Developmental expression of a mucinlike glycoprotein (MUCLIN) in pancreas and small intestine of CF mice

ROBERT C. DE LISLE, MATTHEW PETITT, KATHRYN S. ISOM, AND DONNA ZIEMER
Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas 66160

Developmental expression of a mucinlike glycoprotein (MUCLIN) in pancreas and small intestine of CF mice. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G219–G227, 1998.—The mucinlike glycoprotein MUCLIN, one of two protein products of the CRP-ductin gene, was used to study changes in the expression of sulfated glycoconjugates during the pathogenesis of cystic fibrosis, using the cystic fibrosis transmembrane conductance regulator (CFTR) knockout mouse (CF mouse). We assessed the appearance of dilated lumina containing protein or mucus plugs in pancreatic acini and crypts of the small intestine and quantified MUCLIN protein and CRP-ductin mRNA during postnatal development. In CF mice, the pancreatic acinar lumen was dilated by postnatal day 16 (P16), but MUCLIN protein was first significantly increased by P23 and remained elevated through adulthood compared with normal mice. Similarly, intestinal crypts had CF-like mucus plugs by P16, but MUCLIN protein was first elevated by P23 and remained elevated through adulthood compared with normal mice. In both organs, MUCLIN labeling of the luminal surface was increased concomitantly with dilation and protein or mucus plugging but before upregulation of expression. The morphological changes were then followed by upregulation of MUCLIN protein and CRP-ductin mRNA expression. This is the first direct study of CF pathogenesis and the resultant increase in glycoconjugate gene expression. The data are consistent with CF pathogenesis progressing from an initial alteration in protein secretory dynamics (increased luminal MUCLIN and protein/mucus plugs) to an upregulation of glycoconjugate gene expression, which is expected to exacerbate obstruction of the luminal spaces.

CRP-ductin; cystic fibrosis

Cystic fibrosis (CF) is caused by mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated Cl− channel (19). Although it is clear that mutations in the CFTR gene that affect Cl− channel function of the CFTR protein cause CF, the pathogenesis of this disease is unclear. A hallmark of CF is the alteration in the amount and composition of mucus in exocrine secretions. The increase and altered composition of these glycoconjugates are believed to contribute to obstruction of the lumina, leading to tissue damage (22). How the loss of functional CFTR results in the increase in glycoconjugate secretion and altered carbohydrate composition (17–18), overexpression of MUCLIN in these circumstances may be inappropriate and may contribute to the progression of CF by exacerbating obstruction of these luminal spaces.

MATERIALS AND METHODS

Animals. CFTR(+/−) mice, developed at the University of North Carolina (cftrm1Unc) (24), were obtained from Jackson Laboratories (Bar Harbor, ME) and maintained in our animal care facility. All experiments were performed in accordance with National Institutes of Health guidelines and were approved by our Institutional Animal Care and Use Committee. Heterozygotes were bred to obtain CFTR(+/+) and CFTR(−/−) mice, which are referred to as normal and CF, respectively. The morning of birth was considered postnatal day 1 (P1). Both CF and normal mice were maintained from P10 on Peptamen (Clintek, Deerfield, IL) to prevent intestinal obstruction, which otherwise results in the death of the majority of the CFTR knockout animals (8, 9). Genotypes of the mice were determined by PCR analysis of DNA prepared from tail snips of postweaning animals or from a piece of liver from younger animals (9).

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Tissue homogenization and immuno-dot-blot analysis of MUCLIN. The pancreas and the proximal half of the small intestine (referred to as small intestine) were homogenized in 10 mM Tris, pH 7.4, with added protease inhibitors (phenylmethylsulfonyl fluoride, leupeptin, pepstatin A, and benzamidine). Because the small intestine of young animals is fragile, we homogenized the whole organ from all time points rather than obtaining mucosal scrapings as was done previously (9). This fact accounts for the differences in intestinal MUCLIN levels for adult tissue reported here compared with our previous work. Protein in the homogenates was determined by the Bradford method (5), using reagents from Bio-Rad (Hercules, CA) and BSA as standards. MUCLIN levels were quantified using an immuno-dot-blot assay with a monoclonal antibody (Hercules, CA) and BSA as standards. MUCLIN levels were determined with an Ambis radioanalytic detector (Ambis, Indianapolis, IN). Tissue homogenates were separated by SDS-PAGE, transferred to polyvinylidine difluoride membranes, and probed for the presence of glycan. Briefly, the blots were treated with sodium metaperiodate to oxidize carbohydrates, derivatized with digoxigenin hydrazide, and probed with an antidigoxigenin alkaline phosphatase-conjugated antibody. Color was developed with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. For Western immunoblots, proteins were separated by SDS-PAGE, transferred to nitrocellulose, and detected with the anti-MUCLIN antibody and a goat anti-rabbit alkaline phosphatase second- ary antibody.

RNA extraction and Northern blot analysis of CRP-ductin and CFTR mRNA. Total RNA was prepared, and Northern blots were performed as previously described (9). Briefly, blots were probed sequentially with 32P-labeled single-stranded DNA probes to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and CRP-ductin (the mRNA that encodes MUCLIN and a second smaller protein). Radioactivity on the blots was determined with an Ambis radioanalytic detector (Ambis, San Diego, CA) to quantify 32P. Representative blots were exposed to X-ray film at −70°C with an intensifying screen. Northern blots were also performed with a CFTR single-stranded DNA probe generated from a mouse CFTR cDNA (obtained from the American Type Culture Collection, Rockville, MD; ATCC no. 63165; see ref. 26). Probes were generated by PCR (2), using purified plasmid DNA as a template as previously described (9).

Histology and immunocytochemistry. Tissue was fixed by immersion in 10% neutral buffered Formalin. For standard histology, tissue was processed for paraffin sections, which were stained with hematoxylin and eosin or with periodic acid-Schiff (PAS) for neutral mucus. Immunocytochemistry was performed on 2.5-μm frozen sections prepared at −40°C on an RMC CR-21 cryostat (RMC, Tucson, AZ). Frozen sections were sequentially incubated with anti-MUCLIN antisera (1:500 in 2% normal goat serum) and goat anti-rabbit-FITC (Jackson ImmunoResearch, West Grove, PA). The labeled slides were mounted with SlowFade (Molecular Probes, Eugene, OR) and observed on a Nikon Diaphot with an FITC filter cube.

Statistical analysis. Data were analyzed by one-way ANOVA with a post hoc pairwise Tukey comparison or by paired t-test, using SYSTAT 7.0 software (Evanston, IL).

RESULTS

We have previously reported that in adult CF mice there is a dilatation of some pancreatic acinar lumina and the lumen exhibits protein aggregates (8, 9). Even more striking, most or all intestinal crypts of CF mice are dilated and filled with mucus plugs (9, 12, 23). To determine the time course of CF pathogenesis in the knockout mice, we examined the morphology of these organs in normal and CF mice from late fetal development (embryonic day 18.5) through postnatal development and into adulthood (8–12 wk). Dilations of acinar lumina in CF mice first became apparent at P8 but were more common by P16 (Fig. 1, A’ and B′, respectively). These dilations persisted in the CF pancreas through P40 (Fig. 1D′) and were also observed in the adult, as previously reported (8, 9). It should be noted that dilations of acinar lumina were variable and seemed to occur locally in individual lobules, with some lobules having no evidence of luminal dilations, as previously reported (9).

Our previous work showed that MUCLIN labeling was strongly enhanced on the acinar luminal surface of CF mice (8, 9). To assess when this redistribution of MUCLIN from its predominantly zymogen granule localization in the normal adult mouse (7) to the luminal membrane of CF pancreatic acini occurs, we performed immunostaining for MUCLIN. The tissue was prepared in a manner that does not permeabilize the zymogen granules and reveals primarily luminal membrane MUCLIN. At all postnatal times, the luminal membrane of CF pancreatic acini was more highly immunoreactive for MUCLIN than that of normal pancreatic acini, and the labeling highlighted the distended lumina and protein aggregates in the lumen (Fig. 1, A-E), as previously shown for adult CF mouse pancreas (8, 9).

We also examined the appearance of normal pancreatic tissue at the same developmental times. Unexpectedly, the luminal membrane of normal mice showed an equal degree of dilation at P16 and P23 (Fig. 2, B and C, respectively) compared with the CF mice (Fig. 1, B′ and C′, respectively). By P32 in normal mice, the lumina looked like those seen in normal adult mice, that is, the lumina were of small diameter (Fig. 2D′); this was also true at P40 (Fig. 2E′). As shown in Fig. 2, A-E, throughout normal postnatal pancreatic development MUCLIN is localized to the luminal membrane and the occasionally accessible granule membrane. During the times when the luminal membranes are dilated in the normal mouse pancreas, MUCLIN is more patchy along the dilated luminal surfaces, and there is not a dramatic increase in the level of luminal labeling (Fig. 2, B and C). This is in contrast to the strong labeling observed in the CF mouse pancreas at the same ages along the dilated luminal surfaces (Fig. 1, B and C).

Since the normal and CF mice used were ~95% C57BL/6 and were maintained on an unusual diet (Peptamen), we determined whether the observation of dilatation of the acinar lumen at P16–P23 was related to strain or diet. We evaluated tissue from normal Swiss Webster and 129/SVJ mice maintained on either standard mouse chow or on a fiber-free pellet diet (custom formulation in which the fiber was replaced by starch;
ICN, Costa Mesa, CA). In all cases, observations of dilated acinar lumina at P16–P23 were as common in these other strains and on different diets as in the normal C57BL/6J mice on Peptamen (data not shown). Thus transient dilation of the acinar lumen appears to be a normal postnatal developmental event in mice.

We next measured MUCLIN and CRP-ductin mRNA levels in pancreata from these same developmental time points. Pancreatic MUCLIN levels were identical when normal and CF mice were compared at P8–P16 (Fig. 3). By P23, there was a small but significant increase in MUCLIN in the CF pancreas compared with normal. MUCLIN in the CF mice continued to increase to P40, and the level was still significantly elevated at adulthood (Fig. 3). In normal mice at P32–P40, MUCLIN levels were slightly and significantly elevated compared with normal at P23 and adult mice, but these values were significantly less than MUCLIN levels in CF mice at the same ages (Fig. 3). Likewise, CRP-ductin mRNA in the CF pancreas was slightly elevated at P23, but the increase was only significant at P32, as shown in the representative Northern blot in Fig. 4, and remained significantly elevated through adulthood (Fig. 5). Thus upregulation of MUCLIN and its mRNA (CRP-ductin) occurs after dilation of the acinar lumen.

We next examined crypts of the small intestine during development to determine if the temporal relationship between CF pathology and MUCLIN expression was the same as in the pancreas. In mice, the intestinal crypt develops as a morphologically identifiable entity after birth (13). In our studies, although villi were well developed early on, very few fully formed crypts were observed at P8 in either normal or CF mice (Fig. 6, A and F). By P16 shallow crypts were evident, and they increased in depth with age (Fig. 6, B–E). In normal mice, the crypt lumina were clear and, although the luminal membrane was PAS reactive, the lumen itself was clear of PAS-reactive material (Fig. 6, B–E). In contrast, by P16 the crypt lumina in CF mice were PAS reactive (Fig. 6G). The CF crypts also increased in size, and the degree of mucus accumulation increased with age (Fig. 6, H–J).

As determined by immunocytochemistry, there was little or no MUCLIN labeling of intestine in either normal or CF mice before P16 (data not shown). From P16 through adulthood in the CF intestine, there was strong labeling for MUCLIN along the distended luminal membrane (Fig. 7, A–C), similar to that shown...
previously for adult CF mouse intestine (9). MUCLIN labeling of normal small intestine was consistently low and associated with the luminal surface of crypts and the Golgi area of the crypt enterocytes (data not shown), as in the normal adult intestine (9).

Expression of MUCLIN and CRP-ductin mRNA were quantified at the same developmental times in the intestines of normal and CF mice. MUCLIN levels were identical in normal and CF intestines between P8 and P16 (Fig. 8). Thereafter, MUCLIN remained at a low constant level in normal mice. In the CF intestine, MUCLIN was significantly increased at P23 compared with normal and increased further through P40 (Fig. 8). The adult CF intestine level of MUCLIN was the same as at P40.

CRP-ductin mRNA levels were slightly and significantly increased at P16 comparing CF with normal (Fig. 9). Thereafter, CRP-ductin mRNA levels in the CF intestine remained significantly elevated through adulthood compared with normal.

Thus, as in the pancreas, the appearance of CF pathology in the small intestine preceded increases in MUCLIN and CRP-ductin mRNA. Unlike the pancreas, there was no transient dilation of crypt lumina or increased MUCLIN expression in the normal mouse.

To determine whether glycoconjugates other than MUCLIN were affected in the CF tissues, we used a total glycan detection method on Western blotted protein. In the pancreas, the most obviously changed glycoconjugate was MUCLIN (Fig. 10A). There were some more subtle changes in a broad band of ~155-165 kDa, which was more abundant in P23 CF tissue, but these changes were not observed at all ages. In addition, a band of ~95 kDa appeared to be stronger in CF tissue at P16 through adulthood, although the differ-

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**Fig. 2.** Pancreatic morphology and MUCLIN immunocytochemistry in normal mice during postnatal development. Cryosections were immunostained for MUCLIN at P8 (A), P16 (B), P23 (C), P32 (D), and P40 (E). A’-E’, corresponding phase-contrast micrographs. MUCLIN is expressed at moderate levels along apical plasma membrane and on occasional zymogen granules. Note dilation of the acinar lumen at P16 and P23 (B’ and C’) but no increase in MUCLIN immunoreactivity (B and C). Bar = 20 µm.

**Fig. 3.** MUCLIN protein expression in normal and CF pancreas during postnatal development. MUCLIN protein was quantified by immuno-dot-blot analysis as described in MATERIALS AND METHODS: n = 5 P8, 8 P16, 5 P23, 7 P32, 5 P40, and 8 adult normal mice, and n = 3 P8, 7 P16, 6 P23, 7 P40, and 8 adult CF mice. Data are means ± SE. *P < 0.01 comparing CF with normal mice of same age. #P < 0.05 comparing normal mice with normal P16 and adult mice.
ences between normal and CF mice were less than for MUCLIN. In the intestine, again the most apparent change was in the levels of MUCLIN (Fig. 10B). That the bands near the top of the blot exhibiting the largest changes were MUCLIN was verified by immunoblot analysis (Fig. 10C). As previously reported, the migration of MUCLIN in the CF intestine was slower than in normal intestine, and this apparent increase in the mass of MUCLIN was most apparent at P32–P40. More clearly than in the pancreas, there was an increase in a broad glycoconjugate band of ~155–165 kDa in the CF intestine (Fig. 10B), which, similar to MUCLIN, was strongest at P40. This band was not MUCLIN immunoreactive, so it is likely a distinct glycoprotein. It has been shown that there is an increase in the number of goblet cells in the intestine of CF mice (12, 24), and one of the mucins expressed by these cells is MUC2 (15). The gels we ran were 5% acrylamide, and they resolved proteins up to ~400 kDa. MUC2 is 550–600 kDa and therefore would not be expected to enter the gels we used. We attempted to visualize glycoconjugates with a higher mass using 3% acrylamide gels, but no distinct bands were observed after the blots were probed for glycans (data not shown).

Because the CF pathologies examined in this study are due to loss of the CFTR gene by gene targeting, we wanted to determine the normal developmental time course of CFTR expression in the mouse. In the mouse pancreas, CFTR mRNA levels are very low and require use of RT-PCR to obtain measurable signals (9, 18). In contrast, CFTR mRNA is relatively abundant in the intestine (9, 12, 25) and is therefore readily measured. We quantified CFTR mRNA in normal mouse intestinal development by Northern blot as described in MATERIALS AND METHODS. As shown in the representative samples in Fig. 11 and quantitatively in Fig. 12, CFTR mRNA was not detected by Northern blot in the mouse small intestine at P8. By P16, CFTR mRNA is measurable, and the levels increase strongly by P23 and remain fairly constant thereafter through adulthood (Figs. 11 and 12). Thus a CF-like pathology in the mouse small intestine quickly appears at the time when CFTR would normally be expressed in postnatal development. This indicates that the events we studied are temporally closely related to the time at which CFTR should first be expressed in the mouse intestine.

DISCUSSION
CF is well described as a clinically manifested disease, but the early events in the pathogenesis of CF are largely unknown, principally because the disease in humans begins in utero (17, 22). To begin exploring the earliest events in the pathogenesis of CF, we used the CFTR knockout mouse and examined expression of the mucinlike glycoprotein MUCLIN in the pancreas and intestine during postnatal development. MUCLIN is a sulfated mucinlike glycoprotein, which is abundant in the normal pancreas and intestine (7–9). With the exception of the pancreatic
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In acinar cell, these other cells are not known to have major storage pools of regulated secretory granules. Therefore, it is likely that MUCLIN has an additional function when localized to these luminal membranes. Because of its mucin-like biochemical characteristics (7, 9), it is probable that MUCLIN serves as a protective molecule on the mucosal surfaces of digestive organs. The majority of the peptide motifs in the protein is either predicted to be heavily O-glycosylated or has numerous intrachain disulfide bonds, both of which confer protease resistance (3, 16).

We have documented for the first time that CF-like pathologies first appear in the pancreas and small intestine during postnatal development in the mouse (P16–P23), whereas in humans CF develops in utero (17, 22). The earliest observed changes were morphological: dilation of the luminal plasma membranes and increased labeling for MUCLIN and neutral mucus (PAS reactivity) in the luminal spaces. Subsequent to the morphological changes there were increases in MUCLIN protein and its mRNA (CRP-ductin). The morphological criteria we used cannot reveal any changes in gene expression during the pathogenesis of CF in this mouse model. Although we were not able to measure an increase in MUCLIN expression at early ages, it is also not known whether mucin gene expression was increased at the earliest appearance of CF pathologies. Using glycan detection on Western blots, we attempted to see if mucins were increased, but we

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*Fig. 7. Intestinal morphology and MUCLIN immunocytochemistry in CF mice during postnatal development.* Cryosections were immunostained for MUCLIN at P16 (A), P23 (B), and P32 (C). A’–C’ are cross-sections through the crypts. At all ages past P8 (not shown), MUCLIN is strongly expressed along the crypt apical plasma membrane, and there are mucus plugs in the crypt lumina. Bar = 50 µm.

*Fig. 6. Intestinal morphology during postnatal development and in adult normal and CF mice. Paraffin sections were processed for routine periodic acid Schiff (PAS) staining. A–E: normal mice at P8 (A), P16 (B), P23 (C), P32 (D), and adult (E). F–J: CF mice at P8 (F), P16 (G), P23 (H), P32 (I), and adult (J). Note that although villi are present at P8 (A and F), typical crypts are not apparent until P16. Normal mice have crypts that are small and clear throughout development (arrows in B–E). CF mice from P16 to adult exhibit PAS-reactive mucus plugs (arrows in G–J). Bar = 50 µm.*
were unable to resolve very high molecular weight glycoconjugates (>400 kDa) on polyacrylamide gels (data not shown). This emphasizes the utility of MUCLIN in these studies, as it is readily resolved on gels and its mRNA, unlike those of known mucin genes, is also reasonably easily quantified.

It may be that at these early times both mucins and MUCLIN simply accumulate on the luminal plasma membrane. It has been proposed that an alteration in the balance of exocytosis and endocytosis occurs in CF (4, 21). In CF, endocytosis may not remove luminal plasma membrane to adequately match the insertion of membrane by exocytosis. This imbalance would result in dilation of the lumina. It was proposed that endocytosis is inhibited by abnormal acidity in the luminal spaces due to loss of CFTR function (21). Alternatively, the luminal membrane may become dilated due to the accumulation of aggregated protein/mucus in the lumen which would physically expand this space.

In the CF mouse we observed higher luminal labeling for MUCLIN in both pancreas and intestine than in

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**Fig. 8.** MUCLIN expression in normal and CF small intestine during postnatal development. MUCLIN was quantified by immuno-dot-blot analysis as described in MATERIALS AND METHODS; n = 3 P8, 3 P16, 3 P23, 4 P32, 5 P40, and 6 adult normal mice, and n = 3 P8, 3 P16, 3 P23, 4 P32, 7 P40, and 6 adult CF mice. Data are means ± SE. *P < 0.01 comparing CF with normal mice of same age.

**Fig. 9.** Quantitation of CRP-ductin mRNA expression in normal and CF small intestine during postnatal development. Ambis scanning of Northern blots of total RNA probed with a single-stranded 32P-labeled DNA antisense to CRP-ductin and normalized to GAPDH; n = 3 P16, 4 P23, 8 P32, 6 P40, and 5 adult normal mice, and n = 4 P16, 5 P23, 4 P32, 3 P40, and 4 adult CF mice. Data are means ± SE. *P < 0.05; **P < 0.01 comparing CF with normal mice of same age.

**Fig. 10.** Total glycan detection in normal and CF pancreas and small intestine during postnatal development. Samples were separated on 5% acrylamide reducing gels, blotted, and probed for total glycans or MUCLIN. A: pancreas samples probed for total glycans (lanes 1-12: 10 µg of indicated age and genotype per lane) and for MUCLIN (MUC; lane 13: 1 µg of normal adult pancreas). B: small intestinal samples probed for total glycans (10 µg of indicated age and genotype per lane). C: small intestinal samples probed for MUCLIN (10 µg indicated age and genotype per lane). Molecular weight standards are as indicated. Ad, adult.
normal mice even before there was a measurable increase in total MUCLIN protein. At the same time, there was noticeable mucus plug accumulation in the intestine. In the CF pancreas, MUCLIN labeling appeared more intense than in normal pancreas as early as P8 (Figs. 1A and 2A, respectively). In the intestine MUCLIN labeling was weak or absent at P8 (data not shown) but was strongly elevated along the luminal surface by P16 (Fig. 7A) at the same time that mucus plugs became apparent (Fig. 6G). Thus our data using MUCLIN labeling as a marker of the luminal membrane are consistent with the idea that there is an imbalance in exocytosis and endocytosis in CF. An alternative explanation is that exocytosed MUCLIN is cleared more slowly in the CF organs at these early developmental times. This is less likely because there was no increase in total MUCLIN protein, which would occur if MUCLIN were accumulating rather than being redistributed from an intracellular site to the luminal plasma membrane. In support of this hypothesis, we previously noted that there are fewer zymogen granules in the CF pancreas (8), and this is also apparent by comparison of Figs. 1 and 2. These observations are consistent with a decrease in recycling of zymogen granule membrane in the CF pancreas.

Our analysis of CF-like morphological changes in the pancreas was somewhat less than straightforward in that we discovered that acinar lumina dilations are a normal but transient event in postnatal pancreatic development in the mouse. The cause of this dilation is unknown, but one possibility is that pancreatic ductal function may lag behind that of the acinar tissue and the luminal space may be overwhelmed by protein secretion as the mice go through weaning, a time known to involve significant developmental maturation in mouse gastrointestinal organs (11). If ductal function lags behind acinar function, this might be a time when events occur during normal development that are similar to those that happen in CF, namely, insufficient ductal bicarbonate and fluid secretion and perturbation of the environment at the acinar luminal space. However, the normal pancreas was distinct from the CF in that the dilated lumina of the normal mice did not exhibit increased labeling for MUCLIN. Despite this complication, it is clear that in the CF pancreas the dilation persists and is followed by upregulation of CRP-ductin mRNA and MUCLIN protein expression.

On the basis of these new data on MUCLIN expression and CF, our working hypothesis is that the initial event in CF is altered fluid and electrolyte secretion by the affected epithelia, which probably results in a more acidic luminal environment. Initially, this acidic environment perturbs the dynamics of protein secretion, resulting in protein redistribution from an intracellular site into the luminal space and dilation of the luminal plasma membrane. Then, in some way, this perturbation of the luminal space signals to the epithelial cells to increase their expression of protective molecules such as MUCLIN. The increased expression of such glycoconjugates is probably inappropriate under these circumstances and is believed to contribute to the progression of the CF pathogenesis (22). Eventually, the luminal space becomes occluded, resulting in acinar damage in the pancreas (17, 22) and meconium ileus in the intestine (22).

Future goals are to understand how loss of CFTR alters the cellular distribution of glycoprotein and
mucin gene products from intracellular sites to the luminal plasma membrane and how expression of these genes is subsequently upregulated in the affected epithelial cells. In humans, CF pathogenesis in the gastrointestinal system begins in utero, and CF patients often have severe pancreatic destruction (17, 22) and meconium ileus at birth (22), whereas in the knockout mouse intestinal problems become severe only at weaning. This may be explained by the fact that maturation of the gastrointestinal system is more complete in utero in humans than in rodents (13). The mildness of pancreatic CF in the mouse is likely related to the expression of additional genes in the mouse pancreas, which allows it to be relatively unaffected when CFTR is lost. It has been demonstrated that mouse pancreatic ducts express a Ca\textsuperscript{2+}-regulated Cl\textsuperscript{−} conductance whose activity can compensate for loss of CFTR (1). Also, there is evidence that there are other, as yet unidentified, interacting genes in the mouse that can profoundly modulate the severity of CF in the knockout mice (12, 14, 20). The fact that CF pathogenesis in the mouse occurs postnatally is an experimental advantage, as it allows investigation of the earliest events in CF pathogenesis. Studies of MUCLIN in the CF mouse should continue to be informative about the pathogenesis of CF.

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Address reprint requests to R. C. De Lisle.

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