Cystic Fibrosis: Pathophysiology of Lung Disease

Christelle Bergeron[1] André M. Cantin[1,2]

[1] Respiratory Division, Department of Medicine, Faculty of Medicine and Health Sciences, University of Sherbrooke, Quebec, Canada
[2] Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke, Quebec, Canada

Address for correspondence André M. Cantin, MD, Pulmonary Research Unit, Faculty of Medicine, University of Sherbrooke, 3001, 12ième Avenue Nord, Sherbrooke, QC, Canada J1H 5N4 (e-mail: Andre.Cantin@USherbrooke.ca).

Semin Respir Crit Care Med

Abstract

Cystic fibrosis (CF) is a common, life-threatening, multisystemic, autosomal recessive disorder. In the last few years, giant steps have been made with regard to the understanding of CF pathophysiology, allowing the scientific community to propose mechanisms that cause the myriad of CF clinical manifestations. Following the discovery of the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene in 1989, the structure and function of the CFTR protein were described. Since then, more than 2,000 variants of the *CFTR* gene and their impact on the amount and function of the CFTR protein have been reported. The role of the CFTR protein as an ion channel transporting chloride and bicarbonate and its repercussions on different epithelial cell-lined organs and mucus are now better understood. Mechanisms behind susceptibility to infection in CF have also been proposed and include abnormalities in the composition, volume and acidity of the airway surface liquid, changes in the submucosal gland's anatomy and function, and deficiencies in the mucociliary clearance system. Numerous hypotheses explaining the excessive inflammatory response in CF are also debated and involve impaired mucociliary clearance, persistent hypoxia, lipid abnormalities, protease and antiprotease disproportion, and oxidant and antioxidant imbalance. The purpose of this review is to summarize our current knowledge of CF pathophysiology, including significant historic discoveries and most recent breakthroughs, and to improve understanding and awareness of this fatal disease.

Keywords
- cystic fibrosis
- pathophysiology
- CFTR
- infection
- inflammation
- proteases
- oxidants
- mucins

Cystic fibrosis (CF) is a common, life-limiting, multisystemic, autosomal recessive disorder.[1] Its incidence varies among populations and is the highest in Caucasian communities of Northern European ancestry, with approximately 1 in 3,000 live births.[2] Descriptions of premature deaths in children with high salt concentrations in their sweat first appeared during the 17th century, but the disease was only recognized as a distinct clinical entity by Dorothy H. Andersen in 1938.[3,4] Ten years later, in 1948, Paul di Sant'Agnese introduced the concept of abnormal ion transport and observed disturbances in sweat composition after an important heat wave in New York caused heatstroke in many of Andersen's patients.[4–6] Since then, our understanding of CF pathophysiology has made giant steps, beginning with the discovery of the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene by Lap-Chee Tsui, John R. Riordan, and Francis Collins in 1989 in Toronto.[7–9] In the last three decades, numerous discoveries have been made and have influenced the course and the management of CF. At present, physicians and researchers better understand the normal structure and function of the CFTR protein.[10] It is currently well known that absent or dysfunctional CFTR protein causes damage in different organs lined with epithelial cells and mucus, including the lungs, the pancreas, the gastrointestinal tract, the liver, and the reproductive tract.[1] Moreover, different classes of mutations in CF and their impact on the CFTR protein and on CF phenotypes were discovered.[11] Several hypotheses have been made regarding topics such as the role of mucins, inflammation, proteases, and the balance of

Issue Theme Cystic Fibrosis: Advances in Understanding and Treatment; Guest Editors: Patrick Flume, MD, Christopher H. Goss, MD, MS, FCCP, Donald Van Devanter, PhD

DOI https://doi.org/10.1055/s-0039-1694021.
ISSN 1069-3424.

oxidants and antioxidants in CF.[12] Thanks to our better understanding of the underlying mechanisms in CF, individuals suffering from this fatal disease have seen their life expectancy improve year after year.[13] The purpose of this review is to describe hypotheses proposed to explain the defects causing CF lung disease, to summarize our current knowledge of CF pathophysiology, to discuss notions still under debate, and to explore future avenues of research. Hopefully, further progress will help refine our mastery of CF pathophysiology and lead us toward a definitive cure.

Historical Milestones Leading to the *CFTR* Gene Discovery

The Mucus Abnormality Hypothesis

After initial observations of CF were made during the 1930s and 1940s, the idea that CF was a disorder caused by the accumulation of abnormally thick and sticky mucus became popular in the scientific community.[4,14] Increased amounts of abnormally viscous mucus were observed in different organs such as the pancreas, the lungs, the gastrointestinal tract, and the reproductive tract. This thick mucus was associated with complications such as pancreatic insufficiency, gallstones, meconium ileus, infertility, sinusitis, and bronchiectasis.[4] Although the production of thick and viscid mucus could explain the majority of the clinical manifestations observed in CF, it could not account for the abnormally high salt concentration in the sweat of CF individuals. This discrepancy encouraged investigators to pursue their quest of the understanding of CF pathophysiology.

The Sodium Hyperabsorption Hypothesis

In the early 1980s, Knowles et al explored the path of electrolyte abnormalities in the airways. In 1981, they reported that the electronegative difference across the nasal epithelium was significantly higher in CF subjects than in normal individuals. They postulated that the larger negative potential difference in the airways was due to an increased absorption of sodium from the airway fluids in the lumen, leading to dehydration that resulted in thick mucus.[15] This discovery allowed the scientific community to define the first physiological link between the anomalies described in the lungs, in the pancreas, and in the sweat glands of CF patients. It meant that the basic defect was not in mucus, but in electrolyte transport in the CF cells.[4] It was later proven that the increased negative potential difference was due to an impermeability to chloride rather than to an increased absorption of sodium.[16] Nonetheless, the hypothesis advanced by Knowles et al was not completely incorrect. Indeed, it is currently well recognized that the CFTR protein influences other ion channels, including epithelial sodium channels (ENaC), on which it has an inhibitory effect, generating reduced levels of sodium absorption. In CF, ENaC activity is enhanced because dysfunctional CFTR is unable to play its inhibitory role, leading to increased sodium absorption from the lumen.[10,17] In the end, three major findings with regard to CF epithelia were retained from their work: CF epithelial tissues are characterized by a larger transepithelial potential difference, a greater response to amiloride, and a decreased permeability to chloride at the luminal surface.[18]

The Sweat Glands and the Chloride Impermeability Hypothesis

In 1983, Paul M. Quinton, who was diagnosed himself with CF at the age of 19, was the first to consider that the disease could be related to an abnormal permeability to chloride in the sweat glands. He refuted the previous hypothesis, which suggested that CF was due to increased rates of sodium reabsorption.[4] He proved his theory by dissecting sweat glands and isolating the reabsorption duct of five control subjects and three CF patients. He collected measures of transepithelial potential differences and realized that they were more negative in CF specimens. He explained this phenomenon by a difference in the permeability of anions in the tissues rather than by a defective anion exchange and came to the conclusion that chloride impermeability was the reason of poor reabsorption of sodium chloride in CF sweat ducts and high concentrations of salt in the sweat of these individuals.[16] His theory is still unquestionably recognized today and explains the basic cellular defect at the origin of our understanding of CF pathophysiology.

The CFTR Gene Discovery

The discovery of the CF gene was one of the most important achievements in CF research, and in science in general. In 1989, in Toronto, after extensive research and analyses, Lap-Chee Tsui and his team identified a single CF locus on human chromosome 7 (region q 31).[8] Because the gene product was still unknown, the identification of the gene needed to occur without a chemical marker. Researchers opted for a different approach, called positional cloning, using restriction fragment length polymorphism (RFLP) markers to localize the CF gene.[4,8] Investigators were able to analyze 280 kb of contiguous DNA isolated by chromosome jumping and walking and clone the locus responsible for CF.[9] The most common CF mutation, present in more than two-thirds of CF alleles, was identified during the same period, and defined as a deletion of three base pairs, which results in the loss of a phenylalanine residue at amino acid position 508 (legacy name F508del).[8] Riordan et al eventually identified the CF gene product, a polypeptide made of 1,480 amino acids with a molecular mass of 168,138 daltons. They named it CFTR.[7] The basic defect responsible for CF had been discovered, the structure and the properties of the CFTR protein had been described, and it was becoming more and more evident that CFTR was involved in ion transport across the apical membrane of epithelial cells. It was the beginning of a new era.

Normal Structure and Function of the CFTR Protein

Following the identification, the sequencing, and the cloning of the *CFTR* gene, the CFTR protein structure was described.[7] The CFTR glycoprotein is a member of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter superfamily and is located at the apical surface of epithelial cells, where it regulates ion transport and fluid homeostasis. It comprises two transmembrane domains (TMDs), each arranged in six subunits, which form a pore that controls the passage of

specific anions.[19] Other unique features of the CFTR protein are the presence of a cytoplasmic regulatory domain (R domain) and of two nucleotide (ATP) binding folds (NFB1 and NFB2). The R domain contains phosphorylation sites, which allow, if phosphorylated by protein kinase A, the activation of the channel. Following the phosphorylation of the R domain, the nucleotide binding folds interact with the TMDs and proceed to ATP hydrolysis to modify the conformation of the channel from an open state (active) to a closed one (quiet). Consequently, the level of activity and the probability of channel opening are dependent on both the phosphorylation state of the R domain and ATP hydrolysis.[10]

The primary function of the CFTR protein is to regulate the transport of chloride ions (Cl^-) across apical membranes. Its open state allows chloride anions to cross the epithelial tissues at the apical surface.[19] Bicarbonate ($HCO3^-$) transport also occurs directly across the CFTR channel and its function influences the pH of epithelial cell surfaces and mucus.[20] Moreover, the CFTR protein has been shown to indirectly regulate other ion channels. It has a strong inhibitory effect on ENaCs[21] and it influences $HCO3^-/Cl^-$ exchangers.[10] Finally, CFTR also influences other chloride channels such as calcium-activated chloride channels (CaCC) and outward rectifying chloride channels (ORCC).[10,22]

Abnormal CFTR Protein in Cystic Fibrosis

Impact of the Abnormal CFTR Protein on Ion Transport and on Other Ion Channels

In the lungs, the "isotonic, low-volume" model is currently the favored model to explain the mechanism by which absent or abnormal CFTR protein causes thick and adherent mucus, lung infections, and inflammation.[23] This theory postulates that abnormal CFTR causes a hyperstimulation of ENaC, leading to an increased absorption of sodium and a more negative transepithelial potential difference. This phenomenon generates a relative increase of chloride ion transport toward the interstitium through other non-CFTR channels, leading to a net increase in sodium chloride (NaCl) absorption and water absorption by osmotic gradient. This process results in depletion of volume of the airway surface liquid (ASL), producing thick and viscous mucus and eventually leading to reduced mucociliary clearance, inflammation, infection, and bronchiectasis.[24]

Abnormal CFTR also involves aberrant secretion of bicarbonate ($HCO3^-$) and dysfunction of $HCO3^-/Cl^-$ exchangers, causing abnormally low pH in the airway lumen, which could decrease the activity of antimicrobial peptides and result in further inflammation and risk of bacterial infections.[20,25,26] Defective bicarbonate secretion in the CF airway lumen is compounded by the secretion of protons through the non-gastric adenosine triphosphatase ATP12A resulting in further acidification of ASL.[25] Interestingly, murine airway epithelial cells express low levels of ATP12A and are resistant to lung infection even in the absence of CFTR.

In the pancreas, abnormal CFTR causes diminished $HCO3^-$ secretion from the pancreatic duct cells into the lumen, leading to enzyme precipitation, mucus accumulation, and eventually pancreas destruction.[27] In the sweat glands, abnormal CFTR reduces sweat production via the β-adrenergic pathway. It also diminishes the chloride permeability, leading to decreased reabsorption of chloride ions from the ductular lumen into the interstitium, creating an increased transepithelial potential difference and a high concentration of sodium chloride in the sweat.[28] This principle is used for diagnosis purposes with the sweat chloride test.[29]

The defective CFTR also appears to have an impact on the development of certain tissues, leading to hypoplastic sinuses and malformations of the trachea in neonatal pigs and children.[30,31]

Different Classes of Variants (Mutations) and Their Impact on the CFTR Protein

Presently, more than 2,000 variants of the *CFTR* gene have been reported, although not all of these variants cause CF (http://genet.sickkids.on.ca). Variants can be categorized as CF-causing, variants of varying clinical consequence, nondisease causing, and variants of uncertain significance (https://cftr2.org). Different variants impact the amount or function of the CFTR protein in different ways with variable consequences. Furthermore, there is a relationship between different *CFTR* variants and their diverse effects on the CFTR protein with sweat chloride concentration and with the different phenotypes expressed in CF individuals.[32] For all these reasons, a system categorizing CF variants into different classes based on their impact on CFTR function was suggested by Lap-Chee Tsui[33] and modified by Welsh and Smith.[34] In the last few years, it has become evident that a single CF variant can result in multiple defects that span several classes. In addition, modifications in *CFTR* classes have been proposed in the context of the discovery of new targeted therapies addressing multiple CFTR defects. A recent updated classification was recommended by De Boeck and Amaral[35] and includes seven different classes:

- Class I variants affect the synthesis of the CFTR protein. This class mostly includes nonsense variants with the presence of a premature termination codon in the mRNA transcript. This results in a complete absence of the CFTR protein due to its rapid degradation by the endoplasmic reticulum (ER). However, some rare class I variants caused by a mis-splicing variant result in the production of small amounts of CFTR transcripts and are associated with milder CF phenotypes.[11]
- Class II variants cause folding defects of the CFTR protein. The protein is produced but misfolded and retained in the ER. The protein is prematurely destroyed before it can traffic to the apical surface of the cell. A small amount of the abnormal CFTR protein sometimes reaches the epithelial interface but is malfunctioning. The c.1521_1523delCTT (legacy name F508del) variant, present in approximately 85% of CF alleles worldwide, is a class II variant. The combination of a corrector and a potentiator has been approved for CF treatment in individuals homozygous for the F508del variant.[36,37]
- Class III variants involve impaired gating of the CFTR channel, with a reduced response of the CFTR protein to ATP resulting in a reduced open probability. The variant

G551D, for which the potentiator ivacaftor has been approved, is a class III variant.[38] Several other class III *CFTR* variants have been shown to respond to ivacaftor.

- Class IV variants cause a decrease in the CFTR channel conductance. The flow of chloride and bicarbonate ions is reduced. R117H variant is a class III and IV variant whose function is also improved by ivacaftor.
- Class V variants produce a reduced level of normal and functional CFTR proteins. They are often caused by alternate splicing defects, which produce both normal and aberrant mRNA transcripts. The proportion of functional CFTR protein can vary between CF patients and even in different organs for a same individual.
- Class VI variants generate instability of the CFTR protein at the apical surface of the epithelial cells. It can be secondary to increased endocytosis of the CFTR protein or abnormal recycling mechanisms.

De Boeck and Amaral suggested an additional class of variants in their proposed classification based on the potential of targeted therapies in the treatment of CF. They included a class VII, regrouping unrescuable variants caused by large deletions leading to a complete absence of mRNA transcription.[35] Marson et al proposed to keep this new class, but to rename it class IA because of the severity of its impact on the CFTR protein. Indeed, these variants produce an outcome similar to the one encountered with class I variants in the traditional classification.[39]

Modifier Genes

Environmental and genetic factors other than the *CFTR* genotype influence disease manifestations in different organs. Meconium ileus is a manifestation of CF of which 88% is defined by heredity rather than environment.[40] One of the key gene modifiers for meconium ileus and lung disease other than *CFTR* is *SLC6A14*. The *SLC6A14* gene product is a Na/Cl^- – dependent transporter of neutral and cationic amino acids, particularly arginine, located at the apical membrane of epithelial cells.[41] Disruption of *SLC6A14* expression markedly aggravates the intestinal obstruction phenotype in CF mice and is strongly associated with meconium ileus in CF newborns.[42]

Not only do genes other than *CFTR* define disease expression, but recent studies have also revealed that genetic signals influencing the expression of a modifier gene such as *SLC6A14* are tissue-specific and can be expressed remotely from the site of disease.[43] Furthermore, several genes that modulate the CF lung phenotype have been identified. The estimated heritability of the lung CF phenotype is estimated to be 50% and the *CFTR* genotype correlates poorly with lung disease severity.[44,45] Among the gene modifiers suspected of affecting CF lung disease are *SLC6A14*, mannose-binding lectin (*MBL*), glutathione-*S*-transferase (*GST*), transforming growth factor-β (*TGF-β*), tumor necrosis factor-α (*TNF-α*), β-2 adrenergic receptor, nitric oxide synthases (NOS), and human leukocyte antigen (HLA) class II antigens.[46,47]

These observations highlight that to fully understand the pathophysiology of CF lung disease, research needs to focus not only on the *CFTR* genotype, but also on modifier genes and tissue-specific genetic variants that influence the expression of the modifier genes. More work will be required to confirm specific genetic modifiers and eventually open the door for personalized therapeutic interventions.

Infection and Inflammation Cycle in CF Lung Disease

The Origins of Infection

For a long time, researchers and clinicians working in the CF field wondered if infection preceded inflammation in CF lung disease or if lung inflammation was secondary to an intrinsic defect of the CFTR protein.[48] Nevertheless, susceptibility to infection occurs only in the airways of CF individuals and not at other sites, suggesting that there is no systemic immune defect in CF.[23] However, inflammation response in CF lung disease is persistent, intense, and ineffective at clearing infectious pathogens.[12] More recently, it was demonstrated that immediately after birth, piglets with CF did not show evidence of inflammation in their airways, although they were more predisposed to be colonized with bacterial organisms and they failed to eradicate bacteria after a pulmonary challenge with *Staphylococcus aureus*.[48,49] These observations suggest that impaired clearance of bacteria is the initial phenomenon, leading to inflammation and structural damage of the CF airways.

The underlying mechanisms explaining the predisposition of CF individuals to develop lung infections have been the subject of extensive study for many years and are summarized in ►**Fig. 1**. In 1996, Smith et al hypothesized that CF airway epithelia fail to kill bacteria because of abnormalities in the ASL.[50] They thought that a bactericidal factor was missing in CF, but after collecting ASL from normal and CF epithelia, they realized that both were equally able to kill *Pseudomonas aeruginosa*. These results led them toward the idea that abnormalities in the composition of ASL were inhibiting the activity of a bactericidal factor. They observed that CF ASL had higher chloride concentrations than normal ASL and found a correlation between increased NaCl concentrations and decreased antibacterial activity. They concluded that a bactericidal factor was part of the ASL covering the apical surface of the epithelial cells and that this substance was dependent on low salt concentration to function properly. Their data provided a link between the physiologic defect of CF and the most common clinical manifestation of CF.[50] Although this theory was appealing, it was not confirmed by the work of other investigators and it is no longer thought to explain the host antimicrobial defect characteristic of CF.

Another hypothesis linked reduced pH at the airway surface with CF airway pathology.[51] This concept was recently reinforced after it was tested in CF pig models by Pezzulo and colleagues.[26] CF pigs represent an interesting model to isolate host defense mechanisms against bacteria because it has previously been demonstrated that they exhibit none of the characteristics of CF lung disease such as inflammation, infection, and structural remodeling immediately after birth.[49] The investigators used grids coated with *S. aureus* or *P. aeruginosa* disposed on the airway surface through a small tracheal

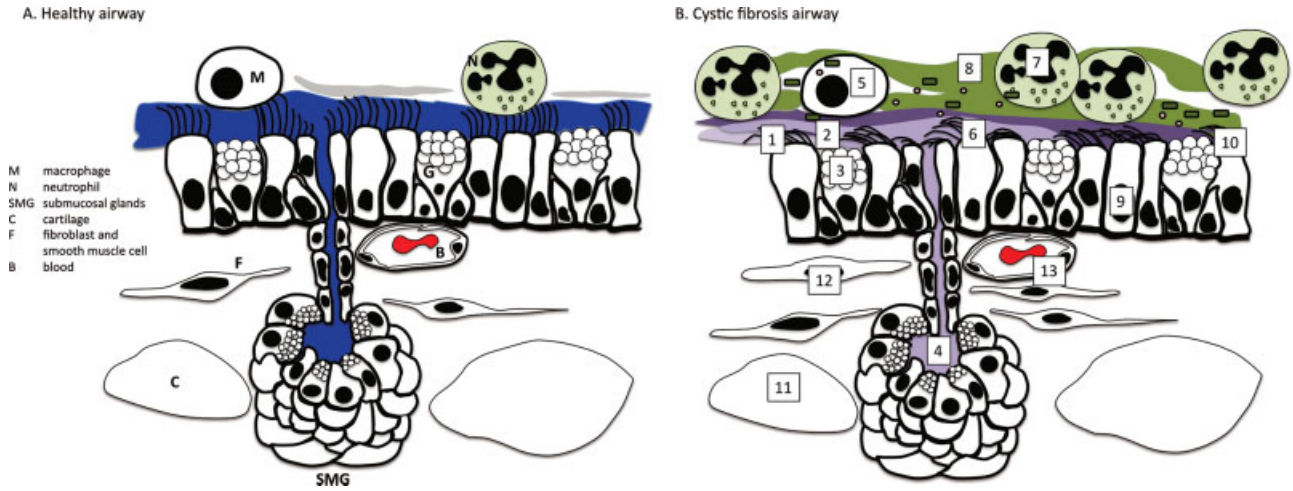

Fig. 1 Representation of (A) the healthy airway and (B) the CF airway. Illustrated are some of the multiple pathophysiological mechanisms associated with CFTR deficiency and CF lung disease. **1**. Poor mucus hydration and mucociliary clearance. **2**. Increased mucus viscosity. **3**. Defective mucin granule expansion and deployment. **4**. Submucosal gland obstruction and deficiency of lactoferrin and lysozyme in airway secretions. **5**. Macrophage phagolysosome defect. **6**. Abnormal arachidonic acid to docosahexaenoic acid (AA/DHA) ratio, ceramide metabolism and altered anti-inflammatory molecules such as resolvins, protectins, maresins and lipoxins. **7**. Neutrophil CFTR-dependent intrinsic defects in oxidant synthesis, granule content release, and increased NET formation. **8**. Hypoxic environment hostile to normal neutrophil function. **9**. Abnormal Nrf2 signaling and glutathione synthesis. **10**. Low glutathione and nitric oxide in CF airway surface liquid. **11**. Structural changes in airway development. **12**. CFTR deficiency leading to hyperresponsiveness of airway smooth muscle cells. **13**. Modifier genes other than CFTR define the CF lung disease phenotype.

incision to prevent or minimize the impact of other mechanisms on bacterial killing such as mucociliary clearance or binding between bacteria and epithelia. They observed that bacteria killing was pH-dependent, with improved antimicrobial activity as pH increased. In non-CF pigs exposed to high carbon dioxide (CO_2) levels, bacterial killing was inhibited and in CF pigs exposed to sodium bicarbonate ($NaHCO_3$), it was enhanced.[26] Because one of the CFTR protein functions is to secrete bicarbonate, these results directly associate the basic CFTR defect to abnormal host defenses against bacteria.

The contribution of airway submucosal glands to impaired host defense mechanisms against bacterial infections has also been a subject of debate.[52] Airway glands are formed by invaginations of the surface epithelium undergoing a series of branchings. A terminal duct, penetrating beneath the epithelial surface, dilates into a collecting duct in which several secretory tubules empty.[53] The terminal duct is lined with an epithelium similar to the surface epithelium (ciliated epithelium), the collecting duct is composed of cells of various types, and the distal ducts are lined with mucous cells and secretory cells.[53] CF lung disease is associated with hypertrophied submucosal glands and hyperplasia of airway surface goblet cells.[54] Engelhardt et al characterized the cellular distribution of *CFTR* gene and protein expression with in situ hybridization and immunocytochemistry. They observed that CFTR mRNA and protein in bronchial tissues were predominantly located in the collecting ducts and serous tubules of the submucosal glands. They hypothesized that the CFTR protein predisposition for collecting ducts could lead to abnormal mucus dilution and flushing by the secretory tubules or to abnormal components of the secretory granules.[54] Other experiments by Engelhardt and colleagues provided strong evidence for a role of submucosal glands in host defense against infection.[52] Gland-free ferret airway epithelial xenografts were created by denuding rabbit tracheas of their surface epithelial cells, seeding the tracheas with ferret airway epithelial cells and transplanting them into immune-deficient mice. Other mice produced gland-containing xenografts. The antibacterial activity of gland-free and gland-containing xenografts was measured, and improved bacterial killing was observed in xenografts containing glands. Higher levels of antimicrobial agents such as lysozyme and lactoferrin were also reported.[52]

Many other theories trying to explain the susceptibility to lung infections in CF have been put forward in the scientific community. Prominent among them is the observation that the reduced volume of ASL prevents adequate ciliary function and interferes with mucociliary clearance. With progression of the disease, mucus impactions occur and adherent mucus plugs and plaques impede bacteria clearance.[23] Airways epithelial cells could also fail to kill bacteria by lacking the major isoform of NOS, NOS-2.[55] Another suggested mechanism with regard to the specific defense against *P. aeruginosa* is that CFTR protein constitutes a receptor for this pathogen, allowing the internalization of the organism, followed by the apoptosis of the epithelial cell involved.[56,57] In the absence of CFTR protein at the epithelial surface, the receptor is also lacking and this process becomes impossible.[23]

Underlying CFTR Defects Leading to Inflammation

In CF lung disease, the inflammatory response to pathogens occurs shortly after birth, exceeds that which is expected for a similar pathogen burden in other diseases, and does not resolve. CFTR-related defects seem to have an impact on this disproportionate inflammatory reaction via different mechanisms (►**Fig. 1**).[12] Absent or dysfunctional CFTR fails to secrete chloride ions and to regulate sodium absorption correctly,

leading to changes in osmotic pressures and dehydration of ASL and mucus layer. Dehydrated ASL impairs mucociliary clearance, favors retention of pathogens in viscous mucus, and precipitates secondary inflammatory reactions.[17]

Hypoxia in the mucus layer lining the CF airways could also trigger an inflammatory response. Abundant, thick, viscous mucus, and extensive mucus plugging have been described in the small airways of CF lungs.[14] Studies provided evidence that the oxygen tension in mucus plaques and plugs in the CF airways infected with *P. aeruginosa* is low.[58] This low oxygen tension could impair normal host defenses against pathogens, encourage bacterial growth, and initiate cell-signaling events leading to a disproportionate inflammatory cascade.[59]

Lipid abnormalities have also been described and linked to excessive inflammation in CF. The omega-3 fatty acids eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (n-3DPA) are present in oily fish and must be absorbed from the gut.[60] EPA, DHA, and n-3DPA are precursors of the potent anti-inflammatory molecules lipoxins, resolvins, protectins, and maresins that are collectively known as "specialized proresolving mediators" or SPM.[61] In contrast, omega-6-derived mediators such as arachidonic acid (AA) are proinflammatory. The usual Western diet has a high omega-6 to omega-3 ratio of free fatty acids. Malabsorption of fatty acids in individuals with CF exposed to a Western diet can lead to omega-3 deficiency.[62] In addition to fatty acid malabsorption, individuals with CF have *CFTR* genotype-dependent increase in the AA to DHA ratio of nasal and rectal tissues, resulting in a proinflammatory profile.[63] Evidence that the increased AA/DHA ratio is linked to the *CFTR* genotype stems from the observations that the AA/DHA ratio is increased regardless of pancreatic sufficiency status and is increased in obligate heterozygotes bearing one F508del *CFTR* mutation.[63,64] A similar increase in the AA/DHA ratio has been reported in CF knockout mice and linked to an increased conversion of linoleic acid to n-6 metabolites in CFTR-deficient cells. However, others have reported that a membrane fatty acid imbalance is not inherent to the *CFTR* genotype.[65]

Some investigators have reported that a deficiency of the sphingomyelin metabolite ceramide is associated with CF. The deficiency in ceramide was associated with low DHA and high AA in CF plasma, and could be corrected with fenretinide.[66] These observations have led to ongoing clinical trials of fenretinide as an anti-inflammatory strategy in CF. Other investigators have reported that the bronchial epithelial cell levels of ceramide are increased, and it has been hypothesized that these CFTR-related abnormalities involve cell death, release of DNA and chemokines, binding of bacteria to extracellular DNA, and impaired bacterial clearance.[67] The sphingomyelinase inhibitor amitriptyline restored normal bronchial epithelial cell membrane levels in CF knockout mice. A small randomized, double-blind placebo-controlled trial of amitriptyline in CF patients resulted in statistically significant increases in lung function (forced expiratory volume in 1 second, FEV_1) and weight.[68] Further clinical trials are needed to determine the role of modifiers of ceramide metabolism in CF inflammation and lung disease.

Protease/Antiprotease Balance

The inflammatory process in CF lung disease appears to be driven by the continuous recruitment and migration of immune cells, mostly neutrophils or polymorphonuclear cells.[69] Bronchiectasis resulting from inflammation and infection can be observed in CF children as early as 3 months of age.[70–73] Armstrong et al confirmed the presence of an abnormal inflammatory response dominated by the persistent presence of an increased number of neutrophils in the airways of CF infants and children.[74] Analysis of bronchoalveolar lavage (BAL) fluid from 70 CF infants (pristine, infected, and uninfected) and 19 disease controls (suffering from chronic stridor, but without CF) confirmed that subjects from the infected CF group had higher neutrophil percentages, free neutrophil elastase activity, and increased cytokine concentrations. The pristine, uninfected, and control groups had similar levels of inflammatory markers.[74]

Although neutrophils are recruited to attack pathogens, their activation can lead to lung tissue destruction through the release of proteases, particularly human leukocyte elastase (HLE). The presence of HLE in the BAL of infants at 3 months of age is associated with a threefold increase in the risk of bronchiectasis.[70] Neutrophils present in CF airways as assessed by BAL have a phenotype typical of an increased exocytosis rate that correlates with lung damage.[75] Recent data indicate that structural lung damage in CF as determined by computed tomography scan more closely correlates with the frequency of detection of BAL HLE than it does with the detection of infection.[76] These results suggest that free HLE in the CF airway is driving much of the CF lung disease (►**Table 1**).

Neutrophils are the principal source of serine proteases, which are stored in the azurophilic (primary) granules in the neutrophil cytoplasm.[12] HLE is the most abundant enzyme in the serine proteases family.[77] These proteases are enzymes playing both destructive and beneficial roles by acting on different substrates, influencing tissue remodeling, chemotaxis, and killing of bacterial and fungal pathogens.[12,78–80] Proteolytic activity is counterbalanced by protease inhibitors, including α-1 antitrypsin, serine leukocyte protease inhibitor (SLPI), elafin, anti-chymotrypsin, and others, to avoid excessive degradation and destruction of surrounding structures.[12] Alpha-1 antitrypsin, an acute phase reactant protein, is markedly increased in the serum of individuals with CF.[81] However, despite an increase in systemic α-1 antitrypsin, HLE is so abundant in the CF airway that naturally occurring antiproteases are overwhelmed.[82,83] The increased amounts of airway HLE spill over into plasma and form plasma α-1 antitrypsin–HLE complexes that correlate with lung function severity and pulmonary exacerbations.[84]

Active elastase in CF can have several consequences that are highly relevant to CF lung pathophysiology and are summarized in ►**Table 1**. Alpha-1 antitrypsin, the most abundant inhibitor of HLE, is cleaved and inactivated in the airway secretions obtained from most CF individuals.[82] Active HLE can also induce *MUC5AC* expression through mechanisms involving oxidants and activation of the TNF-α converting enzyme (TACE)—epidermal growth factor receptor (EGFR)

Table 1 Consequences of human leukocyte elastase activity in cystic fibrosis lungs

Targets	Consequences
Cleavage of α-1 antitrypsin, SLPI, and other antiproteases	Unabated proteolysis
Increased transcription of *MUC5AC*	Altered mucociliary clearance
Goblet cell degranulation	Mucus accumulation
Proteolysis of airway-wall tissue	Loss of tissue elasticity and generation of bronchiectasis
Complement, complement receptor, immunoglobulin, and immunoglobulin receptor cleavage	Opsonophagocytosis defect and bacterial infection
Phosphatidylserine receptor cleavage	Persistent inflammation
Increased IL-8 release	Neutrophil influx

Abbreviation: SLPI, serine leukocyte protease inhibitor.

pathway.[85–88] Opsonin-related mechanisms normally allow recognition, phagocytosis, and killing of pathogenic bacteria. However, in the CF airway, HLE cleaves immunoglobulins, complements, and their receptors leading to severe opsonin-receptor mismatches and deficient bacterial phagocytosis.[89–91] HLE can also cleave the phosphatidylserine receptor present on macrophages, rendering the macrophage unable to ingest and clear apoptotic neutrophils.[92] The delayed clearance of dead neutrophils and their products is associated with severe and sustained inflammation.

It is generally thought that inhibition of proteases, particularly HLE in the CF airway, should reverse many of these mechanisms, help resolve inflammation, and restore host defenses against CF pathogens.[93] However, due to the massive amount of free HLE and other proteases that are not readily accessible to antielastase drugs delivered topically or systemically, it has not yet been possible to observe consistent clinical benefits of HLE inactivation despite numerous studies.[94]

Oxidant/Antioxidant Balance

Neutrophils need to undergo a robust oxidative burst activity to clear pathogens.[95] The oxidative burst is dependent upon the assembly of the protein complex nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[96] It is not clear that the NADPH oxidase activity of neutrophils in CF airway secretions is normal. Houston et al have reported a reduced oxidative burst.[97] Others have observed CFTR-dependent defects in blood-derived neutrophil oxidative metabolism.[98,99] Predilection to *Burkholderia cepacia* complex suggests impairment in oxidative burst function.[100,101] Others have reported that inhibition of CFTR function further delays the association of the NADPH complex with vacuoles in macrophages infected with *Burkholderia cenocepacia*.[102] These observations raise the possibility that in addition to the multiple mucosal host defense abnormalities discussed above, intrinsic defects may exist in bone marrow-derived inflammatory cells deficient in CFTR.[103,104]

Although it has long been believed that the oxidants produced by neutrophils were directly responsible of bacterial killing, it has become clear that interactions between oxidants, cationic peptides, and proteases are essential to allow proper microbial killing.[105] Reeves et al demonstrated that an influx of superoxide in the phagocytic vacuole was caused by the activation of neutrophil NADPH oxidase that led to an influx of potassium ions across the vacuolar membrane and the subsequent release of cationic peptides and proteins from neutrophil granules.[105] Bacteria present in CF airway secretions are encased in anionic polymers such as bacterial alginate, airway mucins, and DNA that may provide protection against cationic peptides.[106] The efficacy of the neutrophil phagolysosome in killing pathogens present in the CF airway remains unknown.

Oxidative stress and sustained imbalance between oxidants and antioxidants could play a role in the pathophysiology of CF lung disease and contribute to tissue damage.[107] Oxidants are essential for adequate host defense, and reactive oxygen species (ROS) produced by neutrophils and epithelial cells have crucial functions such as killing bacteria, stimulating tissue repair, modulating efferocytosis of apoptotic neutrophils, and protein folding.[108] Nonetheless, excessive amounts of oxidants may have an impact on apoptosis, synthesis and secretion of mucins, and ion transport, and lead to disproportionate inflammation and lung damage.[108] How malfunctioning CFTR predisposes CF individuals to redundant inflammation remains unclear, but hypotheses involving glutathione (GSH) and thiocyanate transport have been postulated.[107–109] Glutathione is an anionic tripeptide transported by the CFTR channel which acts as an antioxidant in the epithelial lining fluid (ELF) by scavenging ROS.[110] Measures of glutathione and glutathione sulfonamide, a specific oxidation product of hypochlorous acid, in BALs of CF children and controls confirmed that glutathione concentrations were lower and that levels of glutathione sulfonamide were increased in patients with CF, corroborating an imbalance between oxidants and antioxidants in these individuals.[110] Although initial data were conflicting, an additional recent study reported decreased levels of glutathione and increased concentrations of oxidative markers in the exhaled breath of CF patients, suggesting an abnormal oxidative stress in the CF airways leading to lung damage.[107] While an antioxidant deficiency may explain in part the oxidant/antioxidant imbalance, the potential sources of oxidants in the CF lungs are numerous and include inflammatory cells, abnormal mitochondrial respiration, bacteria, and bacterial products such as pyocyanine.

Mucins and Mucociliary Clearance

Lungs are constantly exposed to particles in inhaled air and their resistance to environmental injury depends highly on mucus in the airways, which helps trap toxins, pathogens, and particles that are subsequently transported out of the respiratory tract by mechanisms involving ciliary beating and cough.[111] Mucus is an extracellular gel mostly composed of water and mucins that has the properties of both a deformable solid and a viscous fluid.[111] Mucins are large glycoproteins rich in serine and threonine residues covalently linked to sugar chains that are highly anionic due to carboxyl or sulfate groups on their terminal extremities. Several genes encode for mucins in the human genome, but only five of them have terminal domains rich in cysteine allowing the formation of disulfide bonds and leading to the creation of polymers in the airways, including MUC5AC and MUC5B.[111] Growing evidence shows that airway mucus is constituted of two different layers: a mobile layer and a periciliary layer.[112] This configuration and the depth of the periciliary layer appear to play a crucial role in the regulation of transport via mucociliary clearance in the airways.[111,112] The exact roles of MUC5AC and MUC5B and the effects of their inhibition remain unclear and were explored by Roy et al.[113] After creating different mice models (*Muc5ac*$^{-/-}$, *Muc5b*$^{-/-}$, and *Mucb*^{Tg}), mucociliary clearance and responses to bacterial infections were studied. It was discovered that MUC5B is required for mucociliary clearance and helps control infections and maintain immune homeostasis. Moreover, MUC5B deficiency causes materials to accumulate in the airways, enhances inflammation, impairs phagocytosis by macrophages, and increases bacterial burden, especially with *S. aureus* organisms. In contrast, MUC5AC deficiency does not appear to result in significant impact on mucociliary clearance.[113] More recently, other studies examined the contribution of mucus concentration and mucin secretion in the development of obstructive lung disease.[112] Again, MUC5B deficiency was associated with reduced mucociliary clearance, enhanced inflammation, and increased incidence of infection. Nonetheless, mucus hypersecretion and adhesion seem to predominate compared with loss of mucus flow in the development of bronchitic lung diseases.[112]

In CF, abnormal regulation of chloride secretion and sodium absorption by dysfunctional CFTR leads to dehydration of the ASL and prevents mucins from playing their role as a water reservoir.[12] The reduction or absence of bicarbonate secretion could also impair decompaction of mucins. Mucins are secreted from granules as highly compact polymers surrounded by cations, mainly calcium and hydrogen, and require bicarbonate for release and disaggregation.[12] Once secreted in the airway lumen, CFTR is required to allow the detachment of mucins and mucociliary clearance.[114]

Henderson et al demonstrated that mucin concentrations in sputum samples fluctuate depending on the different techniques used to measure levels.[115] Using immunologic measurements, MUC5B concentrations were decreased in sputum samples obtained from CF individuals compared with levels in normal sputum. In comparison, analysis with chromatography and refractometry techniques showed increased levels of MUC5B and higher partial osmotic pressures in sputum samples from CF patients. This discrepancy seems to be secondary to the fact that mucins in CF sustain proteolysis and are cleaved at antibody recognition sites, which prevents mucin recognition with immunologic techniques. Increased levels of mucins in CF airways are hypothesized to provoke increased osmotic effects and trigger abnormal viscoelastic properties, leading to mucus stasis, infection, and inflammation.[115]

Animal Models in Cystic Fibrosis

Over the years, animal models have greatly contributed to our understanding of numerous mechanisms involved in the pathophysiology of CF and they helped in the development of new therapies. Animal models of different species are essential and widely used in CF research. Unfortunately, no single animal model reproduces exactly the complexity of CF in humans, but different animal models have been useful to study diverse aspects of the disease.

The first genetically modified mice models of CF disease appeared 3 years after the *CFTR* gene discovery.[116] Although the cloned CFTR protein revealed a 78% amino acid sequence homology to the human CFTR protein, it was later demonstrated that spontaneous colonization with common pathogens encountered in CF lungs was not detected in mice models. Nevertheless, several discoveries were made thanks to these animal models, including observations on mucociliary transport, hyperinflation, and recruitment of inflammatory cells.[117] Murine models have also been used to study the abnormalities in the submucosal glands and ion transport disturbances including hyperabsorption of sodium and chloride permeability in nasal and tracheal epithelium.[117] Additionally, CF mice develop severe intestinal manifestations, but milder pancreatic disease than in CF humans. These discrepancies could be attributed to different densities of submucosal glands in mice airways, alternate activation of chloride channels in the murine lungs, and as discussed above, low expression of the ATP12A proton pump.[117]

Porcine models are also used in CF research due to their similarities with human lungs and their identical ion properties.[117] At birth, the lungs of CF piglets show no sign of pathology, but they rapidly develop infection.[49] The CF piglets also revealed congenital tracheal abnormalities, including narrowed proximal airways with reduced caliber and circularity, prominent smooth muscle, atypical cartilage, and smaller and hypoplastic submucosal glands. When compared with imaging of CF children 2 years of age or younger, similar tracheal findings were observed, suggesting that abnormal CFTR function could induce anatomical changes as early as in fetal life.[30] As in humans, airways of piglets are characterized by an intense recruitment of inflammatory cells, increased inflammatory markers, and acidification of ASL. Submucosal glands show the same defects in chloride and bicarbonate transport, but no evidence of sodium hyperabsorption. Pancreatic disease is similar to that in humans with different degrees of disease severity depending on the genotype.[117] Nevertheless, pig models have not been extensively used in CF research due to their short longevity caused by severe meconium ileus, which is always fatal for these animals.[117]

Lungs of ferrets share similar characteristics with human lungs and CF ferrets develop lung infections early in their life. Recent experimentations showed that these animals are models of interest for the study of innate immunity and inflammatory responses in CF. Meconium ileus is frequent in ferrets, but less lethal than in pigs. Pancreatic disease is also similar to that in humans. However, even if ferrets seem to be promising models, they are recently developed and further studies will be required to discover their full potential.[117]

Other less common animal models were recently generated. The CF rat appears to be a good model to study the long-term complications of CF such as lung disease, growth failure, and bone disease. A zebrafish model was also created to study pancreatic disease pathogenesis and could be useful for studies of mucosal immunology, infection, and personalized medicine.[117–120]

Future Directions

Despite the many important discoveries that have helped to improve our understanding of CF pathophysiology over the last 30 years, much work remains to be done. Luckily, research in CF is blooming in various fields and countless studies are ongoing. Our better comprehension of the *CFTR* gene, its mutations and their impacts on the CFTR protein has led to the development of new promising treatments. At a time when health care is evolving toward a personalized medicine, a real enthusiasm for the development of gene-specific and other targeted therapies is perceptible among the scientific community. Many trials looking at drugs with the potential to restore the CFTR function or impact other ion channels are ongoing. The development of modulators has exploded, and studies of triple combination therapy are promising.[121,122] One can foresee that in the not too distant future, we will be able to choose the right modulator for a person with CF based on the individual's genotype. Several other drugs aimed at improving mucociliary clearance or reducing inflammatory response are also being studied.[123–125] All of these developments have been made possible through major advances in physiology, new technologies such as high-throughput screening, better surrogate readouts for drug discovery, improved animal models, and high-quality clinical trials. Collaborations between investigators in the basic sciences and clinicians have been exemplary in CF research. These are exciting times for CF researchers who are leading the way to provide the much-needed explanations behind the clinical manifestations of CF and to identify new therapies.

Conclusion

From the *CFTR* gene discovery to the development of targeted therapies, work of physicians and researchers around the world has allowed the CF community to gain a better understanding of this fatal disease. The CFTR protein structure, its function in different organs, and its abnormalities in CF are now well known. Mechanisms leading to lung infection and excessive inflammatory reactions, although still not completely understood, are becoming clearer and less debated. Hopefully, the years to come will be as rich in discoveries as the last 3 decades have been and will lead to the development of novel and safe therapies that will change the management of CF and the life of the persons suffering from this disease.

Funding

C.B. has been awarded a Cystic Fibrosis Canada Clinical fellowship grant for 2018–2019 and is currently enrolled in the Adult Cystic Fibrosis Fellowship program at the University of Toronto (St. Michael's Hospital).

A.M.C. is a member of the FQRS-funded Centre de Recherche Clinique du CHUS, and was supported by a grant from the Canadian Institutes of Health Research.

Conflicts of Interest

Dr. Cantin reports personal fees from Vertex Pharmaceuticals, outside the submitted work.

References

1 Elborn JS. Cystic fibrosis. Lancet 2016;388(10059):2519–2531
2 O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet 2009;373 (9678):1891–1904
3 Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease. Am J Dis Child 1938;56:344–399
4 Quinton PM. Physiological basis of cystic fibrosis: a historical perspective. Physiol Rev 1999;79(1, Suppl):S3–S22
5 Di Sant'Agnese P, Darling RC, Perara GA, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. AMA Am J Dis Child 1953;86(05):618–619, discussion, 619
6 Di Sant'Agnese PA, Darling RC, Perera GA, Shea E. Sweat electrolyte disturbances associated with childhood pancreatic disease. Am J Med 1953;15(06):777–784
7 Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989;245(4922):1066–1073
8 Kerem B, Rommens JM, Buchanan JA, et al. Identification of the cystic fibrosis gene: genetic analysis. Science 1989;245(4922): 1073–1080
9 Rommens JM, Iannuzzi MC, Kerem B, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989;245(4922):1059–1065
10 Riordan JR. CFTR function and prospects for therapy. Annu Rev Biochem 2008;77:701–726
11 Castellani C, Cuppens H, Macek M Jr, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 2008;7(03):179–196
12 Cantin AM, Hartl D, Konstan MW, Chmiel JF. Inflammation in cystic fibrosis lung disease: Pathogenesis and therapy. J Cyst Fibros 2015;14(04):419–430
13 Keogh RH, Szczesniak R, Taylor-Robinson D, Bilton D. Up-to-date and projected estimates of survival for people with cystic fibrosis using baseline characteristics: a longitudinal study using UK patient registry data. J Cyst Fibros 2018;17(02):218–227
14 Zuelzer WW, Newton WA Jr. The pathogenesis of fibrocystic disease of the pancreas; a study of 36 cases with special reference to the pulmonary lesions. Pediatrics 1949;4(01):53–69
15 Knowles M, Gatzy J, Boucher R. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. N Engl J Med 1981;305(25):1489–1495
16 Quinton PM. Chloride impermeability in cystic fibrosis. Nature 1983;301(5899):421–422
17 Boucher RC. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu Rev Med 2007;58:157–170

18 Knowles M, Gatzy J, Boucher R. Relative ion permeability of normal and cystic fibrosis nasal epithelium. J Clin Invest 1983;71 (05):1410–1417
19 Cant N, Pollock N, Ford RC. CFTR structure and cystic fibrosis. Int J Biochem Cell Biol 2014;52:15–25
20 Gustafsson JK, Ermund A, Ambort D, et al. Bicarbonate and functional CFTR channel are required for proper mucin secretion and link cystic fibrosis with its mucus phenotype. J Exp Med 2012;209(07):1263–1272
21 Gentzsch M, Dang H, Dang Y, et al. The cystic fibrosis transmembrane conductance regulator impedes proteolytic stimulation of the epithelial Na+ channel. J Biol Chem 2010;285(42):32227–32232
22 Gabriel SE, Clarke LL, Boucher RC, Stutts MJ. CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship. Nature 1993;363(6426):263–268
23 Chmiel JF, Davis PB. State of the art: why do the lungs of patients with cystic fibrosis become infected and why can't they clear the infection? Respir Res 2003;4:(08)1–12
24 Matsui H, Grubb BR, Tarran R, et al. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell 1998;95(07):1005–1015
25 Shah VS, Meyerholz DK, Tang XX, et al. Airway acidification initiates host defense abnormalities in cystic fibrosis mice. Science 2016;351(6272):503–507
26 Pezzulo AA, Tang XX, Hoegger MJ, et al. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature 2012;487(7405):109–113
27 Kopelman H, Corey M, Gaskin K, Durie P, Weizman Z, Forstner G. Impaired chloride secretion, as well as bicarbonate secretion, underlies the fluid secretory defect in the cystic fibrosis pancreas. Gastroenterology 1988;95(02):349–355
28 Quinton PM. Cystic fibrosis: lessons from the sweat gland. Physiology (Bethesda) 2007;22:212–225
29 Farrell PM, White TB, Ren CL, et al. Diagnosis of cystic fibrosis: consensus guidelines from the Cystic Fibrosis Foundation. J Pediatr 2017;181S:S4–S15, 15.e1
30 Meyerholz DK, Stoltz DA, Namati E, et al. Loss of cystic fibrosis transmembrane conductance regulator function produces abnormalities in tracheal development in neonatal pigs and young children. Am J Respir Crit Care Med 2010;182(10):1251–1261
31 Chang EH, Pezzulo AA, Meyerholz DK, et al. Sinus hypoplasia precedes sinus infection in a porcine model of cystic fibrosis. Laryngoscope 2012;122(09):1898–1905
32 Wilschanski M, Zielenski J, Markiewicz D, et al. Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. J Pediatr 1995;127(05):705–710
33 Tsui LC. The spectrum of cystic fibrosis mutations. Trends Genet 1992;8(11):392–398
34 Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 1993;73(07): 1251–1254
35 De Boeck K, Amaral MD. Progress in therapies for cystic fibrosis. Lancet Respir Med 2016;4(08):662–674
36 Taylor-Cousar JL, Munck A, McKone EF, et al. Tezacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del. N Engl J Med 2017;377(21):2013–2023
37 Wainwright CE, Elborn JS, Ramsey BW, et al; TRAFFIC Study Group; TRANSPORT Study Group. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. N Engl J Med 2015;373(03):220–231
38 Ramsey BW, Davies J, McElvaney NG, et al; VX08-770-102 Study Group. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 2011;365(18):1663–1672
39 Marson FAL, Bertuzzo CS, Ribeiro JD. Classification of CFTR mutation classes. Lancet Respir Med 2016;4(08):e37–e38
40 Blackman SM, Deering-Brose R, McWilliams R, et al. Relative contribution of genetic and nongenetic modifiers to intestinal obstruction in cystic fibrosis. Gastroenterology 2006;131(04): 1030–1039
41 Ahmadi S, Xia S, Wu YS, et al. SLC6A14, an amino acid transporter, modifies the primary CF defect in fluid secretion. Elife 2018; 13:7. pii: e37963. doi: 10.7554/eLife.37963
42 Sun L, Rommens JM, Corvol H, et al. Multiple apical plasma membrane constituents are associated with susceptibility to meconium ileus in individuals with cystic fibrosis. Nat Genet 2012;44(05):562–569
43 Gong J, Wang F, Xiao B, et al. Genetic association and transcriptome integration identify contributing genes and tissues at cystic fibrosis modifier loci. PLoS Genet 2019;15(02):e1008007
44 Vanscoy LL, Blackman SM, Collaco JM, et al. Heritability of lung disease severity in cystic fibrosis. Am J Respir Crit Care Med 2007;175(10):1036–1043
45 Corey M, Edwards L, Levison H, Knowles M. Longitudinal analysis of pulmonary function decline in patients with cystic fibrosis. J Pediatr 1997;131(06):809–814
46 Merlo CA, Boyle MP. Modifier genes in cystic fibrosis lung disease. J Lab Clin Med 2003;141(04):237–241
47 Guillot L, Beucher J, Tabary O, Le Rouzic P, Clement A, Corvol H. Lung disease modifier genes in cystic fibrosis. Int J Biochem Cell Biol 2014;52:83–93
48 Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. N Engl J Med 2015;372(04):351–362
49 Stoltz DA, Meyerholz DK, Pezzulo AA, et al. Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth. Sci Transl Med 2010;2(29):29ra31
50 Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell 1996;85(02):229–236
51 Song Y, Salinas D, Nielson DW, Verkman AS. Hyperacidity of secreted fluid from submucosal glands in early cystic fibrosis. Am J Physiol Cell Physiol 2006;290(03):C741–C749
52 Dajani R, Zhang Y, Taft PJ, et al. Lysozyme secretion by submucosal glands protects the airway from bacterial infection. Am J Respir Cell Mol Biol 2005;32(06):548–552
53 Widdicombe JH, Wine JJ. Airway gland structure and function. Physiol Rev 2015;95(04):1241–1319
54 Engelhardt JF, Yankaskas JR, Ernst SA, et al. Submucosal glands are the predominant site of CFTR expression in the human bronchus. Nat Genet 1992;2(03):240–248
55 Kelley TJ, Drumm ML. Inducible nitric oxide synthase expression is reduced in cystic fibrosis murine and human airway epithelial cells. J Clin Invest 1998;102(06):1200–1207
56 Pier GB, Grout M, Zaidi TS. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of Pseudomonas aeruginosa from the lung. Proc Natl Acad Sci U S A 1997;94(22):12088–12093
57 Cannon CL, Kowalski MP, Stopak KS, Pier GB. Pseudomonas aeruginosa-induced apoptosis is defective in respiratory epithelial cells expressing mutant cystic fibrosis transmembrane conductance regulator. Am J Respir Cell Mol Biol 2003;29(02):188–197
58 Worlitzsch D, Tarran R, Ulrich M, et al. Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. J Clin Invest 2002;109(03):317–325
59 Scortegagna M, Cataisson C, Martin RJ, et al. HIF-1alpha regulates epithelial inflammation by cell autonomous NFkappaB activation and paracrine stromal remodeling. Blood 2008;111(07): 3343–3354
60 Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans 2017;45(05):1105–1115
61 Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014;510(7503):92–101
62 Maqbool A, Schall JI, Gallagher PR, Zemel BS, Strandvik B, Stallings VA. Relation between dietary fat intake type and serum fatty acid status in children with cystic fibrosis. J Pediatr Gastroenterol Nutr 2012;55(05):605–611

63 Freedman SD, Blanco PG, Zaman MM, et al. Association of cystic fibrosis with abnormalities in fatty acid metabolism. N Engl J Med 2004;350(06):560–569

64 Strandvik B, Gronowitz E, Enlund F, Martinsson T, Wahlström J. Essential fatty acid deficiency in relation to genotype in patients with cystic fibrosis. J Pediatr 2001;139(05):650–655

65 Werner A, Bongers ME, Bijvelds MJ, de Jonge HR, Verkade HJ. No indications for altered essential fatty acid metabolism in two murine models for cystic fibrosis. J Lipid Res 2004;45(12): 2277–2286

66 Guilbault C, Wojewodka G, Saeed Z, et al. Cystic fibrosis fatty acid imbalance is linked to ceramide deficiency and corrected by fenretinide. Am J Respir Cell Mol Biol 2009;41(01):100–106

67 Teichgräber V, Ulrich M, Endlich N, et al. Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. Nat Med 2008;14(04):382–391

68 Adams C, Icheva V, Deppisch C, et al. Long-term pulmonal therapy of cystic fibrosis-patients with amitriptyline. Cell Physiol Biochem 2016;39(02):565–572

69 Mall MA, Hartl D. CFTR: cystic fibrosis and beyond. Eur Respir J 2014;44(04):1042–1054

70 Sly PD, Gangell CL, Chen L, et al; AREST CF Investigators. Risk factors for bronchiectasis in children with cystic fibrosis. N Engl J Med 2013;368(21):1963–1970

71 Stick SM, Brennan S, Murray C, et al; Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF). Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. J Pediatr 2009;155(05): 623.e1–628.e1

72 Mott LS, Gangell CL, Murray CP, Stick SM, Sly PD; AREST CF. Bronchiectasis in an asymptomatic infant with cystic fibrosis diagnosed following newborn screening. J Cyst Fibros 2009;8 (04):285–287

73 Sly PD, Brennan S, Gangell C, et al; Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF). Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. Am J Respir Crit Care Med 2009;180(02):146–152

74 Armstrong DS, Hook SM, Jamsen KM, et al. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. Pediatr Pulmonol 2005;40(06):500–510

75 Margaroli C, Garratt LW, Horati H, et al. Elastase exocytosis by airway neutrophils is associated with early lung damage in children with cystic fibrosis. Am J Respir Crit Care Med 2019; 199(07):873–881

76 Rosenow T, Mok LC, Turkovic L, et al. The cumulative effect of inflammation and infection on structural lung disease in early CF. Eur Respir J 2019 (e-pub ahead of print). doi:10.1183/ 13993003.01771-2018

77 Lee WL, Downey GP. Leukocyte elastase: physiological functions and role in acute lung injury. Am J Respir Crit Care Med 2001;164 (05):896–904

78 Belaaouaj A, Kim KS, Shapiro SD. Degradation of outer membrane protein A in Escherichia coli killing by neutrophil elastase. Science 2000;289(5482):1185–1188

79 Belaaouaj A, McCarthy R, Baumann M, et al. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. Nat Med 1998;4(05):615–618

80 Benabid R, Wartelle J, Malleret L, et al. Neutrophil elastase modulates cytokine expression: contribution to host defense against Pseudomonas aeruginosa-induced pneumonia. J Biol Chem 2012;287(42):34883–34894

81 Cantin AM, Lafrenaye S, Bégin RO. Antineutrophil elastase activity in cystic fibrosis serum. Pediatr Pulmonol 1991;11(03): 249–253

82 Suter S, Schaad UB, Roux L, Nydegger UE, Waldvogel FA. Granulocyte neutral proteases and Pseudomonas elastase as possible causes of airway damage in patients with cystic fibrosis. J Infect Dis 1984;149(04):523–531

83 Cantin A, Bilodeau G, Bégin R. Granulocyte elastase-mediated proteolysis of alpha 1-antitrypsin in cystic fibrosis bronchopulmonary secretions. Pediatr Pulmonol 1989;7(01):12–17

84 Cantin AM, Bilodeau G, Larivée P, Richter MV. Plasma biomarkers and cystic fibrosis lung disease. Clin Invest Med 2012;35(04): E173–E181

85 Voynow JA, Young LR, Wang Y, Horger T, Rose MC, Fischer BM. Neutrophil elastase increases MUC5AC mRNA and protein expression in respiratory epithelial cells. Am J Physiol 1999;276 (05):L835–L843

86 Griffin S, Carroll TP, Greene CM, O'Neill SJ, Taggart CC, McElvaney NG. Effect of pro-inflammatory stimuli on mucin expression and inhibition by secretory leucoprotease inhibitor. Cell Microbiol 2007;9(03):670–679

87 Shao MX, Nadel JA. Neutrophil elastase induces MUC5AC mucin production in human airway epithelial cells via a cascade involving protein kinase C, reactive oxygen species, and TNF-alpha-converting enzyme. J Immunol 2005;175(06): 4009–4016

88 Shao MX, Nadel JA. Dual oxidase 1-dependent MUC5AC mucin expression in cultured human airway epithelial cells. Proc Natl Acad Sci U S A 2005;102(03):767–772

89 Fick RB Jr, Naegel GP, Squier SU, Wood RE, Gee JB, Reynolds HY. Proteins of the cystic fibrosis respiratory tract. Fragmented immunoglobulin G opsonic antibody causing defective opsonophagocytosis. J Clin Invest 1984;74(01):236–248

90 Berger M, Sorensen RU, Tosi MF, Dearborn DG, Döring G. Complement receptor expression on neutrophils at an inflammatory site, the Pseudomonas-infected lung in cystic fibrosis. J Clin Invest 1989;84(04):1302–1313

91 Tosi MF, Zakem H, Berger M. Neutrophil elastase cleaves C3bi on opsonized pseudomonas as well as CR1 on neutrophils to create a functionally important opsonin receptor mismatch. J Clin Invest 1990;86(01):300–308

92 Vandivier RW, Fadok VA, Hoffmann PR, et al. Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. J Clin Invest 2002;109(05):661–670

93 Kelly E, Greene CM, McElvaney NG. Targeting neutrophil elastase in cystic fibrosis. Expert Opin Ther Targets 2008;12(02): 145–157

94 Polverino E, Rosales-Mayor E, Dale GE, Dembowsky K, Torres A. The role of neutrophil elastase inhibitors in lung diseases. Chest 2017;152(02):249–262

95 Babior BM. Phagocytes and oxidative stress. Am J Med 2000;109 (01):33–44

96 Panday A, Sahoo MK, Osorio D, Batra S. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. Cell Mol Immunol 2015;12(01):5–23

97 Houston N, Stewart N, Smith DS, Bell SC, Champion AC, Reid DW. Sputum neutrophils in cystic fibrosis patients display a reduced respiratory burst. J Cyst Fibros 2013;12(04):352–362

98 Painter RG, Bonvillain RW, Valentine VG, et al. The role of chloride anion and CFTR in killing of Pseudomonas aeruginosa by normal and CF neutrophils. J Leukoc Biol 2008;83(06): 1345–1353

99 Painter RG, Marrero L, Lombard GA, Valentine VG, Nauseef WM, Wang G. CFTR-mediated halide transport in phagosomes of human neutrophils. J Leukoc Biol 2010;87(05):933–942

100 Sousa SA, Ulrich M, Bragonzi A, et al. Virulence of Burkholderia cepacia complex strains in gp91phox-/- mice. Cell Microbiol 2007;9(12):2817–2825

101 Speert DP, Bond M, Woodman RC, Curnutte JT. Infection with Pseudomonas cepacia in chronic granulomatous disease: role of nonoxidative killing by neutrophils in host defense. J Infect Dis 1994;170(06):1524–1531

102 Keith KE, Hynes DW, Sholdice JE, Valvano MA. Delayed association of the NADPH oxidase complex with macrophage vacuoles

containing the opportunistic pathogen Burkholderia cenocepacia. Microbiology 2009;155(Pt 4):1004–1015
103 Pohl K, Hayes E, Keenan J, et al. A neutrophil intrinsic impairment affecting Rab27a and degranulation in cystic fibrosis is corrected by CFTR potentiator therapy. Blood 2014;124(07): 999–1009
104 Lamothe J, Valvano MA. Burkholderia cenocepacia-induced delay of acidification and phagolysosomal fusion in cystic fibrosis transmembrane conductance regulator (CFTR)-defective macrophages. Microbiology 2008;154(Pt 12):3825–3834
105 Reeves EP, Lu H, Jacobs HL, et al. Killing activity of neutrophils is mediated through activation of proteases by K+ flux. Nature 2002;416(6878):291–297
106 Chan C, Burrows LL, Deber CM. Alginate as an auxiliary bacterial membrane: binding of membrane-active peptides by polysaccharides. J Pept Res 2005;65(03):343–351
107 Spicuzza L, Parisi GF, Tardino L, et al. Exhaled markers of antioxidant activity and oxidative stress in stable cystic fibrosis patients with moderate lung disease. J Breath Res 2018;12(02): 026010
108 Cantin AM, White TB, Cross CE, Forman HJ, Sokol RJ, Borowitz D. Antioxidants in cystic fibrosis. Conclusions from the CF antioxidant workshop, Bethesda, Maryland, November 11-12, 2003. Free Radic Biol Med 2007;42(01):15–31
109 Xu Y, Szép S, Lu Z. The antioxidant role of thiocyanate in the pathogenesis of cystic fibrosis and other inflammation-related diseases. Proc Natl Acad Sci U S A 2009;106(48):20515–20519
110 Kettle AJ, Turner R, Gangell CL, et al; AREST CF. Oxidation contributes to low glutathione in the airways of children with cystic fibrosis. Eur Respir J 2014;44(01):122–129
111 Fahy JV, Dickey BF. Airway mucus function and dysfunction. N Engl J Med 2010;363(23):2233–2247
112 Livraghi-Butrico A, Grubb BR, Wilkinson KJ, et al. Contribution of mucus concentration and secreted mucins Muc5ac and Muc5b to the pathogenesis of muco-obstructive lung disease. Mucosal Immunol 2017;10(02):395–407
113 Roy MG, Livraghi-Butrico A, Fletcher AA, et al. Muc5b is required for airway defence. Nature 2014;505(7483):412–416
114 Hoegger MJ, Fischer AJ, McMenimen JD, et al. Impaired mucus detachment disrupts mucociliary transport in a piglet model of cystic fibrosis. Science 2014;345(6198):818–822
115 Henderson AG, Ehre C, Button B, et al. Cystic fibrosis airway secretions exhibit mucin hyperconcentration and increased osmotic pressure. J Clin Invest 2014;124(07):3047–3060
116 Clarke LL, Grubb BR, Gabriel SE, Smithies O, Koller BH, Boucher RC. Defective epithelial chloride transport in a gene-targeted mouse model of cystic fibrosis. Science 1992;257(5073):1125–1128
117 Semaniakou A, Croll RP, Chappe V. Animal models in the pathophysiology of cystic fibrosis. Front Pharmacol 2019;9:1475. doi: 10.3389/fphar.2018.01475
118 Progatzky F, Cook HT, Lamb JR, Bugeon L, Dallman MJ. Mucosal inflammation at the respiratory interface: a zebrafish model. Am J Physiol Lung Cell Mol Physiol 2016;310(06):L551–L561
119 Bernut A, Dupont C, Ogryzko NV, et al. CFTR protects against Mycobacterium abscessus infection by fine-tuning host oxidative defenses. Cell Reports 2019;26(07):1828.e4–1840.e4
120 Leslie M. Zebrafish larvae could help to personalize cancer treatments. Science 2017;357(6353):745
121 Keating D, Marigowda G, Burr L, et al; VX16-445-001 Study Group. VX-445-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. N Engl J Med 2018;379(17): 1612–1620
122 Davies JC, Moskowitz SM, Brown C, et al; VX16-659-101 Study Group. VX-659-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. N Engl J Med 2018;379(17): 1599–1611
123 Rafeeq MM, Murad HAS. Cystic fibrosis: current therapeutic targets and future approaches. J Transl Med 2017;15(01):84. doi: 10.1186/s12967-017-1193-9
124 Scott DW, Walker MP, Sesma J, et al. SPX-101 is a novel epithelial sodium channel-targeted therapeutic for cystic fibrosis that restores mucus transport. Am J Respir Crit Care Med 2017;196 (06):734–744
125 Ehre C, Rushton ZL, Wang B, et al. An improved inhaled mucolytic to treat airway muco-obstructive diseases. Am J Respir Crit Care Med 2019;199(02):171–180