). Pancreatic abnormalities in CF mice have been reported by others and it was considered that some of the effects could be due to the effects of malnutrition in these mice (34). Results of the present study suggest that at least part of the destructive stress on the pancreas in CF is due to excess signaling from the duodenum in an unproductive attempt to increase pancreatic bicarbonate ion secretion. We showed that correction of duodenal pH in the CF mouse significantly reduces expression of these stress-related genes as well as reversing structural changes to the pancreas. Proton pump inhibitors are safe and effective for improving the nutritional status of pancreatic-insufficient CF patients (20). The rationale for proton pump inhibitor therapy is that neutralization of duodenal pH protects acid-sensitive lipase in the oral enzyme supplements these patients use and improves lipid assimilation (20). The data presented here suggest that early use of proton pump inhibitor therapy may be warranted in CF patients who are still pancreatic sufficient but are at significant risk for becoming pancreatic insufficient. Proton pump inhibitor therapy may also be beneficial to CFTR compound heterozygous individuals who are at increased risk for developing pancreatitis. Although it has not been reported whether these individuals have altered duodenal pH, it may be that they have a chronic low level of duodenal acidity. If so, this would contribute to stressing the pancreas and may have a role in their increased risk for developing pancreatitis. Our findings in the CF mouse suggest that CFTR compound heterozygotes may also benefit from treatment with proton pump inhibitor therapy.

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