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Am J Physiol Gastrointest Liver Physiol 301:G528-G536, 2011. First published 9 June 2011; doi:10.1152/ajpgi.00052.2011

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Cystic fibrosis growth retardation is not correlated with loss of Cftr in the intestinal epithelium

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Submitted 9 February 2011; accepted in final form 6 June 2011

Hodges CA, Grady BR, Mishra K, Cotton CU, Drumm ML. Cystic fibrosis growth retardation is not correlated with loss of Cftr in the intestinal epithelium. *Am J Physiol Gastrointest Liver Physiol* 301: G528–G536, 2011. First published June 9, 2011; doi:10.1152/ajpgi.00052.2011.—Maldigestion due to exocrine pancreatic insufficiency leads to intestinal malabsorption and consequent malnutrition, a mechanism proposed to cause growth retardation associated with cystic fibrosis (CF). However, although enzyme replacement therapy combined with increased caloric intake improves weight gain, the effect on stature is not significant, suggesting that growth retardation has a more complex etiology. Mouse models of CF support this, since these animals do not experience exocrine pancreatic insufficiency yet are growth impaired. Cftr absence from the intestinal epithelium has been suggested as a primary source of growth retardation in CF mice, a concept we directly tested by generating mouse models with Cftr selectively inactivated or restored in intestinal epithelium. The relationship between growth and functional characteristics of the intestines, including transepithelial electrophysiology, incidence of intestinal obstruction, and histopathology, were assessed. Absence of Cftr exclusively from intestinal epithelium resulted in loss of cAMP-stimulated short-circuit current, goblet cell hyperplasia, and occurrence of intestinal obstructions but only slight and transient impaired growth. In contrast, specifically restoring Cftr to the intestinal epithelium resulted in restoration of ion transport and completely protected against obstruction and histopathological anomalies, but growth was indistinguishable from CF mice. These results indicate that absence of Cftr in the intestinal epithelium is an important contributor to the intestinal obstruction phenotype in CF but does not correlate with the observed growth reduction in CF.

CF mouse models; weight; length; intestinal obstruction

CYSTIC FIBROSIS (CF) patients are characterized by reduced morphometric indexes; both height and weight for age, as well as percent ideal body weight, are lower for CF patients than the non-CF population (11, 25, 27). Specifically, between one-quarter to one-third of all CF patients are below the tenth percentile in weight and height (18). Body size of CF patients is a positive predictor of pulmonary function and overall health, suggesting that understanding the origin of reduced growth in CF patients has significant clinical importance (10, 11, 26, 27). Since pulmonary function is a robust indicator of predicting mortality in CF patients, understanding the etiology of poor growth in CF is important for therapeutic approaches aimed at correcting it.

Pancreatic obstruction in utero or shortly after birth of most CF patients leads to atrophy and destruction of the pancreatic acinar cells (32, 36). This results in exocrine pancreatic insufficiency (PI), such that pancreatic digestive enzymes chymotrypsin, trypsin, carboxypeptidase A, amylase, lipase, elastase, and trypsin are undetectable in pancreatic secretions (6). As a consequence, maldigestion and subsequent malabsorption occur in the absence of enzyme replacement therapy (43). This CF-related malabsorption has been presumed to account for the characteristic endocrine disruption of the disease (29, 49), akin to malnutrition. It is well established that good nutritional status and long-term survival are correlated, so optimization of nutrient uptake and absorption in CF patients has been a focus of care. To help maintain high body mass index and prevent malnutrition, current clinical treatment of CF includes early diagnosis and aggressive nutritional intervention (16). However, a review of clinical studies examining dietary augmentation and pancreatic enzyme replacement therapy indicates that increasing caloric intake results in weight gain but does not significantly affect stature (50). These observations are inconsistent with malnutrition as an explanation of linear growth retardation associated with CF and suggest that a mechanistic understanding of the reduced body size of CF patients is still not clear.

The CF mouse provides a model to identify origins of growth reduction in CF. Similar to CF patients, the CF mouse experiences growth retardation, typically displaying a 20–50% reduction in weight compared with normal littermates (9, 20, 21, 35, 39, 45, 46, 48, 53, 56). Interestingly, the CF mouse displays only mild pancreatic pathology with little to no exocrine pancreatic dysfunction (12, 22), suggesting an origin other than PI for the observed growth reduction. The reduced growth in the CF mouse without PI supports observations in CF patients, suggesting additional causes of CF growth reduction. Although reduced dietary intake in the mice has also been suggested (22), we found no evidence of reduced caloric intake by these animals (45). One hypothesized origin for the reduced growth of the CF mouse is the intestine. CF mice display impaired gastrointestinal physiology since they experience a high incidence of intestinal obstruction (9, 21, 39, 48) similar to the less frequently occurring meconium ileus and distal intestinal obstruction syndrome observed in CF patients (15). The intestinal dysfunction in CF may include intestinal mucosal defects that interfere with normal nutrient absorption, leading to reduced growth (8, 38).

Using a complementary approach with several CF mouse models, we have delineated the intestinal effects on growth and obstruction and find that absence of Cftr function in the intestinal epithelium *1*) is an important contributor to intestinal obstruction, but likely not the only component, and *2*) does not correlate with the majority of growth impairment in CF mice.

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MATERIAL AND METHODS

Mouse strains. The *Cftr^{tm1Unc}* and the *Cftr^{tm1Unc}*+FABP-CFTR mouse models have previously been described (48, 57). The creation of the *Cftr^{fl10}* conditional Cftr mouse model has been previously described (21). The *Cftr^{invfl10}* conditional Cftr mouse model was created in the exact same manner as described for the *Cftr^{fl10}* mouse with the exception of the inversion of the exon 10 sequence and the placement of *loxP* sites in opposite orientation. These two conditional Cftr mouse models were each crossed to mice carrying the villin-Cre transgene in which Cre recombinase is only expressed in the intestinal epithelium (31). Animals were monitored for survival on a daily basis, and weight was assessed every 5 days from 10 to 40 days of age. Length on 6-wk-old euthanized mice was assessed from nose to anus by use of digital calipers. All experiments were completed on littermates within the same generation to decrease phenotypic variability.

All animals used in this study were cared for according to a Case Western Reserve University approved protocol and Institutional Animal Care and Use Committee guidelines. Animals were housed in standard polysulfone microisolator cages in ventilated units with corncob bedding. Mice were given ad libitum access to chow (Harlan Teklad 3000; Harlan Teklad Global Diets, Madison, WI) and sterile water. All animals were maintained on a 12-h light, 12-h dark schedule at a mean ambient temperature of 22°C.

PCR. Genotyping was completed by PCR analysis using DNA extracts from ear biopsies. To detect the *Cftr^{fl10}* allele (408 bp) and the *Cftr$^{\Delta 10}$* allele (148 bp), primers P1 (5′-GTAGGGGCTCGCTCTTCTTT-3′), P2 (5′-GTACCCGGCATAATCCAAGA-3′), and P3 (5′-AGCCCCTCGAGGGACCTAAT-3′) were used. To detect the *Cftr^{invfl10}* allele (563 bp) and the *Cftr$^{fl10'}$* allele, primers P1, P2, and P4 (5′-CACCCACTCCAGCTTAATCC-3′) were used. PCR reactions were completed for 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s.

Intestinal epithelial isolates were obtained as previously described (5). RNA was isolated from epithelial isolates by use of TRIzol (Invitrogen). One microgram of RNA was reversed transcribed in cDNA by use of QScript cDNA synthesis kit (VWR). Real-time quantitative PCR was performed on a StepOne PCR system (Applied Biosystems). Cftr expression was assessed via a TaqMan expression assay that used primers spanning exon 10 and 11 (Mm01156903_m1, Applied Biosystems). Expression was normalized to β-actin as the endogenous control. Each RNA sample was used to make cDNA in duplicate, and the expression results were then averaged to yield the final result. The average of each sample was then expressed as a percentage of *Cftr^{fl10}* expression.

Bioelectric measurements. Nasal potential difference (NPD) measurements were obtained as previously described (7, 21). Short-circuit measurements on intestinal sections were obtained as previously described (21).

Intestinal histology. A segment of mouse ileum (2–3 cm above the cecum) was isolated and fixed in 10% formalin, embedded in paraffin, and sectioned (5 μm). Periodic acid-Schiff (PAS) and Alcian blue staining of these sections were completed to detect mucus accumulation and identify goblet cells. The number of goblet cells present in the villi were expressed as the total number of PAS-positive cells per 1,000 epithelial cells (3). These counts originate from two independent locations in the ileum per mouse with at least five mice per group.

Statistics. A Kaplan-Meier log-rank test was used to determine differences in survival. All other data comparisons utilized a two-tailed unpaired *t*-test.

RESULTS

The gut-corrected CF mouse displays poor growth. A "gut-corrected" CF mouse model has previously been described in which mice homozygous for a null mutation in the Cftr gene (*Cftr^{tm1Unc}*) (48) also carry a human cystic fibrosis transmembrane regulator (CFTR) transgene expressed from the rat fatty acid binding protein (FABP) gene promoter (57). Expression of CFTR in the intestine provided almost full protection from the intestinal obstruction associated with CF mice, but growth of these mice was not reported (57). To decrease genetic variability, this strain of mice was made congenic on the C57BL/6J background. These mice showed a low incidence of obstruction, consistent to what was reported for the mixed genetic background (Fig. 1*A*) (57). Interestingly, these animals experienced growth retardation similar to CF mice (Fig. 1*B*), raising questions about the relationship between CF growth impairment and intestinal function. However, mechanistic interpretation is difficult because there are many potential variables with this strain of mice. Most notably, the FABP-CFTR transgene is under different transcriptional control compared with the endogenous Cftr gene, and thus FABP-CFTR magnitude and site of expression are different from endogenous Cftr. In addition, although the FABP promoter directs high expression of human CFTR to the gut, the promoter also directs high levels of expression to the brain, pancreas, and kidney and low

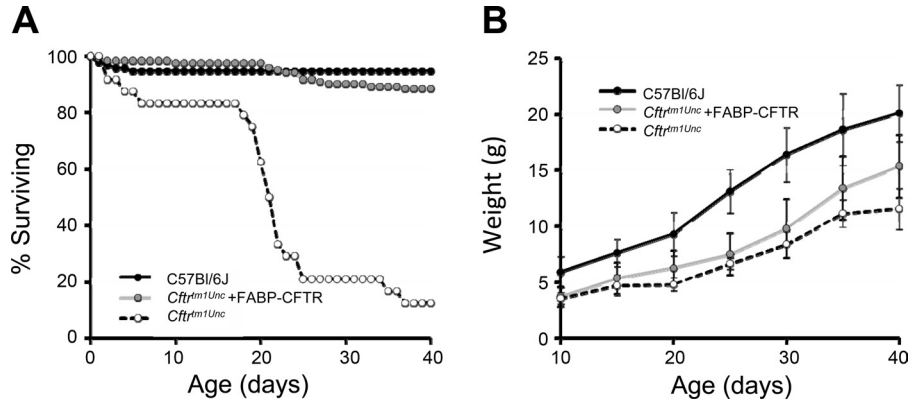

Fig. 1. Survival and growth of cystic fibrosis (CF) mice with and without FABP-CFTR. Survival (*A*) and growth of mice (*B*) were assessed in CF mice (*Cftr^{tm1Unc}*, $n = 24$), CF mice in which human cystic fibrosis transmembrane regulator (CFTR) was expressed by the fatty acid binding protein (FABP) promoter (*Cftr^{tm1Unc}*+FABP-CFTR, $n = 28$), and control littermates (C57Bl/6J, $n = 96$) up to 40 days of age. Weight was significantly decreased in *Cftr^{tm1Unc}* and *Cftr^{tm1Unc}*+FABP-CFTR compared with control animals at every age measured ($P < 0.0001$) with equal numbers of each sex used (data represent means ± SD). Incremental loss of *Cftr^{tm1Unc}* mice led to decreasing numbers of weight measurements for this group (e.g., by *day 40* only 4 mice were available for weights). The *Cftr^{tm1Unc}*+FABP-CFTR and C57Bl/6J groups contained consistent numbers of mice in each group throughout the growth curve.

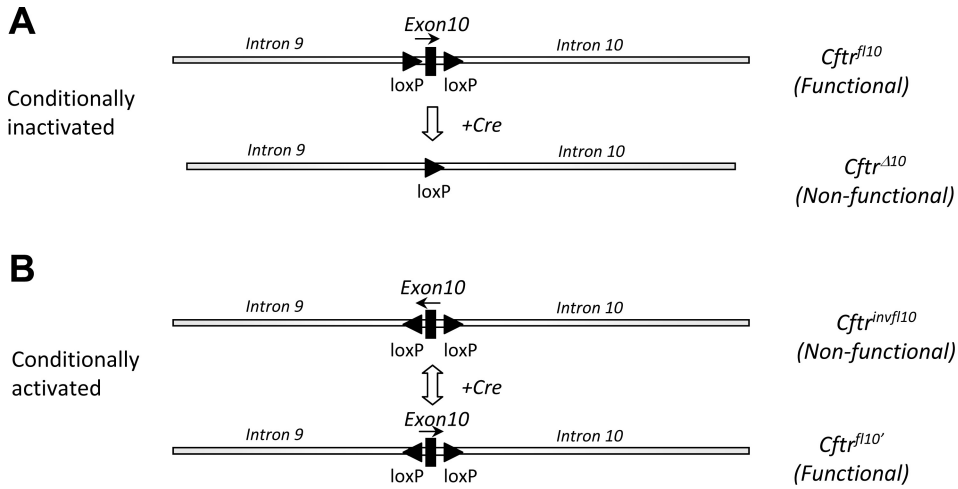

Fig. 2. Schematic of 2 conditional *Cftr* alleles. *A*: conditional *Cftr* allele consisting of exon 10 surrounded by 2 *loxP* sites in the same orientation ($Cftr^{fl10}$). The presence of Cre recombinase induces recombination between the 2 *loxP* sites, resulting in deletion of exon 10 ($Cftr^{\Delta 10}$) and thus nonfunctional Cftr. *B*: conditional *Cftr* allele consisting of an inverted exon 10, and thus nonfunctional Cftr, surrounded by 2 *loxP* sites in opposite orientation ($Cftr^{invfl10}$). The presence of Cre recombinase induces recombination between the 2 *loxP* sites, resulting in the inversion of exon 10 and thus producing functional *Cftr* ($Cftr^{fl10'}$). The inversion of exon 10 is a reversible reaction in the presence of Cre recombinase that results in approximately equal presence of exon 10 sense and antisense alleles (functional and nonfunctional Cftr, respectively).

levels in the lung (57), thus complicating interpretation of the phenotype.

Specific inactivation or activation of Cftr in the intestinal epithelium utilizing new conditional Cftr alleles. To address the limitations of the gut-corrected CF mouse model, we took a complementary approach in which the Cftr gene was "floxed" ($Cftr^{fl10}$) to allow conditional expression of the gene (21). By this approach, the normal transcriptional regulation of the gene is not disrupted, but rather its ability to make a functional product is manipulated in specific cell types. Two varieties of the allele were developed, one that allows targeted inactivation of *Cftr* (21) and one that allows targeted activation, or restoration, of *Cftr*. The alleles are shown schematically in Fig. 2. The $Cftr^{fl10}$ allele allows for deletion of exon 10 in cells that specifically express Cre recombinase, thus inactivating Cftr in these cells while leaving Cftr functional in all other cells. The $Cftr^{invfl10}$ allele consists of an inverted, or antisense, orientation of exon 10 with *loxP* sites in opposite orientation. The $Cftr^{invfl10}$ allele allows for inversion of the antisense exon 10 in cells that specifically express Cre recombinase, thus restoring Cftr activity in these cells while leaving Cftr nonfunctional in all other cells (Fig. 2). The inversion of the $Cftr^{invfl10}$ allele is a reversible reaction in the presence of Cre recombinase that results in approximately equal presence of exon 10 sense and antisense alleles (functional and nonfunctional Cftr, respectively), producing similar levels of *Cftr* found in nonphenotypic heterozygotes.

To further understand the relationship between Cftr function in the intestinal epithelium and growth, each of these alleles was exposed to Cre recombinase expressed specifically in intestinal epithelial cells. Both alleles were made homozygous in combination with a Cre transgene expressed from the villin gene promoter. Intestinal epithelial specificity is retained with Cre expression in the villus and crypt cells along the entire small and large intestine (31). Since these mice do not show Cre expression outside of the intestine, they provide the opportunity to study the effects of Cftr function in the intestine without the confounding effects of expression in other tissues as was reported for the FABP-CFTR mice (57). Both Cftr alleles crossed with the villin-Cre transgene displayed DNA modification only in the intestinal epithelium (Fig. 3). To verify the presence or absence of Cftr expression in the intestinal epithelium from the two alleles, intestinal epithelial cells were isolated from the duodenum, jejunum, ileum, cecum, and colon, and quantitative RT-PCR was performed. Cells from mice homozygous for the $Cftr^{fl10}$ allele with villin-Cre, in which exon 10 should be deleted in intestinal epithelial cells, displayed complete absence of normal Cftr mRNA expression, identical to mice homozygous for the $Cftr^{invfl10}$ allele in which exon 10 is constitutively inverted and thus inactive (Fig. 4). Cells from mice homozygous for the $Cftr^{invfl10}$ allele with villin-Cre, in which Cftr activity should be partially restored in

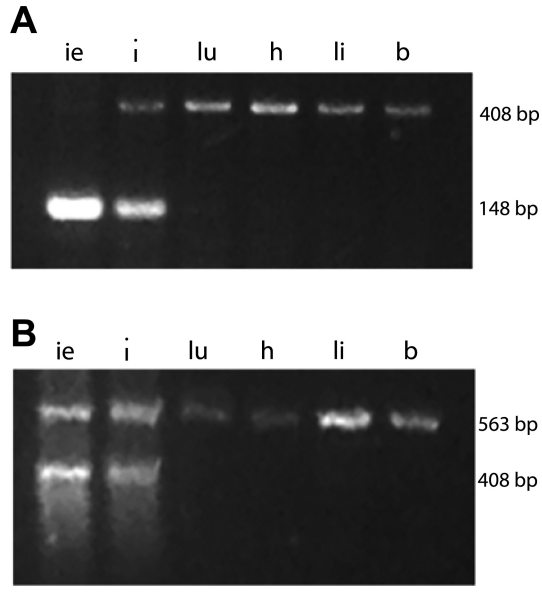

Fig. 3. Intestinal epithelium-specific recombination of conditional Cftr alleles. *A*: DNA amplification of the region surrounding exon 10 from various tissues of a mouse homozygous for $Cftr^{fl10}$ with the villin-Cre transgene. Cre recombinase expression specifically in intestinal epithelium results in deletion of exon 10 ($Cftr^{\Delta 10}$, 148 bp) in the intestinal epithelium (ie) and the whole intestine (i). Exon 10 is still present in lung (lu), heart (h), liver (li), and brain (b), as well as the whole intestine, which includes cell types other than epithelium ($Cftr^{fl10}$- 408 bp). Cre expression was also absent in kidney and bone (data not shown). *B*: DNA amplification of the region surrounding exon 10 from various tissues of a mouse homozygous for $Cftr^{invfl10}$ with the villin-Cre transgene. Cre recombinase expression specifically in intestinal epithelium results in inversion of exon 10 ($Cftr^{fl10'}$, 408 bp) in the intestinal epithelium and the whole intestine. Exon 10 remains inverted ($Cftr^{invfl10}$, 563 bp) in the lung, heart, liver, and brain. The inverted form of exon 10 is also observed in the intestinal tissue due to the reversible nature of this reaction.

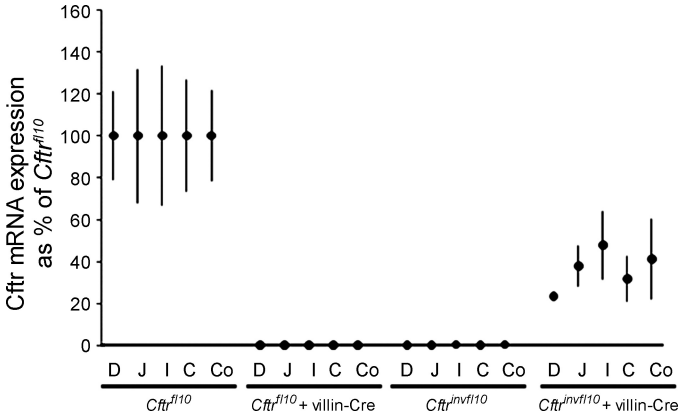

Fig. 4. Cftr mRNA expression in isolated intestinal epithelium from conditional Cftr mice with and without villin-Cre *Cftr* expression in intestinal epithelial isolates from the duodenum (D), jejunum (J), ileum (I), cecum (C), and colon (Co) from $Cftr^{fl10}$, $Cftr^{fl10}$ + villin-Cre, $Cftr^{invfl10}$, and $Cftr^{invfl10}$ +villin-Cre. Expression is reported as a percentage of *Cftr* expression in control mice ($Cftr^{fl10}$) in the corresponding intestinal section. Each measurement is an average of 3 mice completed in replicates (data represent means ± SE).

intestinal epithelial cells, displayed 20–60% *Cftr* expression levels compared with control animals (Fig. 4). This range of Cftr expression is predicted since on average 50% of the $Cftr^{invfl10}$ copies are expected to be in the sense orientation and 50% are expected to be in the antisense orientation in cells with Cre recombinase (Fig. 2).

To determine whether there was detectable "leak" of Cre expression in other epithelial cells, NPD measurements were completed to look for evidence of Cftr function. Mice carrying the $Cftr^{fl10}$ allele showed readily detectable forskolin-induced potential difference changes, regardless of Cre status, and mice carrying $Cftr^{invfl10}$ showed no response to forskolin, regardless of Cre status (Fig. 5 and Table 1). These results, in combination with the RNA and DNA analyses of nonintestine tissues, suggest that inactivation or activation of Cftr by using the two Cftr conditional alleles in combination with villin-Cre does not occur in other epithelial cells but is specific to the intestine.

Because tissue specificity appeared to be restricted to intestinal epithelium, the physiological consequences of these manipulations were assessed by measuring cAMP-induced short-circuit current. Examples are shown in Fig. 6, and mean values are tabulated in Table 1. In the absence of Cre, mice with the $Cftr^{fl10}$ allele produced forskolin-stimulated transepithelial short-circuit currents comparable to mice without the *loxP* sites (21). In the presence of villin-Cre, the $Cftr^{fl10}$ allele generated forskolin-stimulated currents that were undetectable and indistinguishable from CF mice, which carry *Cftr* null alleles systemically. The same process was carried out for mice with the $Cftr^{invfl10}$ allele. In the absence of villin-Cre, this allele behaves like other *Cftr* null alleles, displaying no detectable forskolin-stimulated currents. With villin-Cre, tissues from these mice showed currents reduced relative to $Cftr^{fl10}$ but dramatically greater than for $Cftr^{invfl10}$ and $Cftr^{fl10}$ with villin-Cre which displayed little to no forskolin-stimulated response (Fig. 6 and Table 1). As expected, these results indicate that Cftr function is present in the intestinal epithelium of the mice with $Cftr^{fl10}$ and $Cftr^{invfl10}$ with villin-Cre but not in the intestinal epithelium of mice with $Cftr^{invfl10}$ and $Cftr^{fl10}$ with villin-Cre.

The presence or absence of Cftr in the intestinal epithelium correlates with intestinal dysfunction. One consequence of loss of Cftr in the intestine is abnormal goblet cell number and distribution. Intestinal sections from mice with functional Cftr in the intestine, $Cftr^{fl10}$ and $Cftr^{invfl10}$ with villin-Cre, displayed normal goblet cell numbers and distribution, whereas intestinal sections from mice without functional Cftr in the intestine, $Cftr^{invfl10}$ and $Cftr^{fl10}$ with villin-Cre, displayed an increased number of goblet cells in the villi (Fig. 7 and Table 2). Inactivation of Cftr in the intestinal epithelium resulted in a CF-like goblet cell profile, whereas restoration of Cftr to the intestinal epithelium resulted in a non-CF phenotype.

The FABP-CFTR transgene clearly protects against intestinal obstruction (Fig. 1 and Ref. 57), but expression in tissues other than the gastrointestinal tract confounds interpretation of the mechanisms involved. The models presented here are specific to the gut and maintain endogenous regulation of Cftr expression. We have found that removing Cftr from intestinal epithelium reduces electrophysiological responses to that of CF mice and histological characteristics of the intestines mimic CF mice as well. Thus if the ileal obstructions characteristic of CF mice are due to loss of Cftr function in the intestinal epithelium, these mice should develop obstructions. As Fig. 8*A* demonstrates, the loss of Cftr function in the intestinal epithelium resulted in ~25% of the animals experiencing lethal obstruction around the time

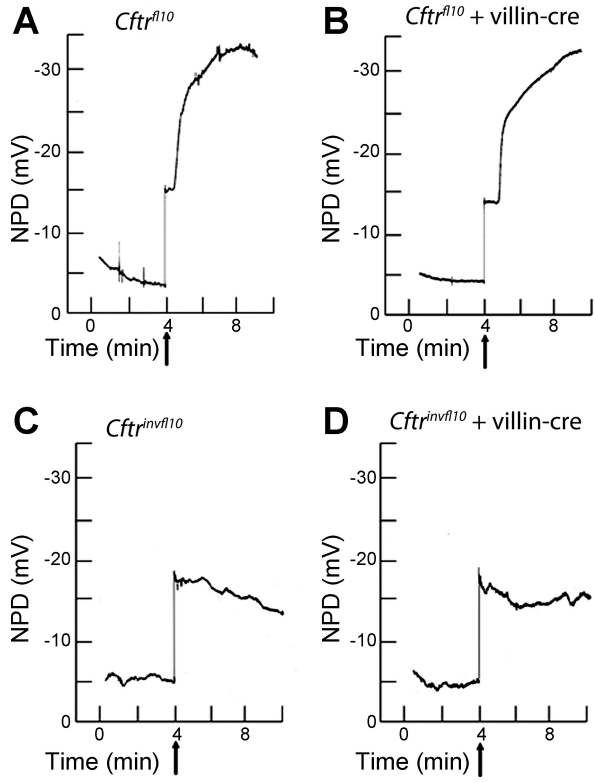

Fig. 5. Exon 10 manipulation in the intestinal epithelium does not alter nasal potential difference (NPD) of mice with Cftr function [$Cftr^{fl10}$ (*A*) and $Cftr^{fl10}$ + villin-Cre (*B*)] and without Cftr function [$Cftr^{invfl10}$ (*C*) and $Cftr^{invfl10}$ + villin-Cre (*D*)] in the nasal epithelium. Arrow indicates addition of forskolin (10 μM) and chloride-free HEPES-buffered Ringer solution. A bi-ionic junction potential of −12.5 mV was observed (step increase in voltage coincident with solution change) independent of Cftr function and was excluded from the calculation of change in NPD.

Table 1. *Intestinal short-circuit currents and nasal potential differences in conditional Cftr strains*

	ΔI_{sc}, μA/cm²†					PD, mV‡
Cftr allele (Cre)	Duodenum	Jejunum	Ileum	Cecum	Colon	Nose
$Cftr^{fl10/fl10}$	64.3 ± 12	144.9 ± 28	196.6 ± 26	173.7 ± 14	188.3 ± 20	−15.6 ± 4
$Cftr^{fl10/fl10}$ (villin-Cre)	2.4 ± 2*	3.21 ± 2*	2.0 ± 1*	1.2 ± 0.2*	0.7 ± 1*	−17.3 ± 5
$Cftr^{invfl10/invfl10}$	−0.4 ± 1*	1.2 ± 1*	0.3 ± 1*	1.39 ± 1*	0.5 ± 1*	2.3 ± 2*
$Cftr^{invfl10/invfl10}$ (villin-Cre)	55.2 ± 10	58 ± 10*	67.0 ± 16*	127.45 ± 19	87.6 ± 29*	1.7 ± 2*

ΔI_{sc}, cAMP-induced peak increase in short-circuit current ($n \geq 5$; mean±SE); PD, response to low-chloride forskolin ($n \geq 4$; mean±SE). *$P < 0.05$ vs. $Cftr^{fl10/fl10}$.

of weaning. However, this is much lower than the 60–70% incidence of obstruction we find when Cftr is inactivated systemically by deleting exon 10, $Cftr^{\Delta 10}$ (21), or by inverting it, $Cftr^{invfl10}$ (Fig. 8*B*). Restoration of Cftr activity in the intestinal epithelium completely protected against obstruction (Fig. 8*B*), as predicted by the electrophysiology and histology of these mice.

The presence or absence of Cftr in the intestinal epithelium does not correlate with growth. Although Cftr function in intestinal epithelium correlates well with protection against obstruction, the results observed from the FABP-CFTR mice suggested that it may not predict growth. Weight profiles of mice with or without functional Cftr in their intestinal epithelium were monitored and compared with CF and non-CF animals. Inactivating Cftr in the intestinal epithelium ($Cftr^{fl10}$ with villin-Cre) has little effect on growth, despite the marked effect on obstruction (Fig. 8*C*). In contrast, restoring Cftr function to the intestinal epithelium ($Cftr^{invfl10}$ with villin-Cre) has no significant effect toward increasing growth, since these animals were substantially smaller than non-CF mice and nearly identical to CF mice (Fig. 8*D*) despite complete protection against obstruction. A similar effect on growth was observed in these mice when body length was measured at 6 wk of age. There was no difference in body length between $Cftr^{fl10}$ with villin-Cre and control littermates (Fig. 8*E*), but $Cftr^{invfl10}$ with villin-Cre mice were significantly reduced from control littermates and no different than CF littermates $Cftr^{invfl10}$ (Fig. 8*F*).

DISCUSSION

Improving growth may be important to the health of CF patients due to its association with pulmonary function, overall health and even mortality, but the causal relationships behind these associations are not clear. Understanding the origins of reduced growth in CF has been difficult because of the systemic nature of the disease. PI is a clear contributor to poor growth in CF patients but cannot fully explain the growth problems as evidenced by the addition of pancreatic enzyme supplements and improved nutrition having no significant impact on stature (50). The presence of similar growth deficits in CF mouse models in the absence of PI also suggests additional origins for the reduced growth in CF. In this study we focused on the possible relationship between intestinal

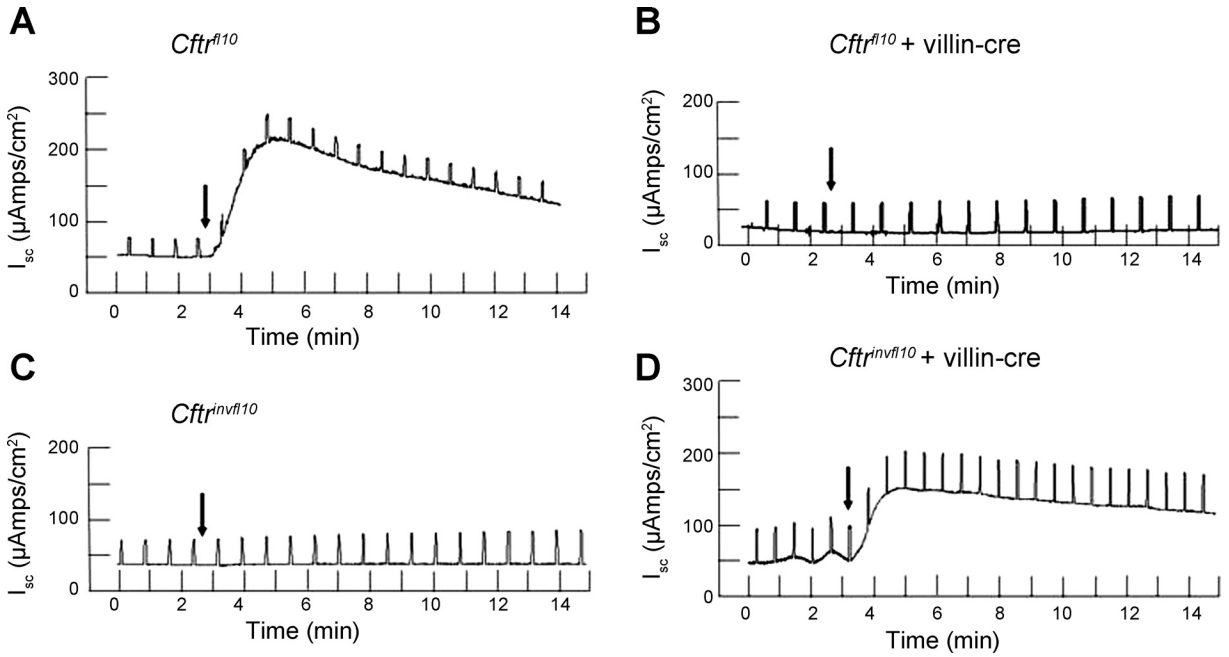

Fig. 6. Exon 10 manipulation in the intestinal epithelium leads to either inactivation or activation of Cftr function as assessed by change in intestinal short-circuit current (I_{sc}). I_{sc} response to the addition of forskolin (10 μM) and IBMX (100 μM) (indicated by arrows) in ileal sections from $Cftr^{fl10}$ (*A*), $Cftr^{fl10}$ + villin-Cre (*B*), $Cftr^{invfl10}$ (*C*), and $Cftr^{invfl10}$ + villin-Cre (*D*). Vertical deflections (at 1-min intervals) result from voltage-clamp to nonzero values to measure transepithelial resistance.

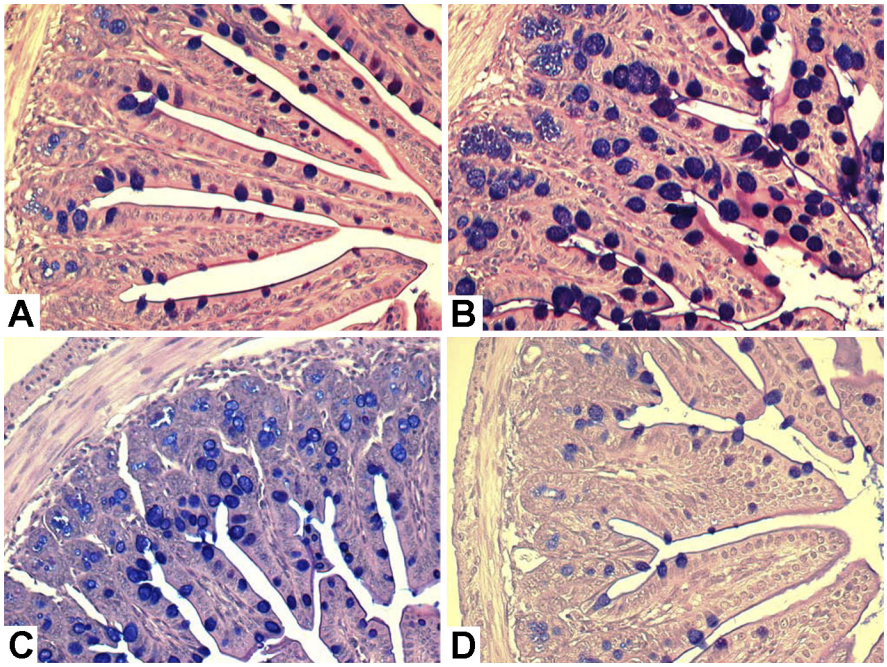

Fig. 7. Histology of ileal sections in conditional Cftr mice with and without Cftr in the intestinal epithelium. Ileal sections from mice with functional Cftr in the intestinal epithelium, $Cftr^{fl10}$ (*A*) and $Cftr^{invfl10}$ + villin-Cre (*D*), displayed a normal number of goblet cells (purple circles) in the crypts and villi. Intestinal sections from mice without functional Cftr in the intestinal epithelium, $Cftr^{fl10}$ + villin-Cre (*B*) and $Cftr^{invfl10}$ (*C*), displayed goblet cell hyperplasia along the villi and within the crypts. Ileal photomicrographs are ×200 magnification.

dysfunction in CF and growth by utilizing several CF mouse models that modulate Cftr activity in the intestinal epithelium.

The $Cftr^{tm1Unc}$ mice carrying the FABP-CFTR transgene are often referred to as gut-corrected CF mice and, because of the increased survival profile and recapitulation of CF nasal electrophysiology (17), they have been used to assess many CF-relevant processes, such as strategies of ion transport correction (30), antimicrobial drugs (52), and CF immunological phenotypes (34, 54). In this study, we show that these mice, when congenic on the C57BL/6J background, displayed poor growth despite significant improvement of survival and nearly complete absence of intestinal obstruction. However, despite their utility in various studies, responses of these mice to various conditions or challenges must be interpreted with caution because of the unintended expression of human CFTR in tissues aside from the gut (57). To restrict Cftr expression to specific sites, and to do so without altering native *Cftr* transcriptional regulation, we created two conditional Cftr alleles by modifying the endogenous murine *Cftr* gene. With these alleles, transcription occurs as it would with the native allele, but the generation of functional Cftr from the mRNA is dictated by Cre expression. When Cre expression is limited to a specific cell type by a tissue-specific promoter (such as villin-Cre), those Cre-expressing cells will produce Cftr mRNA that is lacking exon 10 in the inactivating strategy or produce Cftr mRNA with exon 10 restored to its normal, functional, orientation in the activating strategy. Cre will have no impact on cells not expressing Cftr mRNA and thus Cftr function is only lost or restored in tissues in which Cftr is normally expressed. In the present study, we used this system to manipulate Cftr expression specifically in the intestinal epithelium but, considering the numerous cell types that express Cftr and the vast number of Cre-expressing mice that currently exist, these alleles can be utilized to study the role of Cftr function in almost any tissue or cell type of interest.

Table 2. *Goblet cell number in the villi of conditional Cftr strains*

Cftr allele (Cre)	Average No. of Goblet Cells
$Cftr^{fl10/fl10}$	139.8 ± 12
$Cftr^{fl10/fl10}$ (villin-Cre)	259.4 ± 20*
$Cftr^{invfl10/invfl10}$	262.2 ± 23*
$Cftr^{invfl10/invfl10}$ (villin-Cre)	169.0 ± 20

Number of goblet cells is averaged per 1,000 villus epithelial cells. *$P < 0.05$ vs. $Cftr^{fl10/fl10}$.

We used these new Cftr alleles to specifically modulate Cftr expression and function in the intestinal epithelium, with the goal of better understanding the role of the intestinal epithelium on the overall phenotype of CF mice. The results lead to two major conclusions. First, they indicate that intestinal obstruction requires loss of Cftr from the intestinal epithelium, but epithelial dysfunction is not the sole cause. Although inactivation of Cftr in intestinal epithelium leads to complete loss of detectable Cftr activity in these cells, the incidence of intestinal obstruction is only about half that found in mice in which Cftr is inactive in all tissues (Fig. 8, *A* and *B*). In contrast, restoring Cftr to the intestinal epithelium completely protects against obstruction. A model to explain these observations is that loss of Cftr from the intestinal epithelium is sufficient to cause goblet cell hyperplasia and hypertrophy, such that the animals are at or near a threshold for lethal obstruction. Although most or all will experience some level of obstruction, only those reaching the threshold will succumb to the obstruction. Support for this model can be observed in the slight but significant relative reduction in weight gain of mice with inactive Cftr in the intestinal epithelium about the time at which the animals transition to solid food and the majority of obstruction is observed (20–30 days; Fig. 8, *A* and *C*). This small transient reduction in growth is observed whether the mice succumb to intestinal obstruction or not, consistent with transient weight loss due to intestinal trauma. In addition, there is evidence that loss of Cftr from other cell types in the gut

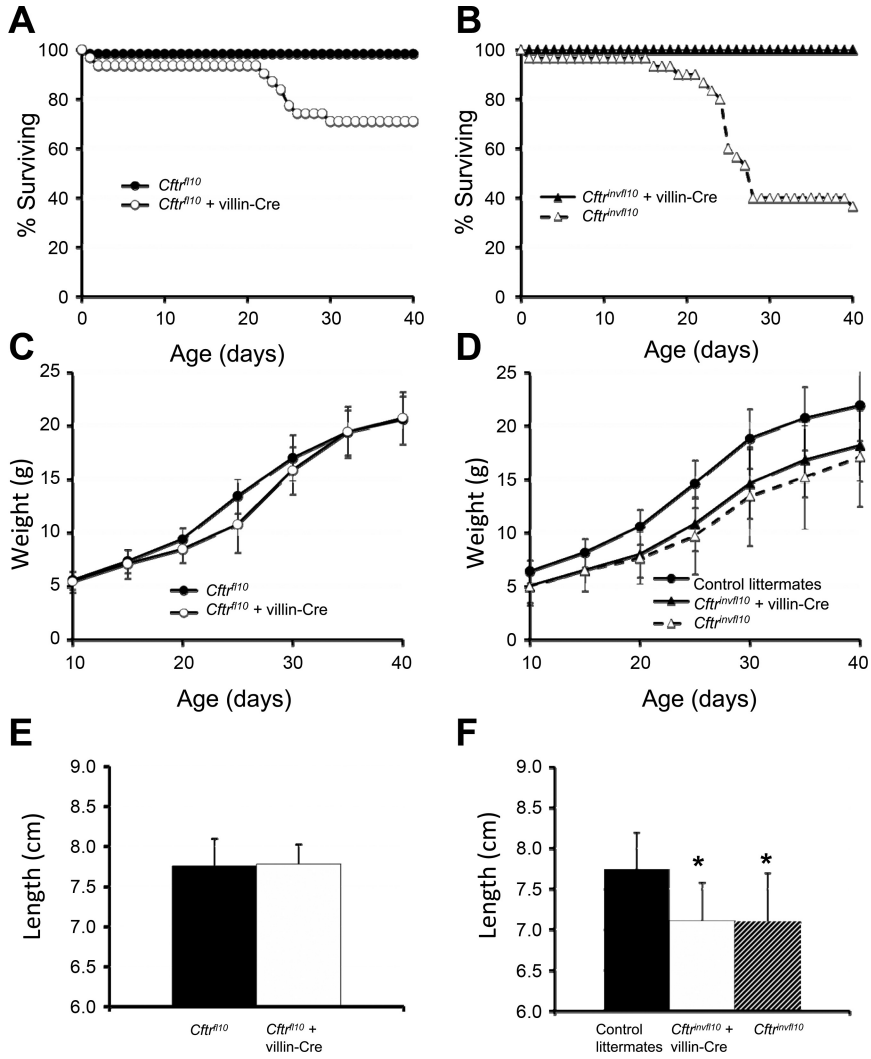

Fig. 8. Survival and growth of conditional Cftr mice with and without Cftr in the intestinal epithelium. Percentage of mice surviving up to 40 days of age for $Cftr^{fl10}$ + villin-Cre ($n = 31$) and $Cftr^{fl10}$ ($n = 60$) (*A*) and $Cftr^{invfl10}$ + villin-Cre ($n = 28$) and $Cftr^{invfl10}$ ($n = 31$) (*B*). Survival of $Cftr^{fl10}$ + villin-Cre and $Cftr^{invfl10}$ was significantly reduced compared with littermates ($P < 0.0001$). Average weight up to 40 days of age from $Cftr^{fl10}$ + villin-Cre and $Cftr^{fl10}$ (*C*) and $Cftr^{invfl10}$ + villin-Cre, $Cftr^{invfl10}$, and control littermates (*D*). $Cftr^{fl10}$ + villin-Cre mice displayed significantly reduced weight from 20–30 days of age compared with control littermates ($P < 0.05$). $Cftr^{invfl10}$ + villin-Cre and $Cftr^{invfl10}$ mice displayed significantly reduced weight at every age compared with control littermates ($P < 0.001$) but were not significantly different from each other. Weights include mice that died before 40 days of age. Thus the incremental loss of $Cftr^{invfl10}$ mice led to decreasing numbers of weight measurements for this group (e.g., by *day 40* only 10 mice were available for weights). Average body length from 6-wk-old mice ($n \geq 8$ for each group) from $Cftr^{fl10}$ + villin-Cre and $Cftr^{fl10}$ (*E*) and $Cftr^{invfl10}$ + villin-Cre, $Cftr^{invfl10}$, and control littermates (*F*). $Cftr^{invfl10}$ + villin-Cre and $Cftr^{invfl10}$ mice displayed significantly reduced body length compared with control littermates (*$P < 0.005$) but were not significantly different from each other (all data represent means ± SD).

could have a deleterious effect on intestinal function and further increase the proportion of animals that cross the lethality threshold. Recent studies have observed the presence of Cftr in smooth muscle cells, identified a possible role for Cftr in relaxation and/or contraction of smooth muscle cells, and observed a difference in intestinal smooth muscle contraction between CF and non-CF mice (13, 33, 40–42, 55). These results suggest that absence of Cftr in intestinal smooth muscle in CF patients may contribute to gastric dysmotility and intestinal obstruction by altering peristalsis and is currently being evaluated by this tissue-specific system.

Second, the results show that restoration of Cftr in intestinal epithelium of the $Cftr^{invfl10}$ mice with villin-Cre produces Cftr activity in the intestine (Table 1) and completely protects against CF intestinal pathology and lethal intestinal obstructions (Fig. 8*B*) but does not improve growth (Fig. 8, *D* and *F*). The failure of a Cftr-competent gastrointestinal tract to impart improved growth in an exocrine pancreatic sufficient state provides strong evidence that CF growth retardation is not likely a consequence of malnutrition. This may explain the ambiguous results observed with regards to fat malabsorption in CF mice. A previous study by Bijvelds and colleagues (4) observed no fat malabsorption in one CF model and a negligible reduction in fat malabsorption in another CF model but no attempt was made to correlate this with growth. Interestingly, another study observed possible fat malabsorption in CF mice, as assessed by lower triglyceride levels, but found this was independent of weight (8), suggesting that the level of malabsorption in CF mice may not be the source of growth reduction. Although malabsorption clearly occurs in CF patients and contributes to reduced growth, the extent of malabsorption that occurs in pancreatic-sufficient CF mice and how this contributes to growth is unclear. Current studies in our laboratory involving the nutrient absorption and metabolic profile in CF mice and the mice in this study will provide a better understanding of the level of malabsorption that occurs in CF mice and its direct consequence in growth.

We have used two novel, conditional alleles of *Cftr* to examine the role of the intestinal epithelium in CF phenotypic manifestations. These manipulations confirm a model in which intestinal Cftr function is necessary for normal transit of intestinal contents. However, these models have also provided insight into the sources of CF-related growth retardation. By inactivating Cftr in the intestinal epithelium of otherwise non-CF mice, and by restoring Cftr to the intestinal epithelium of otherwise CF mice, we have excluded intestinal epithelial

Cftr by itself as a significant contributor to CF-related growth retardation. In the absence of a pancreatic or intestinal origin for the reduced growth, absence of Cftr from the endocrine system may provide an alternative explanation. The various endocrine abnormalities observed in CF patients have been attributed to malnutrition or lung infections, but patients with good clinical and nutritional status still display endocrine abnormalities (23, 24). Among these abnormalities is a reduction in insulin-like growth factor I (IGF-I), an important endocrine player in somatic growth, that has been reported in both humans and mice with CF (2, 19, 28, 45, 51). In addition, a recent study found that IGF-I was reduced in newborn pigs and humans with CF, suggesting that additional factors other than malnutrition and chronic inflammation contribute to reduced growth at this stage (44). Thus the CF-associated effects on endocrine function may involve other tissues and/or cell types, such as effector cell types (e.g., hypothalamus or pituitary) or endocrine target cells, such as liver, bone, or muscle.

The *Cftr* conditional alleles presented here demonstrate their utility for identifying the cell type or tissue ultimately responsible for a particular phenotype and thus determine which effects of Cftr's absence are primary and which are secondary. For example, osteoporosis is a common complication in CF patients and osteopenia has been observed in CF mouse models (14, 19, 37). Whether this decrease in bone mineral density is directly due to absence of CFTR from cell types found in bone (47) or a secondary effect due to endocrine dysfunction or even perturbations in calcium homeostasis due to absence of CFTR from the gut, kidney (1), or other tissue is unknown. Similar questions could potentially be approached for phenotypes involving numerous tissues and organs, such as lung, liver, pancreas, and immune cells, to name just a few. For those phenotypes of interest, such studies are limited only by the availability of Cre-expressing strains and the number of such strains is quite large and should therefore facilitate dissecting the etiology of CF-associated phenotypes.

ACKNOWLEDGMENTS

The authors thank Nicole Brown and Christine Zhang for assistance with the bioelectric measurements and Bill Marcus for assistance with genotyping. We also thank Alma Wilson and Molly Halligan of the Case Western Reserve University CF animal core for their assistance in maintaining the mouse strains.

GRANTS

This work was supported by National Institutes of Health Grants P30DK27651 (M. L. Drumm) and R01GM088823 (M. L. Drumm) and grants from the Cystic Fibrosis Foundation (M. L. Drumm).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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