CHAPTER 32
Microbial therapy for cystic fibrosis

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32.1 Introduction: pathophysiology of cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive inherited disease in Caucasians, caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which leads to a deficiency or absence of functional CFTR proteins at the apical membrane of epithelial cells in several body systems[1]. CFTR protein is an ion channel that controls Cl^- and H_2O transepithelial flux. CFTR loss results in electrolyte abnormalities and an acidic pH that has consequences on mucus consistency in the extracellular milieu. As a result of CFTR impairment, epithelial cells in lungs, pancreas and intestine produce abnormally thick, sticky mucus. In addition to its role as a chloride channel, CFTR protein also affects bicarbonate transport, inducing the formation of dehydrated and viscous mucus in the lungs and digestive tract, with an increased risk of recurrent and chronic pulmonary infections and inflammation, pancreatic insufficiency (PI), CF-related liver disease (CFRLD) and diabetes (CFRD). The prevalence of CF-related chronic comorbidities has increased in line with the substantial extension of life expectancy of CF subjects[2].

The clinical hallmark of CF is recurrent severe pulmonary inflammation and infection, beginning in early childhood and responsible for progressive respiratory failure defined as pulmonary exacerbation. The most common respiratory pathogen in CF patients is *Pseudomonas aeruginosa*. Other bacteria originating from the lungs or gastrointestinal tract may act as pathogens in CF. Patients colonized with *P. aeruginosa* are at increased risk for pulmonary infections and persistent inflammation and frequently require multiple antibiotic treatment. Pulmonary exacerbations are the leading cause of morbidity with a progressive decline in lung function. The pathogenesis of intestinal involvement in CF is multi-factorial and several mechanisms are involved[3]: (i) loss of the functional CFTR chloride channel with poorly hydrated and acidic luminal fluid; (ii) reduced digestive enzyme activity from the exocrine pancreas, which impairs digestion and absorption; (iii) increased mucus production with viscous mucus, and (iv) impaired mucus clearance secondary to disturbed motility.

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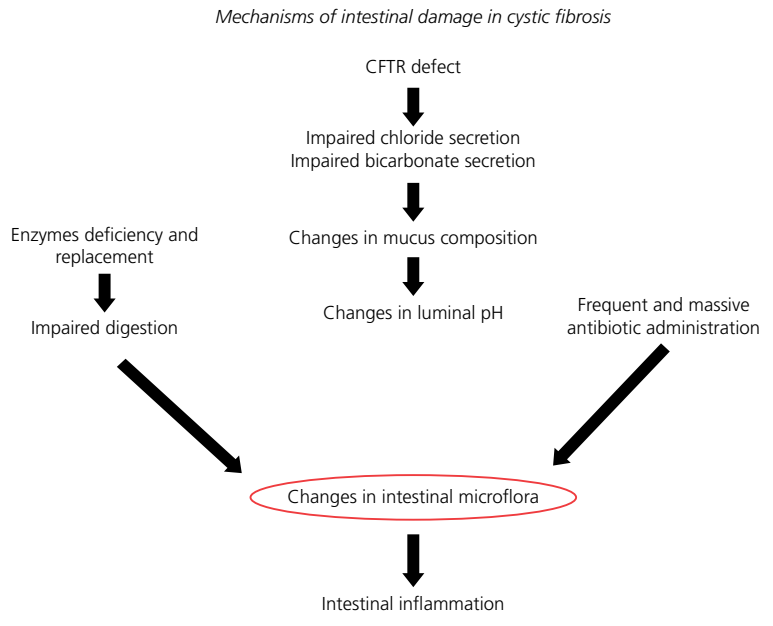

Figure 1 The intestine is a target organ in CF and a major role may be played by the disrupted microflora. This contributes to the intestinal inflammation and may affect the rates and severity of respiratory involvement. Probiotic administration may in part restore intestinal microbiota, reduce intestinal inflammation and, together with other treatment, reduce the risk of pulmunary exacerbations, ultimately improving the long-term outcome of this progressive disease.

Although CF is typically considered a disease targeting the respiratory tract, CFTR defects also affect organs such as the intestine[3]. The intestinal epithelium shows a proximal-to-distal gradient with the highest CFTR expression in the duodenum and decreasing distally along the small intestine to the ileum[4]. The gastrointestinal tract may be strongly affected by CF, with negative impact on nutritional status and poor nutritional status, associated with pulmonary deterioration and predicting a fatal outcome[5]. Mouse models of CF have greatly contributed to understanding of the physiopathology of gut involvement. In all affected epithelia, including the CF intestine, mutations in the *CFTR* gene result in total or partial loss of chloride channel function. Combined loss of fluid volume and abnormal acidity in the intestinal lumen[6] lead to the accumulation of mucus, which will affect digestion, nutrient absorption, motility, the gut microbiota and gut inflammation (Figure 1).

32.2 Intestinal inflammation in CF

Chronic intestinal inflammation has been demonstrated in cystic fibrosis patients, even in the absence of gastrointestinal symptoms. In a study comparing duodenal endoscopy, specimens from 14 pancreatic-insufficient patients and 20 healthy controls, the mucosal morphology appeared normal, but increased infiltration of the lamina propria by mononuclear cells expressing inflammatory markers, such as the intercellular adhesion molecules ICAM-1, CD-25, IL-2 and IFN-gamma, was described[7]. Considering that patients with chronic pancreatitis but not CF do

not show duodenal inflammation, the inflammation in CF could be the direct result of an abnormal immune response or might be linked to the CFTR defect. Gut lavage collected from 21 asymptomatic pancreatic-insufficient children with CF showed significant increases in the concentrations of albumin, IgG and IgM, eosinophilic cationic protein, neutrophil elastase, IL-8 and IL-1 compared to 12 controls[8]. This data supports the hypothesis that intestinal immune activation could be linked to the CFTR defect. More recently, using wireless capsule endoscopy in 41 patients with CF, including 13 pancreatic-sufficient patients, signs of small-bowel inflammation were identified in 60% of the CF cohort[9]. In a study of unselected CF children the authors evaluated fecal calprotectin, a non-specific marker of intestinal inflammation that is increased in inflammatory bowel diseases, and found that 29 out of 30 pancreatic-insufficient CF patients showed an increased fecal calprotectin concentration, significantly higher than that found in healthy controls,[10] raising the hypothesis that intestinal inflammation could be a major feature of CF, independent of other features. However, fecal calprotectin was increased in pancreatic-insufficient patients but was in the normal range in pancreatic-sufficient patients in a subset of the Werlin *et al*. study[9].

More recently, an increase in fecal calprotectin concentration was confirmed in CF patients, although the authors were unable to demonstrate a correlation between increased fecal calprotectin concentration and pancreatic insufficiency[11]. The authors found that 10 of 16 CF patients with pancreatic sufficiency had elevated fecal calprotectin concentration, with increase comparable to those of patients with pancreatic insufficiency. This supports the hypothesis that intestinal inflammation is a feature of CF regardless of pancreatic status and that "CF enteropathy" is independent of pancreatic involvement[11].

The course of CF-related intestinal inflammation is as yet unclear. Patients with lower forced expiratory volume in 1 second (FEV1) showed higher fecal calprotectin concentrations. Lower FEV1 is associated with worse pulmonary status. The increase in respiratory secretions probably contributes to increased intestinal inflammation[8]. Bacteria and other contents of sputum may be involved in direct stimulation of the intestinal mucosa and calprotectin in the sputum may itself increase the fecal levels. However, another hypothesis to explain intestinal inflammation in CF is that inflammatory triggers such as cytokines or bacterial products (e.g. lipopolysaccharide (LPS)) activate intestinal monocytes and epithelial cells. Increased levels of fecal calprotectin concentration were found in children with CF and with aberrant intestinal microbial composition. CF children showed an abundance of *E. coli* in fecal microbiota compared to healthy controls. In particular, a significant correlation was found between *E. coli* abundance and fecal calprotectin and the fractional amount of fat in the stools, suggesting that *E. coli* may directly contribute to cystic fibrosis gastrointestinal dysfunction[12].

32.3 Dysbiosis in CF

CF patients have multiple risk factors for altered intestinal microbiota, including thick intestinal mucus, constipation and slow intestinal motility. In addition, they must frequently undergo heavy courses of antibiotics[13]. Fecal microbiota population structure showed a temporal instability and reduced species richness in CF children

compared with healthy controls[14,15]. Dysbiosis in CF patients included a reduced presence of Bifidobacteria, Clostridium cluster IV and XIVa and Firmicutes and increased concentrations of Proteobacteria[16,17]. In CF children, dysbiosis is an aberrant early event due to a very unstable gut microbial community. An aberrant microbial structure was observed in CF children compared with healthy controls. In particular, the levels of *Eubacterium rectale, Bacteroides uniformis, Bacteroides vulgatus, Bifidobacterium adolescentis, Bifidobacterium catenulatum*, and *Faecalibacterium prausnitzii* were decreased in children with CF[16,17].

Salami and Marsland in chapter 24 suggested that through pharyngeal reflux and microaspiration of gastro-intestinal and breastmilk contents in neonates, gut-derived bacteria could be the colonizers of airways in humans. Along these lines, the existence of a gut-lung axis in CF has recently been hypothesized (Figure 2). However, experimental support for a link between intestinal microbiota and bacterial colonization of the respiratory tract is far from being obtained. A very interesting aspect is the early development of gut and airway microbiota in CF infants. A recent study analyzed gut and airway microbiota in CF newborns approximately every three months up to the first 21 months of age. Interestingly, the gut and respiratory tract microbiota showed similar results with a clear association of a large number of genera that were present in the gut prior to colonization of the respiratory tract[18]. Also, the severity of the disease correlates with intestinal dysbiosis in CF[19]. The severity of CF depends on host genetic variation. There are several genetic mutations responsible for the maturation process and transfer of CFTR to the apical compartment of cells. One of the most common mutations, the

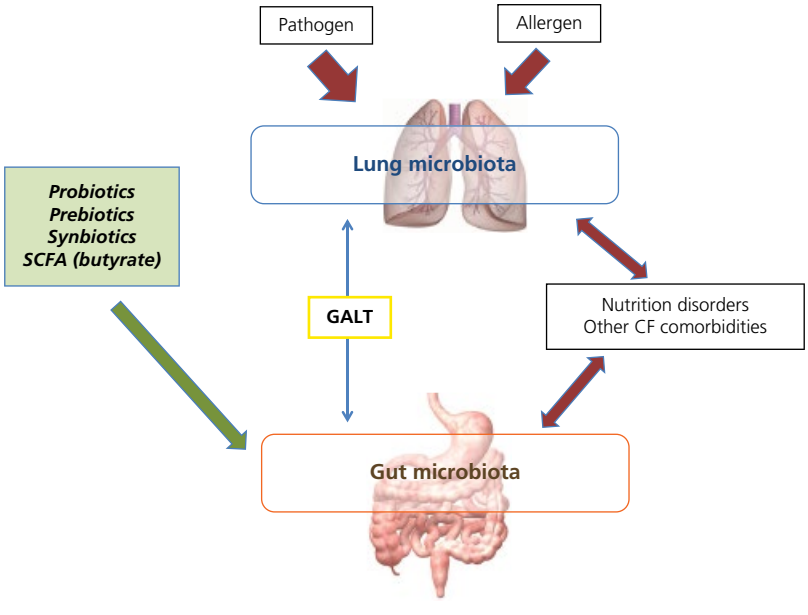

Figure 2 Gut-lung axis in CF. A link between intestinal microbiota and bacterial colonization of the respiratory tract has been hypothesized. Manipulation of intestinal microbiota through the administration of probiotics, prebiotics and synbiotics may influence lung function and nutritional status in patients with CF (modified by reference 25). SCFA: short-chain fatty acids; GALT: gut-associated lymphatic tissue.

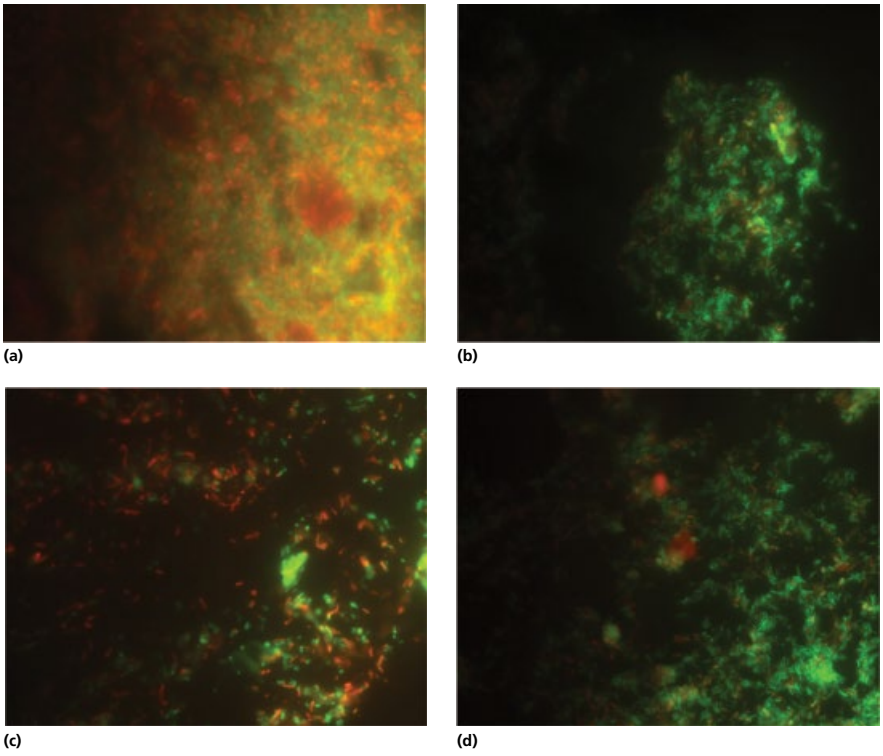

Figure 3 Habitually constitutive bacteria intestinal microflora evaluated by FISH (fluorescence in situ hybridization). Top: Total bacteria and Faecalibacterium prausnitzii were evaluted by EUB (green) and Fpra (red) probes in healthy (a) and cystic fibrosis (b) children. Bottom: Total bacteria and Eubacterium rectale were evaluted by EUB (green) and Erec (red) probes in healthy (c) and cystic fibrosis (d) children. Images kindly provided by Dr. V. Buccigrossi. (*see color plate section for color details*).

F508del, is associated with the most severe phenotypes of CF. It was reported that dysbiosis is enhanced in homozygous F508del patients as shown by the increase in harmful bacteria, such as *Escherichia coli* and *Eubacterium biforme*, and a decrease in beneficial species such as *Faecalibacterium prausnitzii, Bifidobacterium spp* and *Eubacterium limosum*[19].

CF is characterized by an increased susceptibility to respiratory infections, and as a consequence CF patients frequently undergo antibiotic therapy. Hence, dysbiosis also depends on the massive antibiotic use (Figure 3). The pressure of high doses of multiple antimicrobial agents to which CF patients are frequently exposed has a dramatic impact on the bacterial colonization, especially in the intestines[20]. Antibiotics induce the loss of *Oxalobacterformigenes*, an important commensal agent that metabolizes oxalate[21]. As a consequence, CF patients are at risk of hyperoxaluria and formation of calcium-oxalate kidney stones. On the other hand, *Clostridium difficile* frequently colonizes CF patients[22]. A reduced concentration of *Bacteroides uniformis, Bacteroides vulgates, Eubacterium rectale, Bacteroidetes* and *Faecalibacterium prausnitzii* was observed compared to healthy controls[23]. In addition, E*scherichia coli* was more abundant in children with CF and was correlated with intestinal inflammation and with intestinal dysfunction in children with CF[12].

Several bacterial species are associated with mucosal inflammation. For example, butyrate is an important energy source for the colonic mucosa, protecting it from colitis and colorectal cancer and promoting the normal development of colonic epithelial cells (discussed in chapter 27 by Sobhani). *Eubacterium rectale* may have a protective role against intestinal inflammation through production of butyrate. In addition, the reduction of *Faecalibacterium prausnitzii* is considered a hallmark of chronic inflammatory disease[24]. A significant inverse correlation between the richness of CF microbiota and intestinal inflammation was observed[23] and children with fecal calprotectin concentrations greater than 200 mg/g showed a significantly lower number of Bacteroidetes in DGGE analysis[23].

In summary, several lines of evidence support the concept that the intestinal environment is abnormal in CF and, together with massive antibiotic therapy, promotes the development of an aberrant microbiota. This is associated with intestinal and respiratory inflammation.

32.4 Microbial therapy in CF

Dietary approaches (mainly manipulation of fat and indigestible carbohydrates) or probiotic and/or prebiotic administration may provide an effective early intervention to restore the abnormal intestinal microbiota in CF, namely to enhance gut microbiota diversity in order to modify the course of lung colonization, thereby ultimately improving patient outcomes. In particular, oral administration of probiotics and prebiotics or a combination of both (the so called "synbiotic approach" described in chapter 30) could influence the composition of the airway microbiota, either indirectly, through the release of bacterial products or metabolites that reach the lung and favor the outgrowth of probiotic bacteria, or directly, via microaspiration of the probiotic strain from the intestinal tract to the airways. These theoretical mechanisms may restore a health-promoting microbiota and have a beneficial effect on the course of the disease[25]. Given the continuous increase of bacterial resistance to antibiotics and the lack of novel antibiotics, the interest in these bacteria for management of broncho-pulmonary exacerbations in CF is increasing. Neither breastfeeding nor the introduction of non-solid food had effects on biodiversity of gut or airway microbiota in CF over time. On the other hand, statistical clustering of the gut and airway microbiota samples demonstrated that specific groups of bacteria are associated with solid-food introduction and breast-feeding[18]. There is increasing evidence that probiotics modulate immune response in the lung; in particular, gut microbial stimulation can enhance T regulatory response in the airway through an interaction with the gut-associated lymphatic tissue (GALT), such as Peyer's patch cells, influencing pulmonary inflammatory response[25,26]. The mechanisms underlying these effects of probiotics are far from being understood. Intranasal and oral probiotics induced up-regulation of natural killer cells and macrophage activity in the airway mucosa, expansion of T-regulatory cells, increase of the IgA-secretory cells in the bronchial mucosa, production of antibacterial compounds, and inhibition of virulence factors[27,28].

Probiotics have an anti-inflammatory effect activating specific microbe-derived ligands signaling pathways[26]. However, few probiotic strains have a role in modifying the course in lung involvement. In animal studies lactobacilli have profound

immunoregulatory effects on the lung, but results of clinical trials in humans have been highly variable. Strain differences may in part explain the observed variability. In humans administration of Lactobacillus GG (LGG) negatively influences the incidence of ventilator-associated pneumonia[29] and reduced respiratory infections in healthy[30] and hospitalized children[31]. The first evidence of the potential benefits of probiotic administration in CF came from a prospective randomized placebo-controlled cross-over trial performed in two groups of patients with CF chronically colonized by *Pseudomonas aeruginosa* (PA). Nineteen children were given LGG for six months followed by placebo (oral rehydration solution) for the subsequent six months. At the same time, 19 children were given the placebo for six months and then the probiotic for the same period of time. The patients on LGG had a significant reduction in intestinal inflammation and of episodes of pulmonary exacerbations and hospitalization rates, with a decrease in IgG, suggesting that there is a relationship between intestinal and pulmonary inflammation. The intake of this probiotic was associated to a significant increase of the maximal FEV1 compared to the placebo as well as to a significant increase in body weight[32].

Those important findings were in part confirmed by a study with a commercially available mixture of probiotics (*Lactobacilllus, Bifidobacterium and Streptococcus thermophiles* spp.) given to 10 patients chronically infected with PA for six months with a significant reduction in the pulmonary exacerbation rate compared to the two previous years[33]. In addition, administration of *Lactobacillus reuteri* (LR) was effective in reducing the number of pulmonary exacerbations and of upper-respiratory-tract infection in patients with CF[34]. In this prospective randomized, double-blind, placebo-controlled study, 61 patients with CF were randomly assigned to receive 10^{10} colony-forming units LR in drops per day or placebo for six months. However, probiotics did not change hospitalization rates, FEV1 or fecal calprotectin and cytokines[34]. In a population of non-selected children and adolescents with CF, LGG supplementation reduced intestinal inflammation as assessed by fecal calprotectin concentration and rectal nitric oxide[10,23]. LGG also restored intestinal microbiota as shown by an increase in *Bacteroides* counts[23]. Therefore LGG significantly decreases intestinal inflammation (in particular calprotectin levels) and increases digestive comfort, restoring in part the normal microbial homeostasis as shown by the total bacterial density and the increase in microbial diversity. Similar results were reported with *Lactobacillus reuteri* [17]. However, the effects of probiotics are likely to be time-, dose- as well as strain-dependent, indicating the need for comparative clinical trials on probiotic therapy in CF. Future work is required in order to identify reliable biomarkers of intestinal inflammation to be used in long term clinical trials on targeted anti-inflammatory therapies in the gut[35].

Less is known about the potential use of prebiotics in CF management. In one study in rats, administration of prebiotics such as the non-digestible carbohydrate fructo-oligosaccharide increased cecal active GLP-1 levels acting via modulation of gut hormones induced by gut microbiota variations[36,37]. Active GLP-1 levels are influenced by pro-inflammatory cytokines and reduced levels of active GLP-1 may be associated with CFRD onset[36]. Modification of gut microbiota by changing dietary content of indigestible carbohydrate may improve under-nutrition in human CF[38]. Prebiotic supplementation of probiotic products is proposed to prolong the intestinal survival of the probiotic strain and therefore enhance immunomodulatory capacity. Human breast milk contains oligosaccharides as well as lactic

acid bacteria, which makes it a natural synbiotic. Synbiotics have produced some positive results in the treatment of conditions associated with chronic inflammation[39]. Thus, synbiotic approaches might be more effective in controlling CF than probiotic or prebiotic treatment alone and might therefore be an effective prophylactic or therapeutic intervention. Finally, manipulating gut microbiota by changing dietary content of indigestible carbohydrate and short-chain fatty acids (butyrate in particular) may both improve undernutrition and have an anti-inflammatory effect in gut microbiota in CF and indirectly on the lung[25].

32.5 Conclusion

In conclusion, the gastrointestinal tract may be strongly affected by CF with a negative impact on nutritional status. CFTR dysfunction results in chronic inflammation and in an altered microbial composition in lung and intestine. Some evidence for the gut-lung axis in CF comes from the link between intestinal microbiota and bacterial colonization of the respiratory tract. In addition, the effect of a restructure of intestinal microflora on intestinal inflammation and respiratory lung function provides new and compelling proof. Probiotics and/or prebiotics in CF have shown some promise, including potential benefits in nutritional status, energy intake and respiratory function. However, their effect requires further clarification before therapeutic implementation.

TAKE-HOME MESSAGE

- The intestine is a target organ in cystic fibrosis and intestinal inflammation has been demonstrated in patients even in the absence of gastrointestinal symptoms.
- The pathogenesis of intestinal involvement in CF is multifactorial and several mechanisms are involved.
- Dysbiosis is a feature of CF children as a consequence of CFTR dysfunction, frequent use of antibiotics, pancreatic enzyme supplementation and modification of intraluminal pH.
- Intestinal inflammation and respiratory lung function are associated with aberrant gut microbiota composition.
- A restructure of gut microbiota, through probiotics and/or probiotics, might improve nutritional status, energy intake and respiratory function.

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