Altered transit and bacterial overgrowth in the cystic fibrosis mouse small intestine

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Submitted 28 November 2006; accepted in final form 8 March 2007

De Lisle RC. Altered transit and bacterial overgrowth in the cystic fibrosis mouse small intestine. Am J Physiol Gastrointest Liver Physiol 293: G104–G111, 2007. First published March 15, 2007; doi:10.1152/ajpgi.00548.2006.—Small intestinal bacterial overgrowth (SIBO) may play an important role in the gastrointestinal complications of cystic fibrosis (CF). This work explored two potential factors in development of SIBO in the CF cfrm<sup>hunc</sup> mouse: impaired Paneth cell innate defenses and altered gastrointestinal motility. Postnatal differentiation of Paneth cells was followed by Defcr, Lyz<sub>5</sub>, and Ang4 gene expression, and SIBO was measured by quantitative PCR of the bacterial 16S rRNA gene. Paneth cell gene expression was low in 4-day-old CF and wild-type (WT) mice and increased similarly in both groups of mice between 12 and 16 days. Peak Paneth cell gene expression was reached by 40 days of age and was less for Defcr and Lys<sub>5</sub> in CF mice compared with WT, whereas Ang4 levels were greater in CF mice. SIBO occurred by postnatal day 8 in CF mice, which is before Paneth cell development. With the use of gavaged rhodamine-dextran to follow motility, gastric emptying in CF mice was slightly decreased compared with WT, and small intestinal transit was dramatically less. Since antibiotics improve weight gain in CF mice, their effects on gastric emptying and small intestinal transit were determined. Antibiotics did not affect gastric emptying or transit in CF mice but did significantly slow intestinal transit in WT mice, suggesting a potential role of normal microflora in regulating transit. In conclusion, small intestinal transit was significantly slower in CF mice, and this is likely a major factor in SIBO in CF.

antibiotic; cryptdin; Paneth cell; motility

IN CYSTIC FIBROSIS (CF), the small intestine is an early site of disease manifestation and the consequences may be serious. As many as 25% of CF infants present with meconium ileus, an obstruction of the distal small intestine by dehydrated mucificulent material (12, 19). A similar proportion of older CF patients experience a comparable condition, termed distal intestinal obstructive syndrome (19). In addition, CF patients often have other gastrointestinal symptoms, including abdominal pain, bloating, flatulence, and failure to thrive. Failure to thrive is an important gastrointestinal symptom that is common in CF and that is associated with deteriorating airway function (44).

In human CF, the exocrine pancreas is largely destroyed in those individuals with mutations on both alleles of the cystic fibrosis transmembrane conductance regulator (CFTR) gene that result in little or no CFTR Cl<sup>−</sup> channel activity (1). Loss of pancreatic function in CF, if untreated, is the major cause of maldigestion and malabsorption, especially of fats (steatorrhea), leading to severe malnutrition. Fortunately, pancreatic insufficiency is fairly effectively treated with oral enzymes. However, even with optimal pancreatic enzyme therapy, gastrointestinal complaints are often not fully corrected, and failure to thrive continues to be common in CF patients (7). In addition, mice with targeted mutations in the Cftr gene are pancreatic sufficient but display failure to thrive (38). These facts argue that in addition to pancreatic insufficiency there is altered function of the intestinal tract in CF that has important effects on growth and nutrition.

A potential unifying factor in the various gastrointestinal complaints that are common in CF may be small intestinal bacterial overgrowth (SIBO). SIBO can result in steatorrhea, abdominal pain, bloating, flatulence, nausea, and anorexia (48). Failure to thrive may also be a consequence of SIBO, attributable to bacterial competition for ingested nutrients as well as intestinal inflammation from enterotoxic metabolites. Also, certain bacteria can interfere with fat digestion/assimilation by producing hydrolyses that deconjugate bile salts (14) making them less amphiphilic. This will impair their ability to emulsify fats and to form the mixed micelles that are important for passage through the unstirred layer and for absorption across the enterocyte microvillus membrane (50).

SIBO has been reported to occur in 30–50% of CF patients, mainly on the basis of breath tests that detect hydrogen gas or methane produced by microbial fermentation (21, 30, 40). SIBO was demonstrated with more direct techniques in the CF mouse (38). On the basis of quantitative PCR results for the bacterial 16S rRNA gene, CF mice have a 40-fold increase in bacterial load in the small intestine (38). CF mice also have other gastrointestinal effects of CF, including excessive mucus accumulation, inflammation, and failure to thrive (17, 39). Importantly, eradication of SIBO in CF mice with broad-spectrum antibiotics significantly improved body-weight gain (38), demonstrating the key role of SIBO in failure to thrive.

In the absence of anatomic abnormalities (e.g., blind loop) or loss of gastric acid production (e.g., proton pump inhibitor use), slowed small intestinal transit is the most frequent cause of SIBO (5). Normally, the interdigestive migrating motor complex (MMC) serves to clean out the intestinal lumen between meals and is in large part responsible for the bacterial load at a low level in the small intestine (46). Consistent with reports of SIBO, there are several studies using breath testing that indicate there is delayed transit through the small intestine in CF patients (3, 15, 20, 30, 36). A more recent study using <sup>99m</sup>Tc as a scintigraphic tracer in a milkshake-type test meal showed that small intestinal transit after 6 h in adult CF patients was reduced by almost 50% compared with healthy controls (34).

In addition to the possibility of slower small intestinal transit, a potential contributing factor to SIBO in CF may be...
dysfunction of Paneth cell innate defenses. Paneth cells release a variety of antimicrobial agents (cryptdins, lysozyme, etc.) through the crypt of Lieberkühn into the intestinal lumen, and these Paneth cell products have an important role in controlling microbial colonization and growth in the small intestine (41). In the CF mouse, excessive accumulation of mucus appears to occlude intestinal crypts, and it has been proposed that this prevents Paneth cell products from reaching the intestinal lumen (38). It has also been suggested that the abnormal environment of the CF small intestine (altered electrolyte concentrations, acidic pH) reduces dissolution of Paneth cell granules once they are released and that this negatively affects their function (13). Therefore, impairment of Paneth cell-mediated innate defenses by the altered luminal environment of the CF intestine could contribute to the development of SIBO.

The aims of the current study were to investigate the potential causes of SIBO in the CF mouse. It was determined whether impaired Paneth cell function could account for development of SIBO in the CF mouse. Also, gastrointestinal transit was investigated as a possible causative factor in SIBO in CF.

MATERIALS AND METHODS

Animals. Cftr(+/−) mice (cftr<sup>−/−/Unc</sup>) were originally obtained from Jackson Laboratory (Bar Harbor, ME) and have been back-crossed on the C57Bl/6 background until congenic (39). Cftr(+/−) mice were bred to obtain wild-type (WT) [Cftr(+/+)] and CF [Cftr(−/−)] mice. Except where otherwise noted, mice of both sexes were used between 6 and 12 wk of age. To prevent lethal intestinal obstruction, all mice (including WT controls) were maintained on a complete elemental liquid diet (Peptamen; Nestle, Deerfield, IL) (18). Where indicated, mice were treated for 3 wk with ciprofloxacin (50 mg·kg<sup>−1·day<sup>−1</sup>) and metronidazole (100 mg·kg<sup>−1·day<sup>−1</sup>) beginning at weaning by addition of the antibiotics to the liquid diet as described (38). All animal procedures were approved by the University of Kansas Medical Center IACUC.

Measurement of Paneth cell gene expression by quantitative real-time RT-PCR. Total RNA was prepared from the entire small intestine by the Trizol method as previously described (38). Real-time quantitative RT-PCR (qRT-PCR) was performed with an iCycler instrument (Bio-Rad, Hercules, CA) using a one-step RT-PCR kit (Qiagen, Valencia, CA). The consensus primers for defensin-related cryptdin, Defcr p130 and Defcr m380 (13), were used to amplify all known Defcr mRNAs (also known as cryptdin). The primers for lysozyme (Lyzs) were forward 5′-GAA TGG AAT GGC TGG CTA CT-3′, reverse 5′-CGT GCT GAG CTA AAC ACA CC-3′, and for angioten- gen 4 (Ang4) were forward 5′-GGG AAT GGG TCC TGG TGA TTT CGG CGT CTA CT-3′, reverse 5′-TGG TGG TGA TTT CGG CGT CTA CT-3′. The mRNA for ribosomal protein L26 (Rpl26) was used as a housekeeping gene for normalization. The Rpl26 primers were forward 5′-AAT GGC ACC ACA GTG CAT ACC-3′, reverse 5′-CTT GGC GCT CCT TCC TCC-3′. Relative expression levels were calculated by using the ΔΔCt method after correcting for minor differences in PCR efficiencies (33) and were expressed relative to 4-day-old WT levels.

Measurement of bacterial load by qPCR of the bacterial 16S rRNA gene. Phosphate-buffered saline containing 10 mM dithiothreitol was flushed through the lumen of the small intestine, and the flushed material was centrifuged at 20,000 g for 30 min to pellet bacteria. The pellet was processed to extract microbial genomic DNA by using the Stool DNA kit (Qiagen) with minor modifications as described (38). Microbial load was measured by quantitative PCR (qPCR) by using universal bacterial 16S rRNA-specific primers as described (37). The 16S rRNA PCR product from a lab strain of Escherichia coli (XL1-Blue; Stratagene, La Jolla, CA) was cloned into the pDRIVE plasmid (Qiagen), and linearized plasmid was used to generate a standard curve for copy number determinations in the real-time qPCR assays as described (38).

Measurement of gastric emptying and small intestinal transit. Mice were fasted overnight with free access to water. In the morning between 8 and 9 AM, the mice were gavaged with the nonabsorbable, indigestible tracer rhodamine-dextran (mol wt = 70,000; Sigma, St. Louis, MO). Each mouse received a 100-µl bolus of 25 mg/ml rhodamine-dextran prepared in 1.5% methylcellulose in saline (11). After gavage, the mice were left with access to water until death. In preliminary experiments, 20 min was found to be optimal for entry of the fluorescent tracer into the small intestine without reaching the cecum or colon in any of the mice. At 20 min postgavage, mice were killed with CO<sub>2</sub> and cervical dislocation. The entire gastrointestinal tract was removed, placed into ice-cold saline to inhibit motility, and divided into stomach and 10 equal segments of small intestine. The stomach and intestinal segments were opened, and the luminal contents were rinsed out into 2 ml saline. The released material was counted (500 g for 1 ml), and the supernatants were used to determine rhodamine fluorescence in a Turner Quantech fluorometer (Barnstead International, Dubuque, IA) with 540-nm excitation and 585-nm emission filters. Gastric emptying was calculated as the percent of tracer remaining in the stomach at 20 min relative to the total amount recovered from the stomach and small intestine. For measurement of small intestinal transit, the raw fluorescence data were transformed by calculating the geometric center of the fluorescence (GCF) distribution present in the small intestine only (not including the fluorescence that remained in the stomach) (11, 42): GCF = ∑ [(fraction of fluorescence per segment) × (segment number)]. To document the distribution of rhodamine-dextran in the small intestine, the tissue was imaged by using a Bio-Rad ChemiDoc XRS with standard ultraviolet transillumination.

In some experiments, a solid-phase tracer was mixed with the rhodamine-dextran. Yellow-green fluorescently labeled polystyrene beads (FluoSpheres; Invitrogen, Carlsbad, CA) were added at a ratio of 2 × 10<sup>5</sup> beads per 100 µl rhodamine-dextran and were administered as above. After 20 min the tissues were harvested and flushed as above. The flushed material was mixed with an equal volume of xylene and was incubated overnight in the dark with gentle agitation to solubilize the polystyrene beads and to liberate the yellow-green fluorochrome. The mixture was centrifuged to separate the xylene (yellow-green fluorochrome) and aqueous (rhodamine) phases, and fluorescence in each phase was measured. The yellow-green fluorescence was measured with 490-nm excitation and 515-nm emission filters.

Statistics. Data are presented as means ± SE. Statistical analysis was performed with Systat software (Chicago, IL). The t-test was used for normally distributed values, and Kruskal-Wallis one-way analysis of variance was used for nonnormally distributed data.

Results

The aim of this study was to examine two factors that could contribute to the development of SIBO in the CF mouse: impaired Paneth cell innate defenses and altered gastrointestinal transit.

SIBO develops early in postnatal life in CF mice, before Paneth cells differentiate. It is morphologically apparent that the mucus and/or altered electrolyte composition in the crypts of Lieberkühn in the CF mouse small intestine impede the dissolution of Paneth cell granules (13, 38). Impaired Paneth cell function may then allow SIBO to occur. To investigate this possibility, I took advantage of the fact that, in the mouse, intestinal crypts appear during the second week of postnatal life. During this postnatal period, Paneth cells differentiate and express their antimicrobial products (9). By determining when

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SIBO occurs relative to expression of Paneth cell antimicrobial products, it is possible to tell if they are related. Bacterial genomic DNA was isolated and used for qPCR to measure levels of the bacterial 16S rRNA gene as an estimate of bacterial load as previously described (38). As shown in Fig. 1, SIBO occurs in CF mice as early as 8 days of age, and the elevated bacterial load is maintained into adulthood. Thus SIBO develops before Paneth cells are known to differentiate.

To verify that Paneth cells develop similarly in CF mice and in WT mice, expression of Paneth cell-specific genes was measured. Defcr gene expression was measured by qRT-PCR using consensus primers that amplify all the known Defcr mRNAs (13). Expression of Lyzs and Ang4 were also measured by using specific PCR primers (see MATERIALS AND METHODS). At 4 days of age, using about 3 ng of input total RNA, Ct values in the qRT-PCR reactions averaged 29.2, 29.2, and 27.3 cycles for Defcr, Lyzs, and Ang4, respectively. Defcr, Lyzs, and Ang4 expression levels were very low until after 12–16 days of age in both WT and CF mice (Fig. 2, A, B, and C, respectively). Expression levels then rose dramatically, until they reached adult values around 40 days of age. The adult level of Defcr expression tended to be less in CF mice, but the difference was not statistically significant (Fig. 2A). There was significantly less Lyzs mRNA expression in the CF tissue at 40 days of age compared with WT (Fig. 2B). In contrast to the other Paneth cell markers, expression of Ang4 tended to be greater in the CF tissue compared with WT, but there was much variability and the differences were not significant. One of the six WT samples used had a more than sevenfold greater value for Ang4 than the other five, and if this outlier were removed from the analysis, the CF value would be statistically greater than WT (P = 0.034). These results show that SIBO occurs by 8 days of age when crypts and Paneth cells have not yet differentiated and Defcr, Lyzs, and Ang4 gene expression levels are very low. Thus development of SIBO is not likely related to impaired Paneth cell function because SIBO occurs before significant expression of Paneth cell antimicrobial factors.

SIBO is most pronounced in the proximal regions of the small intestine. The regional distribution of bacteria in the CF small intestine can provide important information about SIBO. A frequent complication of CF is obstruction of the terminal ileum (meconium ileus and distal intestinal obstructive syndrome), and intestinal obstruction is the most frequent and
severe phenotype in mouse models of CF (22). A possibility is that SIBO reflects the association of bacteria with the mass of mucofeculent material that accumulates in the distal ileum. If this is so, one would predict that the increase in bacterial load in CF would be most pronounced in the distal small intestine.

The bacterial load was determined in equal quarters along the length of the small intestines of WT and CF mice. As shown in Fig. 3, the difference in bacterial load is smallest in the most distal segment of the CF mouse small intestine compared with WT. The bacterial load in CF mice relative to WT was 20-fold in the first quarter, 107-fold in the second quarter, 73-fold in the third quarter, and only 7-fold in the last quarter of the small intestine. These data are not consistent with the idea that bacteria collect in the terminal ileum along with the accumulated mucus. They are more compatible with altered gastrointestinal transit, which was tested next.

Gastric emptying is slightly less in CF mice. It has been reported that gastrointestinal transit is altered in CF, and reduced small intestinal motility is known to be a leading causative factor in SIBO. To investigate gastrointestinal transit in the CF mouse, the indigestible, nonabsorbable tracer rhodamine-dextran was gavaged into overnight-fasted WT and CF mice. After 20 min, the mice were killed and the distribution of fluorescence in the gastrointestinal tract was measured. Gastric emptying was calculated from the fluorescence remaining in the stomach relative to the overall recovered fluorescence (recovery was ±10% of the gavaged amount). The degree of gastric emptying at 20 min in the CF mouse was about four-fifths that of WT (Fig. 4A).

Small intestinal transit is reduced in CF mice. Mice were fasted before use so that measurements would reflect the MMC activity, which is most pronounced during the interdigestive period (24). Also, the tracer used was nonnutritive to avoid postprandial effects on transit. The MMC activity of the small intestine is responsible for the housekeeping function that clears the small intestinal lumen and maintains a low bacterial load under normal conditions. The distribution of fluorescence in the small intestine 20 min postgavage was used to estimate transit in WT and CF mice. The rhodamine-dextran fluorescence was easily observed, and representative examples of the fluorescence patterns in WT and CF mice are shown in Fig. 5. In the example of the WT small intestine, there are two peaks of fluorescence, one centered near the first 25% and the other centered ~65% of the distance from duodenum to ileum (Fig. 5). This pattern was observed in 12 out of 13 WT mice, and only 1 WT mouse had a single peak of fluorescence. This pattern suggests that two boluses exited the stomach during the 20-min postgavage period and that the first bolus traversed 60–70% of the small intestinal length during that period.

In contrast to WT, the CF intestine had a broad distribution of fluorescence over the first third of the small intestine in the example shown (Fig. 5). Only 1 CF mouse out of 10 had fluorescence past the 4th segment of the small intestine. Because gastric emptying was only slightly less in the CF mouse, the fluorescence pattern in the small intestine suggests that two separate boluses had exited the stomach. However, due to slowed transit, the two boluses appear to have merged in the proximal region of the CF intestine.

To confirm that the rhodamine-dextran, which is a fluid-phase marker, was accurately reflecting transit in the gastrointestinal tract, a second solid-phase tracer was also used. Yellow-green fluorescent polystyrene beads were mixed with the rhodamine-dextran, and the mixture was gavaged into WT and CF mice. As shown in Fig. 6, A and B, the two tracers showed identical transit patterns at 20 min postgavage. Therefore, the majority of the studies were performed using only rhodamine-dextran.

Data from several WT and CF mice were analyzed, and the average distributions of fluorescence along the small intestine were plotted. It is apparent that the tracer did not progress nearly as far along the small intestine in CF mice (Fig. 6D) compared with WT (Fig. 6C). To perform a statistical comparison of the extent of small intestinal transit, the data were transformed into GCF values as described in MATERIALS AND METHODS. The difference in GCF between WT and CF was very statistically significant (Fig. 7A), demonstrating that small intestinal transit is much less in the CF mouse compared with WT.

Effects of broad-spectrum antibiotics on gastric emptying and small intestinal transit. It was previously shown that SIBO could be eradicated in CF mice by using oral broad-spectrum antibiotics and that this treatment dramatically improved body-weight gain (38). To test whether eradication of SIBO affected gastric emptying and small intestinal transit, WT and CF mice were treated orally with ciprofloxacin and metronidazole. As shown before, antibiotic treatment improved body-weight gain of CF mice compared with untreated CF mice (treated CF male mice in the current study were 93 ± 4.2% of WT body weight and untreated CF mice were 75 ± 3.8% of WT at 6 wk of age; P = 0.006). Gastric emptying was not significantly affected by antibiotic treatment in either WT or CF mice (Fig. 4B) compared with untreated mice (Fig. 4A).

In contrast to gastric emptying, there was an effect of antibiotics on small intestinal transit, primarily in WT mice. The distribution of rhodamine-dextran fluorescence in the small intestine 20 min postgavage was shifted toward more proximal segments in antibiotic-treated WT mice (Fig. 6E).
Fig. 4. Gastric emptying in WT and CF mice and effects of antibiotic treatment. Mice were fasted overnight, and between 8 and 9 AM they were gavaged with a bolus of rhodamine-dextran (see MATERIALS AND METHODS). Twenty minutes later, the mice were killed and fluorescence in the stomach and intestines was measured. Gastric emptying was calculated from the fluorescence remaining in the stomach and the total fluorescence recovered. A: untreated mice (n = 12 WT; n = 9 CF; *P = 0.049). B: antibiotic-treated mice. Mice were treated with oral antibiotics (ciprofloxacin and metronidazole) for 3 wk (n = 6 WT; n = 7 CF; P = 0.39). There were no significant differences comparing untreated with antibiotic-treated WT or comparing untreated with antibiotic-treated CF. Data are means ± SE.

Fig. 5. Visualization of small intestinal transit in WT and CF mice. Mice were gavaged with rhodamine-dextran. Twenty minutes later, the small intestine was imaged on a Bio-Rad ChemiDoc XRS imager using ultraviolet transillumination. WT small intestine exhibited 2 peaks of fluorescence, whereas CF intestine had a single broad peak in the proximal region.

Fig. 6. Visualization of gastric emptying in WT and CF mice and effects of antibiotic treatment. Mice were fasted overnight, and between 8 and 9 AM they were gavaged with rhodamine-dextran (see MATERIALS AND METHODS). Twenty minutes later, the mice were killed and fluorescence in the stomach and intestines was measured. Gastric emptying was calculated from the fluorescence remaining in the stomach and the total fluorescence recovered. A: untreated mice (n = 12 WT; n = 9 CF; *P = 0.049). B: antibiotic-treated mice. Mice were treated with oral antibiotics (ciprofloxacin and metronidazole) for 3 wk (n = 6 WT; n = 7 CF; P = 0.39). There were no significant differences comparing untreated with antibiotic-treated WT or comparing untreated with antibiotic-treated CF. Data are means ± SE.

DISCUSSION

In this study, SIBO in the CF mouse was investigated to gain insight into how it develops. First, the potential role of impaired Paneth cell function was tested. In the normal intestine, Paneth cells are important in controlling bacterial levels in the small intestine. For example, when rats are treated with the zinc chelator dithizone to transiently deplete Paneth cells, the intestine is more heavily colonized by gavaged E. coli than in untreated animals (47). In the CF mouse, the egress of Paneth cell secretions appears to be blocked by occlusion of the intestinal crypts with mucus (38) and released Paneth cell granules do not dissolve normally (13). This makes it reasonable to propose that impaired Paneth cell function could be involved in development of SIBO in CF. However, it was found that SIBO occurred in the CF mouse as early as 8 days after birth, which is before postnatal differentiation of Paneth cells. There was not a difference in the timing of Paneth cell development in the CF intestine, as revealed by measuring expression of Paneth cell-specific genes. The increased level of expression of these Paneth cell genes during the postnatal period measured in this work is consistent with previous studies (16, 23). Interestingly, of the Paneth cell genes measured, only Ang4 was expressed at higher levels in the adult CF intestine. This can be explained by the fact that expression of most known Paneth cell antibacterial genes is not regulated by the presence of bacteria (41), but Ang4 expression is induced by bacteria (23) and the CF mice have bacterial overgrowth.

Thus impaired Paneth cell defenses do not appear to be a factor in development of SIBO in this case. The measured Paneth cell genes are expressed at very low levels in the young mouse intestine and are not detectable by Northern blot but can be detected by RT-PCR. By immunohistochemistry, defensins were shown to be expressed in neonates in sporadic cells of the villus epithelium before Paneth cells differentiate (16). It is conceivable that the low levels expressed in these scattered cells function in innate defenses during the perinatal period. If so, one would have to postulate that the altered intestinal environment in CF somehow interfered with their function for this to be involved in SIBO in the CF mouse.

The other possible factor considered in development of SIBO is altered small intestinal transit, which is a frequent cause of SIBO (5) and which has been reported to be common in CF patients (3, 15, 20, 30, 34, 35). With the use of a fluorescent tracer method, it was found that gastric emptying was similar in CF mice to that of WT mice but that small intestinal transit was dramatically less. There are several possible effects of CF on the intestine that could play roles in altered transit. One is impaired fat digestion and assimilation, which in humans is in part due to pancreatic insufficiency (8, 45). However, even with optimal pancreatic enzyme therapy, lipid assimilation is not fully restored (7). The presence of unabsorbed nutrients, especially lipids, in the ileum can activate the “ileal brake,” which slows small intestinal transit (31). Although CF mice are pancreatic sufficient they have defective lipid assimilation because of a combination of the acidic
intestinal environment (6) and fecal bile salt loss, similar to human CF patients (28, 32). Poor lipid assimilation may also be due to the deconjugation of bile salts by bacteria. *Clostridium perfringens* comprise ∼6% of the bacteria that overgrow the small intestine of CF mice (38), and they produce hydrolyses that deconjugate bile salts (14). Bile salts are important for fat digestion and for absorption of the breakdown products, and deconjugation may contribute to unabsorbed lipids reaching the ileum in CF, which may then activate the ileal brake.

The ileal brake is one possible mechanism that could link bacteria and altered intestinal transit in CF. However, the relationship between luminal bacteria and intestinal motility is complex. There is an intricate bidirectional interplay between luminal microbes and intestinal motility (5, 25). It is well known that the intestine responds to pathogenic microbes by increasing secretion and motility as a defense mechanism to expel the pathogen (49). Even in health, there are important interactions between the gut and its commensal microflora.

**Fig. 6.** Small intestinal transit in WT and CF mice and effects of antibiotic treatment. Mice were fasted overnight, and between 8 and 9 AM they were gavaged with a bolus of rhodamine-dextran (see MATERIALS AND METHODS). Twenty minutes later, mice were killed and small intestine was divided into 10 equal segments. A and B: comparison of a fluid-phase tracer (rhodamine-dextran, Dextran) to a solid-phase tracer (yellow-green fluorescent polystyrene beads, Beads) in untreated WT (A) and CF (B) mice. Representative examples are shown from 3 mice of each genotype. Both tracers transit in parallel. C and D: rhodamine-dextran transit in untreated WT (C) and CF (D) mice (n = 12 WT; n = 9 CF). E and F: rhodamine-dextran transit in antibiotic-treated WT (E) and CF (F) mice (n = 6 WT; n = 7 CF). See Fig. 7 for geometric center of fluorescence (GCF) calculations and statistical analysis. Data are means ± SE of fluorescence in each segment relative to total fluorescence in the small intestine.

**Fig. 7.** Small intestinal transit in WT and CF mice and effects of antibiotic treatment. The distributions of rhodamine-dextran fluorescence shown in Fig. 6 were used to calculate the GCF in the small intestine by using the formula GCF = \[ \text{GCF} = \sum \left( \frac{\text{fraction of fluorescence per segment}}{\text{segment number}} \right) \] A: untreated mice (n = 12 WT; n = 9 CF; *P < 0.001). B: antibiotic-treated mice. Mice were treated with oral antibiotics (ciprofloxacin and metronidazole) for 3 wk (n = 6 WT; n = 7 CF; P = 0.456 for antibiotic-treated CF vs. antibiotic-treated WT; +P < 0.001 for antibiotic-treated WT vs. untreated WT; P = 0.276 for antibiotic-treated CF vs. untreated CF). Data are means ± SE.
MMC during the interdigestive period plays a major role in keeping bacterial levels low in the proximal small intestine. At the same time, the composition of bacterial species in the intestine affects motility. The most detailed information has come from studies of germ-free and gnotobiotic rats (10, 26). Germ-free rats have decreased small intestinal transit compared with those with conventional microbial flora. After monospecies association with individual bacterial strains, there are specific effects on motility depending on the bacterial species used. Some species stimulate small intestinal transit, especially primitive fermenting anaerobes, whereas others, including the facultative aerobe *E. coli*, inhibit transit compared with conventional microflora (26).

In humans with radiation therapy-induced enteropathy, there is a strong correlation between the extent of decreased MMC activity and the level of SIBO (27). Furthermore, the most prominent bacteria that overgrow in patients with impaired transit are Gram-negative organisms, mostly *E. coli* (25). We previously showed that the vast majority of bacteria that overgrow the CF mouse small intestine are Gram negative (38). From sequencing the 450-bp 16S rRNA PCR product in that study, it was not possible to distinguish between the closely related Enterobacteriaceae family members *E. coli*, *Escherichia fergusonii*, *Escherichia vulneris*, and *Photorhabdus luminescens*. We have since used primers that span most of the 16S rRNA gene and were able to classify the overgrown species as *E. coli*. Thus, in the CF mice as in humans with impaired small intestinal transit, the predominant bacterial species that overgrows the small intestine is *E. coli*.

The link between bacteria and intestinal motility is strengthened by my novel finding that broad-spectrum antibiotic treatment of WT mice significantly slowed intestinal transit. This result is similar to the slowed transit observed in germ-free animals (26). Whether the effect of antibiotics on transit in WT mice is due to a reduction in bacterial load is an open question. It is conceivable that the antibiotics used (ciprofloxacin, metronidazole) could have effects on intestinal motility. An example of an antibiotic that affects motility is erythromycin, which is prokinetic because of its ability to activate the motilin receptor. Another possible explanation is that, because the antibiotic treatment was fairly long (3 wk), the intestines could have been colonized by microbes that are resistant to these antibiotics and have inhibitory effects on intestinal transit. This issue will require further investigation.

It should be noted that although the GCF values were similar comparing antibiotic-treated WT mice (Fig. 7B) with either untreated (Fig. 7A) or treated CF mice (Fig. 7B), the distribution of the fluorescent tracer was somewhat different in the untreated WT mice. In untreated or antibiotic-treated CF mice, rhodamine-dextran was most concentrated in the first tenth of the small intestine and rapidly decreased in the next two or three tenths (Fig. 6, A and B). The distribution of fluorescence in the antibiotic-treated WT small intestine was distinct in that the fluorescence was rather evenly spread over the first four tenths, with a sharp drop-off by the fifth tenth (Fig. 6B). So, although intestinal transit was lessened by antibiotic treatment of WT mice, the pattern was still different from that of antibiotic-treated CF mice, indicating that there are other factors besides bacteria that alter transit in the CF intestine.

The initial cause of the bacterial overgrowth in CF is not yet known. One possibility is that abnormal accumulation of mucin in the lumen of the small intestine provides a niche for bacterial colonization. Mucus is believed to accumulate in the CF small intestine due to the dehydrated acidic environment that occurs when CFTR function is absent (29, 38). Following the initial colonization, the bacteria may then cause a mild inflammation that stimulates more mucus secretion (17), which increases the niche they can occupy. As bacteria overgrow, they can then slow intestinal transit, further enhancing their ability to remain in the small intestine. This scenario would explain SIBO in CF as a secondary effect of loss of CFTR and the decrease in fluid secretion and improper regulation of luminal pH that follows.

An alternative explanation could be a more direct effect of loss of CFTR on gastrointestinal motility. It was recently reported that there are alterations in the structure and innervation of smooth muscle cells in the CF mouse lung (43). It was suggested that the changes were related to the fact that CFTR is expressed in the neuroendocrine cells of the lungs, and these cells have developmental roles that are perturbed in CF. There have been no reports of CFTR expression in the muscularis or enteroendocrine cells of the intestine (2), so it is unclear whether a similar mechanism can be proposed to play a role in slowed intestinal transit in CF.

In summary, the current study provides evidence that slowed small intestinal transit occurs in the CF mouse, and this likely has a prominent role in SIBO. Because many of the gastrointestinal complications of CF can be explained by SIBO, it is important to understand how this occurs and what its effects are on gastrointestinal function and nutrition. It remains to be determined whether there is a direct effect of loss of CFTR on the enteric nervous system and musculature, or if the effects are secondary to an altered microbial ecology in the CF gut.

ACKNOWLEDGMENTS

I thank Oxana Norkina and Donna Ziemer for assistance with 16S rRNA qPCR analysis, Eileen Roach for figure preparation, and Lauren Meldi for managing the mouse colony.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-56791 and Cystic Fibrosis Foundation Grants DELISL05G0 and DELISL06G0.

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