Spring 2014

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Abstract

The following literature review provides an account in support of the premise that the cystic fibrosis (CF) disease affects widespread areas of the body primarily due to the defective CFTR protein. Mutations in the CFTR gene lead to defects in CFTR protein that causes the disease. Lack of protein function or lack of functional protein cause variability in severity of the phenotype. The defective CFTR protein changes ion influx and efflux across the body's cell membranes, which ultimately changes the internal environment of these cells. This change contributes to each cell's production of proteins through transcription and translation. The simple changes in ion movement in and out of these cells have detrimental effects on the overall function of the cells. The cells create a system for maintenance of the body's health, and if the cells do not function appropriately, the body fails to manage viral and bacterial infections. In this review, I will discuss how the defective CFTR channel works in the cells of the bodies of CF patients, and explain why the normal CFTR channel is essential for proper health and maintenance of the human body. In addition to the CFTR channel, other factors affect the severity of the disease. Genome-wide association studies explore other factors attributed to CF, including environmental factors, modifier genes, transcriptional regulation factors, and post-translational modifications. Potentiators and correctors target the CFTR channel in order to increase CFTR function, working alongside therapies in order to combat the effects of defective channels in CF patients.

Introduction

Cystic Fibrosis is an autosomal recessive disorder caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein (1, 2). It is most common in individuals of Caucasian descent, and diagnosed most in childhood (3). Screening for CF occurs in infants and in the U.S, 1 in 3,500 neonates is diagnosed with Cystic Fibrosis (3). In order to manifest this CF phenotype, an individual must be homozygous for a defective CFTR gene, inheriting one mutated copy, or allele, of the gene from each parent (3). The disease causes a widespread effect in the body among organs such as the skin, lungs, pancreas, liver, and gastrointestinal tracts, and could cause multisystem organ failure in the body (1).

In people with CF, the CFTR gene is defective due to mutations, which occur on chromosome 7, and this gene then affects the protein called CFTR (1). The defective CFTR protein results in the manifestation of CF (1). Dehydration of airway surface liquid causes persistent inflammation of the airways in the lungs (2). Dehydration leads to a depletion of the periciliary layer and production of highly viscoelastic mucus, making the mucociliary clearance system ineffective (2). The CFTR protein, which normally shuttles sodium and chloride ions across the membranes of cells, is defective, acting as a

dysfunctional chloride channel which fails to appropriately balance water and electrolyte composition in the cells of the body (1, 2).

The imbalance of essential ions in the body's cells has deleterious effects, and could eventually destroy affected organs (1). Patients with CF become more prone to repeated infections by organisms due to the buildup of secretions in the lungs; these secretions lead to a reduction in bacterial clearance and lung damage (1). In the pancreas, a deficiency of digestive enzymes occurs, leading to badly absorbed undigested foods and malnutrition (1). Islet cells in the pancreas could become damaged with time, leading to a decrease in insulin and glucagon secretion; proper secretion of these essential hormones is important for the regulation of the body's blood-glucose levels (1). In addition, cirrhosis, or the result of advanced liver disease could occur, as well as infertility in males (1).

CFTR Gene History

CF was first linked to another gene, called a PON gene, which confirmed that CF was a homogeneous genetic disease and that the CF gene could be identified by genetic linkage analysis with a high number of two-generation families (4). After the first DNA marker linked to CF was found, localization of CF to the long arm of chromosome 7 was then confirmed (4). In order to isolate this gene, techniques such as "chromosome walking," "positional cloning," and "gene hopping" were used to isolate and identify the CFTR gene (3). Evidence for the CFTR gene exists in the presence of the most common mutation, ΔF508, renamed F508del (4). This 3-base pair deletion was found on a rare extended chromosome (4).

CFTR Gene Structure

The CFTR protein is a cyclic adenosine monophosphate-regulated (cAMP-regulated) chloride ion channel composed of 1,480 amino acids (3). It is a member of the ATP-binding cassette (ABC) family and it spans 189 kb of genomic DNA at chromosome 7q31 (5, 6). CFTR has tight tissue-specific regulation of expression (5). The active locus is in a looped conformation that incorporates distal regulatory elements into close proximity with the gene promoter (5). The cAMP-regulated anion channel is expressed primarily at the apical plasma membrane of secretory epithelia; by understanding CFTR, one can understand defects in cellular processing, translation, and chloride channel gating (4).

CFTR Protein Channel Conductance

The promotion of the CFTR gene normally results in expression of CFTR protein, which is a transmembrane channel that works to move chloride and bicarbonate ions into and out of the body's cells (3). Cellular processing and chloride channel function are studied in order to understand the basic defect that causes CF (4).

The CFTR protein is structurally unique. The large multi-domain glycoprotein is an ATP-binding cassette (ABC) transporter composed of five domains: two transmembrane domains (TMD1/TMD2) that form the channel pore, one regulatory domain (R) that gates the channel by phosphorylation, and two nucleotide-binding domains

(NBD1/NBD2) that bind and hydrolyze ATP (3, 4). Mature, wild type CFTR appears compact and folded and is known for protease resistance (7). In contrast, an immature CFTR protein, or even one mutated by the F508del CFTR mutation, has an open conformation, and shows increased protease sensitivity (7).[1] Classical channel opening occurs by phosphorylation of the R domain by protein kinase A and recruitment of ATP to the nucleotide binding domains NBD1 and NBD2 (3).[2] When ATP binds at the interface between NBD1 and NBD2, the channel opens (8). When ATP hydrolyzes, the NBDs dissociate to close the channel (3, 8). This concludes the process of channel "gating," ultimately allowing for chloride transport and conductance in and out of the cell as shown in Figure 1 (3).

The architecture of the CFTR protein results in ion transport via the channel and causes voltage-dependent open channel blockage (9). A wide range of organic anions are encountered in the cytoplasm of the cell (9). The channel is usually involved in secreting chloride and bicarbonate ions at hyperpolarized potentials, so it contains fixed positive charges that allow a capture of anions from the cytoplasm by electrostatic attraction (9). At the center of the pore lies a positive charge, which allows for attraction of anions (9). Beyond this point, anions pass into a narrow uncharged pore region that allows discrimination between different anions (9). At this point, anions are selectively filtered; the channel stops organic anions from leaving the cell, and inhibits especially large anions from passing into the central pore region (9).

Normal CFTR Function

The CFTR protein channel functions in conductance of ions in and out of the body's cells, which is important for the body's maintenance of water and solute concentrations. Active chloride efflux drives fluid secretion, while bicarbonate influx drives fluid transport across the cells of tissues such as pancreatic ducts, the gastrointestinal tract, and the duodenum (9). In the duodenum and pancreatic duct, the sodium bicarbonate in these secretions is very high and the bicarbonate drives fluid secretion (9). Water then follows this high solute transport into the epithelia (9). Anion secretion in the airways contributes to the formation and maintenance of a thin layer of liquid called airway surface liquid (ASL), which consists of a lower-viscosity perciliary liquid (PCL) next to the membrane which has beating cilia, and a higher-viscosity gel layer where mucins trap inhaled debris and microbes (9). Anion secretion becomes important here because it maintains the volume and composition of PCL (9). Sodium is absorbed via an epithelial sodium channel, while chloride is secreted and shuttled out of the cell by CFTR; both of these transport processes help to maintain this periciliary layer on which the essential mucociliary clearance of debris and bacteria depends (9).

In the intestines, the secretion of salt and water is important for the maintenance of an aqueous environment that supports enzymatic activity, absorption of nutrients, and clearance of luminal contents (9). The proximal small intestine is the main site for

[1]Protease-an enzyme that breaks down proteins and peptides; proteolysis-the breakdown of proteins or peptides into amino acids by the action of enzymes

[2]Phosphorylation-adding phosphate group(s); protein kinase A phosphorylates the CFTR channel so that ATP can bind to NBD1 and NBD2, opening the channel during gating

absorption; it takes in salt and secretes bicarbonate in order to act as a buffer for gastric acid (9). The sodium-hydrogen and chloride-bicarbonate exchanges handle salt and water balance (9). In the more distal part of the small intestine, CFTR is localized to the crypt cells and villi, and in the large intestine CFTR is localized to the crypt epithelium; sodium moves through the pathway just as it does in airway cells (9). In the pancreatic duct, CFTR couples with anion exchangers called SLC26A3 and SLC26A3 to generate sodium bicarbonate secretion for alkalinizing the duodenum, which drives pancreatic digestive enzymes to the lumen of the duodenum and allows for salivary clearance of mucins and enzymes (9).

Defective CFTR Protein

In 1983, Paul Quinton discovered that the chloride transport defect causes CF, and follow-up studies show evidence that sodium reabsorption was elevated in airways, and thus that sodium and chloride transport were both altered in CF airways (10). The equilibrium of absorption and secretion of sodium and chloride respectively is disrupted by mutations in the CFTR gene, resulting in the absence of functional CFTR-dependent chloride secretion (11). While chloride transport is defective, sodium absorption is functional and persistent, causing ASL dehydration (11). Meanwhile, the sodium channel becomes hyperactive, causing increased sodium and water absorption (12).

In CF patients, the CFTR proteins on the plasma membranes of cells are defective or absent, and this compromises the formation of sufficient PCL (9). Chloride is not properly shuttled out of the cells, but the epithelial sodium channel continues to absorb sodium, resulting in a paucity of low-viscosity fluid on which effective mucus clearance thrives (9). Due to the lack of an effective system of mucociliary clearance, mucus accumulates and so does bacteria (9). These bacteria and infections block ducts of glands in the body and colonize airways, causing further loss of the clearance system and leading to ineffective innate immunity (9).

The main function of the CFTR anion channel in the airway epithelia and glands of the submucosa is to control electrolyte transport, as shown in Figure 2, and subsequently regulate pulmonary host defenses (13). This electrolyte transport controls the necessary airway surface liquid of airway epithelia (13). Studies show that patients with CF have reduced anion conductance, or chloride conductance, but that their sodium conductance does not increase at all. Increased voltage and current of Cystic Fibrosis responses to amiloride, an inhibitor of epithelial sodium channels (ENaC) are usually interpreted as epithelial hyperabsorption (13). However, a 2011 study by Itani et al attributed epithelial hyperabsorption to the loss of chloride rather than to increased activity of sodium channels (13). This study corresponds to previous studies of newborn CF pigs manifesting a defect in host defense against bacteria but not acquiring secondary manifestations of the disease (13). The study by Itani et al shows that CF sweat gland ducts and airway glands of the submucosa having reduced anion transport but not sodium hyperabsorption (13).

Disease pathology within affected organ systems depends on the contribution of CFTR function in salt and water secretion (9). It also depends on the extent of the consequences

of deficient CFTR and how CFTR is expressed in the body's tissues (9). For example, as little as 20% impairment of chloride secretion is enough to cause obstruction of the vas deferens, whereas 50% impairment of CFTR activity is not detectable as a change in sweat chloride, and little CFTR activity produces a large decrease in sweat chloride levels (9). The function of the cells is important for the regulation of the body's organ systems, and the core defect in the dysfunctional CFTR channel that affects proper cell function throughout the body is anion transport (9).

CFTR protein synthesis

Under usual circumstances, extracellular signals stimulate CFTR gene expression by promoting transcription of the CFTR gene into mRNA (3). The single strand template moves through nuclear pores in order to interact with ribosomes in the cytoplasm or on the rough endoplasmic reticulum (ER) (3). Transfer RNA is translated into nascent chains of amino acids (3). Polypeptides are formed and assembled into the immature CFTR protein product, which is then folded within the lipid bilayer of the ER (3). The protein matures in the ER and the final CFTR protein is then transferred to the Golgi apparatus for post-translational modification and packaging into transport vesicles (3). The channel is moved or "trafficked" to the surface of the cell for final expression on the apical membrane of cells (3).

Based on how long it takes for the CFTR protein to leave the ER, CFTR domain assemblage takes between 30 and 120 minutes, meaning that there has to be a distinct process in which folding occurs (14). Folding occurs by two steps that require ATP, one being integration into the bilayer of the ER and the other being conversion from an incompletely folded, ER-associated version of a protein to a properly folded, mature conformation that exits the ER and goes to the Golgi apparatus for processing (14). Mutations in the CFTR gene have an effect on every step in protein synthesis. For example, the F508del mutation prevents the completion of protein folding (14). Therefore, protein synthesis must be understood in order to recognize mutations, which cause phenotypic symptoms of the disease of CF (3).

Causes of the Defective Channel: Mutations

A mutation is a permanent change in the DNA sequence that makes up a gene; mutations of the CFTR gene lessen the proper function of the CFTR protein (7). There are over 1,000 mutations identified thus far for the CFTR gene, and these are categorized in five different classes (1, 15):

- Class I
    - Defective protein production; few or not functioning CFTR chloride channels (1, 15)
    - Nonsense mutation or frameshift mutation in DNA that causes an in-frame premature termination codon in the protein-coding region (8)
    - Results in premature end to translation of protein (8)
    - Unstable truncated and nonfunctional proteins are degraded before they can reach the cell membrane (8)
    - Accounts for 10% of all CF mutations worldwide (8)

- Class II
    - Defective processing so that CFTR does not reach the surface membrane where it normally functions (1,15)
    - Result in a protein whose processing is blocked in the ER, failing to progress to the Golgi apparatus (1, 15)
    - Improperly folded CFTR, resulting in premature degradation (8)
    - F508del is the most common mutation at 90% (1, 15, 18)
        - Incompletely glycosylated (1, 15)[3]
        - Results in an improperly folded protein that is retained in the ER, causing its breakdown by the ubiquitin proteosomal system, and a small amount of the mature protein reaches the cell surface (16)
- Class III
    - Produces a protein that reaches its site of action on the cell surface but does not conduct chloride due to defective regulation (1, 8, 15)
    - Typically these mutations result in defects in CFTR regulation by ATP and phosphorylation (8)
    - Third most common G551D mutation is in 3% of all CF patients (8)
        - Substitutes glycine for aspartate at amino acid residue 551 (8)
        - G551D-CFTR protein is adequately folded and inserts appropriately into the plasma membrane, but fails to open because of defective regulation (8)
        - Interferes with NBD1 and NBD2 dimerization, ATP binding, and hydrolysis (8)
    - When rescued and inserted into the plasma membrane, F508del exhibits defective regulation and can also be classified as Class III (8)

- Class IV
    - Reduced amounts of functional CFTR protein (1)
    - Causes defects in chloride channel conductance due to reduced single-channel chloride ion conductance and open channel probability (8, 15)
        - R117H missense mutation affects 0.5% of CF patients worldwide (8)
            - Causes a substitution of arginine to histidine at residue 117
            - R domain is usually phosphorylated, and NBD1 and NBD2 bind to ATP, but the opening time of the channel is reduced, therefore reducing chloride transport (8)
- Class V
    - Less than 1% of patients with CF have this mutation type (8)
    - Normal plasma membrane CFTR is produced, but less protein made due to lack of transcriptional regulation (8, 15)
    - Influences the splicing machinery, or mRNA processing, and generates both abnormal and normal spliced mRNA, which vary in levels among patients and even organs within the same patients (15)

[3]glycosylation-an enzymatic process that attaches glycans to proteins, lipids, or other organic molecules

- Spliced variants cause a reduced number of functioning CFTR in the plasma membrane of the cell (15)
- A455E is a common mutation (15)

Less functioning CFTR in the body's cells leads to a more severe phenotype of CF (1). Classes I-III are associated with more severe disease and higher mortality, as shown in Table 1 (1). Some mutations are more detrimental to the body's cells than others (17). For example, the class II mutation type, which includes the most common F508del mutation, affects the actual amount of CFTR at the cell surface because an immature protein will be created (17). The ER retains and fails to fully process the protein, which is instead degraded by ER quality control (17). Not enough CFTR protein will be made, posing a detrimental problem for regulation and conductance of ions into and out of the cells (17). The F508del mutation can be contrasted to the R117H mutation in Class IV, which is a milder mutation, because the CFTR protein is available at the cell surface, yet it decreases channel conductance of chloride ions (17). CFTR has reached the cell surface and there is enough of it, yet it cannot function to its full capacity and poorly conducts ions instead (17). The varying clinical consequences of the R117H mutation may not be as detrimental as those of the disease-causing F508del mutation (17).

Disease-causing mutations result in defective cAMP-regulated choride secretion in the body's cells (17). Restoring the function of CFTR-mediated chloride transport could improve CF (17). There are mutation-specific therapies for each class that seek to repair CFTR function:

- Class I: These treatments consist of aminoglycoside antibiotics and small molecules that camouflage premature stop codons, incorporating an amino acid into the sequence and allowing for translation to continue over premature stop codons to the normal termination of the transcript (17). The mechanism allows successful translation of mRNA into a full-length protein (17).

- Class II: Correctors are used to treat this mutation type. Correctors are specific chaperones or small molecules that allow mutants to escape ER degradation and reach the cell surface (17).

- Class III: Potentiators are CFTR activators that can overcome the channel gating and regulatory defects of CFTR mutants, which localize to the cell membrane (17). Potentiators are compounds that increase chloride secretion only in the background of normal physiological control; ideally these should not regulate the cAMP pathway, but rather act on the channel itself (17).

- Class IV: In order to compensate for less CFTR conductance, treatments include either increasing the cell surface density of these mutants in conjunction with Class II correctors, or increasing stimulation of the existing channels with class III potentiators (17).

- Class V: Splicing factors that correct missplicing or manipulate those that alter the balance of different splice forms must be increased (17). Increased levels of correct transcripts are promoted in CF patients who bear CFTR mutations that occur due to splicing errors (17).

Internal Environment of Cells

In addition to the regulation of chloride conductance, the CFTR protein also plays a role in fluid homeostasis and influences the inner workings of the cells (18). Cellular maintenance includes the transport of electrolytes (18). The main pathway that regulates CFTR activity is the elevation of cAMP and activation of protein kinase A (PKA), since PKA-mediated phosphorylation of CFTR opens the anion pathway in the channel and allows exit of chloride ions (18). The second messenger cAMP mediates the intracellular response to a wide range of cellular processes, some of which include gene expression, metabolism, and growth and division of the cell (19). The main effector of cAMP is PKA, a tetrameric enzyme in its inactive form that has two catalytic subunits (C) and one regulatory subunit (R) dimer. Once the R subunits are bound to cAMP, the C subunits are released and begin phosphorylation of downstream targets (19). Different stimuli can cause an increase in intracellular cAMP, but in order for the cell to execute the appropriate job of responding to a specific stimulus, the proper subset of downstream targets must be phosphorylated. Therefore, compartmentalization of the cell's components is important for successful stimulation and completion of the cAMP-signaling pathway (19).

An example of the importance of this pathway occurs in the F508del mutation. The protein is unable to reach the cell membrane and gets degraded by the ER, causing a lack of CFTR protein at the cell surface, thus failing to keep the cAMP-dependent chloride conductance going in affected tissues (18). Even with correctors of the F508del mutation, the channel has been shown to have regulatory defects like reduced channel gating. It has also been shown that when isolated, the F508del mutation maintains normal PKA-dependent regulation (18). These data suggest that the intracellular milieu must play an important role in the ability of CFTR to respond to cAMP regulation (18). Therefore, the mutation does not simply cause a change in stability of CFTR but also is associated with a change in the activity of intact cells (18).

In particular, CFTR activity depends on a high level of organization of cytoskeletal F-actin (18). The multiprotein complex specifically in epithelial cells involves F-actin and scaffolding proteins, NHERF1 and ezrin. This complex maintains CFTR in restricted areas of the plasma membrane of cells, and also controls CFTR function (18). Ezrin is a kinase A anchoring protein, which tethers PKA in the vicinity of CFTR, allowing cAMP-dependent chloride efflux to occur (18). The complex also ties mutant CFTR to the actin cytoskeleton, keeping the CFTR stable and delaying internalization, allowing efflux to occur (18). Altered cytoskeletal organization in cells results in excessive cAMP in the cytosol, and less cAMP in the subcortical compartment of the cell (18). Excessive cytosolic cAMP might have debilitating effects such as an increased activation of the nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB, and cells in CF airway epithelial cells have been found to have the effect of constitutive NF-κB,

signaling hyper inflammation (18). The lesser amount of cAMP in the subcortical region of the cell may cause a deficient regulation of the movement of chloride ions out of the cell (18).

Clinical Manifestations of CF

Normally, the CFTR protein is effective at mucociliary transport, assisted by hydration of the ASL (3). Hydration of the ASL occurs through a gradient by efflux of chloride ions through CFTR channels, in addition to influx of sodium through epithelial sodium channels in the membrane (3). Lack of CFTR or defective protein causes less chloride efflux and unregulated hyperabsorption of sodium ions (3). An imbalance of osmotic gradient causes dehydration of ASL, increased mucus thickness and damaged mucociliary transport as shown in Figure 3 (3).

Mucus is a complex fluid containing immunoglobulins, antiseptic enzymes, salts, proteins, glycoproteins and water (20). Mucus is secreted by mucous cells and has many important functions, one being defending against infectious agents (20). It is a dense, viscoelastic material that is stabilized by hydrogen bonding, electrostatic interactions, and hydrophobic interactions (20). The mucus of CF patients contains less water than normal, a high amount of debris, and is more viscous due to its high molecular weight (20). The thickening of mucus leads to abnormal clearance and also bacterial growth (20). Thick mucus can block airways, leading to bacterial infection, chronic local inflammation, and a decline of lung function (3).

The goblet cells in the epithelia release these mucins, forming double-layered attached mucus also found in the stomach, colon, lungs and small intestine because it is easily removable mucus (21). Mucins are proteins that possess long domains with amino acids and O-glycans (21). Several types of mucins form the actual mucus gel, and these have one or more domains involved in binding (21). Bicarbonate may be the missing link between CFTR and blocked mucus (21). Bicarbonate is an ion that can pass through the CFTR channel; it neutralizes pH and removes Ca^{2+} in order to unpack mucin during secretion (21). High amounts of bicarbonate ion, which are normally provided by functional CFTR are necessary for proper unfolding of mucins during secretion (21). In patients with CF who have the F508del mutation, the mucus becomes dense, difficult to move, and remains stagnant (21).

Defective CFTR affects the lungs not only with lung disease, but also with conditions such as acute lung injury (ALI) (22). One of the major mechanisms that causes the removal of edema fluid from the alveolar air space of the lungs in people with ALI is the active transport of sodium and chloride ions across the alveolar type I and type II cells in the lungs (21). This creates an osmotic gradient for the reabsorption of water to occur (22). A 2013 study by Roux et al provides a link between CFTR and other conditions such as ALI that can occur as a result of main dysfunctional protein channels. Results show that if there is inhibition of the CFTR channel and the epithelial sodium channel (ENac), failure to produce alveolar fluid clearance causes morbidity and mortality in affected individuals (22).

In the absence of CFTR, mucus accumulates in the intestines, providing a home for microbes to colonize (23). In previous studies using CFTR knockout animals, mycobacterial species have been found in antibiotic-resistant infections of the skin, lungs, and gastrointestinal tract, showing that without the CFTR channel, bacteria flourish in areas of mucus that have accumulated in the body (23). Defective secretion of digestive enzymes and resulting fat malabsorption in the gastrointestinal system demonstrate a failure to thrive in addition to excess fat in the feces (3). Also, ionic imbalance in the biliary tract may lead to increased risk of gallstone and hepatobiliary disease (3). Again, the CFTR channel is necessary for proper conductance within cells of the human body (23).

In addition to the airways, lungs, and gastrointestinal tract, the glands of the body also require proper chloride conductance. In a healthy person, the CFTR protein is responsible for reabsorption of chloride, and subsequently, sodium in the reabsorptive duct of the sweat gland (3). Lack of CFTR protein or protein function causes salty beads of sweat to rise in individuals with CF (3). Sodium is excessively secreted by sweat glands, which is why most patients diagnosed with CF have high sweat chloride levels (3).

The defective CFTR channel and effect on improper ion conductance in and out of the body's cells affects not only the respiratory, digestive, and endocrine systems but also the reproductive system. Females with CF may experience the thickening of cervical mucus, which could cause infertility (3). Most males with CF have a developmental defect, which blocks the transport of spermatozoa from the testes or epididymis to the vas deferens (5). This congenital bilateral absence of the vas deferens causes azoospermia, or lack of sperm in the semen of males (3).

Nervous System

The defective CFTR protein affects the peripheral and central nervous systems. CF patients have been found to have peripheral nervous system abnormalities such as neuropathy, and the CFTR gene has been expressed and active in Schwann cells (24). Mutations cause a loss of CFTR, which results in myelin sheath abnormalities of these Schwann cells (24). A 2013 study by Reznikov et al shows similar abnormalities to those found in neuropathies in knockout CFTR pigs (24). Chloride channels stabilize membrane potential in Schwann cells, so if the CFTR channel were removed or defective, changes in ionic flow across these cells would rapidly occur (24). In addition to abnormalities in Schwann cells, a delay in conduction of the vestibulocochlear nerve was found, as were axon density reduction and a decrease in speed of the trigeminal and sciatic nerve impulses (24). People with CF have also had an unusual cholinergic and adrenergic sensitivity in pupil constrictions, sweat and salivation, regulation of blood pressure levels, and constriction of the bronchioles (24).

The peripheral nervous system also contains neurotransmitters, which allow the transmission of signals from one neuron to the next (25). Acetylcholine is a neurotransmitter that participates in paracrine and autocrine signaling in uptake of choline; signaling occurs by mediation mechanisms such as facilitated diffusion, sodium-independent transporters including organic cation transporters, and sodium-dependent

choline transporters (25). Organic cation transporters are involved in choline transport across the plasma membrane; CFTR function might contribute to altered choline transport in non-neuronal cells through interference with organic cation transporters (25). Cholinergic activity is related to immune dysfunction and an up-regulation of cytokine production (25). Acetylcholine is also a major regulator of airway function by controlling contact between cells, stimulation of fluid secretion, ciliary beat frequency, and the overall regulation of the mucociliary clearance system (MCC), which are all major proponents of symptoms of CF disease (25).

In addition to neurotransmitters, Schwann cells, and their effects on the peripheral nervous system, Sphingosine-1-phosphate (S1P) is a signaling pathway that occurs via the central nervous system and CFTR function aids in proper regulation of its expression (26). A 2012 study by Meissner et al shows an inverse relationship between microvascular CFTR activity and S1P signaling in vasomotor responses and myogenic tone (26). Sphingosine-1-phosphate is a signaling mediator that regulates artery tone and vasoconstriction, controlling blood flow, blood pressure, and transport of blood to the tissues (26). CFTR moves extracellular S1P across the plasma membrane for degradation, and if CFTR function is interrupted in any way, the S1P signaling will also be affected (26). During heart failure, S1P signaling is enhanced in the resistance arteries, and improper function of CFTR may be a reason for these conditions (26). The CFTR protein could act as an S1P transporter, limiting S1P receptor-mediated effects (26). CFTR dysfunction could cause conditions that are associated with S1P signaling (26).

Immune Cells

In addition to the role of the CFTR protein in the nervous system, defective CFTR also largely affects the body's immune response. The body's immune response can be divided into two different segments, the innate immune system and the adaptive immune system, as shown in Figure 4 (27). The innate immune system contains leukocytes that interact with the environment, in addition to opsonins and antimicrobials that lack memory (27). Innate immune cells have receptors for foreign molecules like the mannose receptor and formyl-peptide receptors that activate TLRs (27). The adaptive immune system includes B and T lymphocytes, both of which contain single antigen-specific receptors. Adaptive immune cells have antigenic memory that causes them to respond rapidly and in response to re-exposure to a foreign antigen (27).

In a healthy person, alveolar macrophages are built for the innate immune response; in the lungs, they maintain a regulated, suppressive environment and prevent overreaction to antigens that enter the body (28). Macrophages work to clear pathogens by moving from protection to immunopathology by patrolling the airways (28). They produce cytokines and chemokines, kill and digest infected cells, and clear debris from the lung (28). However, their unique ability to produce a cytokine storm in response to bacterial or viral infections can lead to clogged airways, epithelial cell death, and unregulated repair of damaged tissue (28). Macrophages pose a problem for CF patients. Instead of producing inflammatory cytokines, alveolar macrophages produce IL-10 in CF lung disease; severe damage occurs due to persistent rounds of infection, clearance, inflammation, and remodeling (28).

Since the CFTR protein alters function of immune cells, an abnormal immune response could cause CF patients to become more immunocompromised (29). Neutrophils provide the first line of defense against airway infection by killing and digesting phagocytized bacteria and fungi in the airways (30). CF airways contain an abundance of neutrophils, which can be attributed to the clearance of *P. aeruginosa* (31). Over time, the neutrophils fail to get rid of *P. aeruginosa* and the release of intracellular components becomes improperly regulated; this leads to development of bronchiectasis, or damage and scarring done to the airways (30). Neutrophilic dysfunction can be attributed to both the intense inflammation of the airways and to the lack of CFTR protein within the cell (30). A lack of CFTR function has been linked to decreased phagocytic capacity and in a cascade effect that results in lack of destruction of the *P. aeruginosa* bacteria, chronic infection, and accelerated injury to the site of infection (30). Both mutation and inhibition of CFTR could cause neutrophils to produce more pro-inflammatory cytokines in response to the lipopolysaccharide (LPS) on the bacteria (31). CFTR reduces bactericidal ability in neutrophils of bronchoalveolar lavage cells, further exacerbating lung infection (32).

The problem in CF patients is the quality of their immune response. In normal neutrophils, efficiency of microbicidal action requires the production of hypochlorous acid (HOCl); it is produced in the presence of hydrogen peroxide (H_2O_2) by an enzyme called myeloperoxidase (MPO) (33). The production of HOCl is necessary for proper host defense, especially since MPO-deficient humans and mice are likely to get fungal and bacterial infections (33). Neutrophils of CF patients have chloride-deficient phagosomes, causing them to lack the amount of necessary HOCl to kill an organism such as *P. aeruginosa* (33).

Cystic fibrosis is a disease that leads to ineffective management of viruses and bacteria. The most common pathogen found in CF patients is *Pseudomonas aeruginosa* (*P. aeruginosa*), which produces a neutrophil-dominated host response that fails to clear the infection, causing a cycle of inflammation and infection to occur (34). A persistent inflammatory response contributes to progressive lung injury, and colonization of *P. aeruginosa* results in an antibody response, which unfortunately is not protective (34). Cell-mediated immunity against these bacteria is critical to host defense, and a subset of T helper cells called the T helper 17 (Th17) cells come into play (34). Th17 cells produce a cytokine called Interleukin-17 (IL-17), which causes the generation and recruitment of neutrophils to sites of infection (34). Murine models infected with acute pulmonary *P. aeruginosa*, Th17 plays a role in vaccine-induced protection; human models show that Th17 is in the submucosa of airways, and that IL-17 is produced from innate immune cells in the CF lung (34).

In typical Th17 responses, IL-17 has been co-expressed with Interleukin-22 (IL-22), which is a cytokine that promotes repair of epithelial surfaces and elicits an anti-microbial slate, thus becoming important for non-immune cells (34). IL-22 has been found to provide host defense upon inflammation of the gut following bacterial infection (34). T helper cells that produce IL-22 without IL-17 are called Th22 cells, which are

found to infiltrate the skin in inflammatory disorders such as psoriasis while also releasing antimicrobial peptides; Th22 cells are strong elements in host defense against lung pathogens such as *Klebsiella pneumoniae* (34). A 2014 study by Bayes et al shows that the presence of Th22, Th17, and Th1 memory CD4+ cells in CF patients are important for the host defense by the immune system in CF patients (34).

In addition to IL-22 and IL-17, Interleukin-13 (IL-13) is also important in inflammatory responses, specifically in intestinal regulation (35). IL-13 has effects on hematopoietic and non-hematopoietic cells, such as macrophages, epithelial cells, and enteric neurons, while also mediating smooth muscle contraction, and regulating intestinal epithelial differentiation and apoptosis (35). IL-13 is an important factor in regulating the intestinal epithelium and more relevantly, CFTR-mediated chloride ion conductance (35). IL-13-induced chloride secretion is CFTR-dependent and is associated with increased expression of CFTR (35).

The airways of those affected by CF fail to manage bacteria efficiently, causing infection (32). Two cytokines, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), are released upon response to *P. aeruginosa* infection and are released in higher amounts in Cystic Fibrosis airways (36). IL-1β and TNF-α and stimulate fluid secretion by submucosal glands that can be blocked by the CFTR inhibitor (36). A 2012 study by Baniak et al in swine airway submucosal glands shows that IL-1β stimulates CFTR-mediated chloride movement across submucosal gland serous cells (36). Bacteria-triggered gland secretion occurs as part of the normal innate immune response to bacterial inhalation (36).

The role of CFTR in non-epithelial cells has somehow received less attention, but it has been established that the absence of CFTR in lymphocytes leads to a divergence in the adaptive immune response in living cells (29). CFTR expression in lymphocytes is associated with volume regulation and regulation of CD8+ T cells, which are cytotoxic T cells that make cytokines (29). In addition, a defect in cyclic AMP-dependent chloride currents in CF-derived lymphocytes shows evidence of the role of CFTR in lymphocytes (33). Studies also show that regardless of B cell function in Cystic Fibrosis patients, the T cell activation events and helper function provide a strong antibody response (29). By testing small molecule regulators of the immune response, researchers can use these findings to help CF patients (29).

In a 2011 study by Abu-El-Haija et al, an analysis of the abundance of neutrophils and macrophages in healthy versus diseased pig pancreatic blood was completed by comparing CD14 antigen levels in the blood (37).[4] Overall, the CF pig pancreas exhibited a significantly increased level of monocytes, macrophages and neutrophils than non-CF pigs and it is even more striking that neutrophils were almost nonexistent in the non-CF pig pancreas (37). The innate immune system response proves to be exclusive in the CF pig pancreas, as macrophages and neutrophils both infiltrated the pancreas, signaling pancreatitis (37). The same study shows that T helper cells and B cells were found in

[4]CD-cluster of differentiation; CD14 detects LPS wall of bacteria

higher numbers in the pancreas of CF pigs versus non-CF pigs (37). It can be concluded that there was indeed activation of adaptive immune system cells (B and T cells) in the pancreas of newborn CF pigs (37).

In summary, macrophages express CFTR and their defect by contributing to an inappropriate host response to opportunistic bacterial pathogens; this response consists of to chronic inflammation and remodeling of the site of inflammation (38). Bacteria target neutrophils, and this causes apoptosis or programmed cell death, in addition to a massive release of toxins and pro-inflammatory products (38). Neutrophils end up doing more damage than good to the airways due to their domination in the inflammatory response (38). T helper cells recruit cytokines to the scene in order to promote this inflammatory response (34). The unique presence of both innate and adaptive systems in order to combat CF shows the extensiveness and complexity of the disease (37).

The Relevance of Animal Models

Animal models have been used to study the effects of CF on live organisms and to come to more clear conclusions about the disease. The following animal models show the effects of CF on target areas of the body including the gastrointestinal tract, pancreas, liver, and gall bladder. A 2011 study by Keiser and Engelhardt shows similarities between phenotypic effects of CF on animals and humans.

Gastrointestinal (GI) Tract

Pigs and ferrets with CF show a phenotype of a condition called meconium ileus, an in-utero intestinal obstruction present in about 15% of newborn infants with CF, when the earliest stool of an infant becomes blocked in the ileum of the small intestine (39). Meconium ileus is fatal within the first 48 hours after birth in piglets with the dysfunctional CFTR gene if no surgery is performed, and is similar to what is exhibited in the phenotype of infants with cystic fibrosis (39). Intestinal atresias, diverticulosis, and microcolon are all conditions that occur in CF infants that can be seen in pigs and ferrets that have the dysfunctional gene as well (39). Meconium ileus in the cystic fibrosis ferret has been found to have a significant genetic influence, as also observed in infants with CF (39). The pig with the CFTR-F508del protein exhibits residual processing to the plasma membrane and partial function, demonstrating that the pig likely has high levels of functional CFTR in the intestine to clear meconium after birth (39). Unlike the CF ferret or pig, the CFTR knockout rat does not present with meconium ileus at birth (40). Animals develop intestinal blockage only after weaning off their mother's milk and nutrients, which is similar to intestinal obstruction syndrome observed in children and adults with the disease of CF (40).

Pancreas

In addition to the gastrointestinal tract, the pancreas is a severely affected organ in people with CF (39). In newborn pigs with the knockout CFTR gene, the destruction of the exocrine pancreas compares to more severe cases, while the pigs with the mutated F508del mutation have a less severe exocrine pancreatic phenotype than the one with the lack of the gene (39). Dysfunctional protein could actually reduce the force of the disease progression (39). In ferrets with CF, the exocrine pancreas undergoes destruction over the

course of the first few months of life, leading to pancreatic dysfunction and a need for pancreatic enzymes (39). Both pig and ferret models demonstrate the decline of the exocrine pancreas in patients with CF.

Liver and Gall Bladder
In addition to pancreatic and GI tract problems, CF also affects the liver and gallbladder (39). Biliary cirrhosis is a common cause of morbidity in humans with CF; pig and ferret models have been studied to explore hepatic lesions, cellular inflammation, and fibrosis, which are all major signs of biliary cirrhosis (39). Newborn ferrets with CF have unusually high levels of bilirubin and plasma alanine aminotransferase, both of which are indicative of liver disease, and this finding is similar to children with CF who have unusually elevated liver enzymes in their blood (39). In addition to the liver, gallbladder disease is observed in 15-30% of older CF patients during the autopsy (39). This disease is extremely severe in pigs with the CFTR-F508del protein (39). In the gall bladder of affected pigs, aggregation of neutrophils, mononuclear inflammation, and luminal obstruction by bile and mucus have all been found (39).

Growth and Nutrition
Nutritional defects are also seen in people with CF (39). It has been found that chloride secretion is defective in the thyroids of pigs without the CFTR gene, possibly demonstrating a mechanism for CF-linked hypothyroidism (39). The serum of newborn pigs and infants with CF show reduced levels of the hormone called insulin-like growth factor 1 (IGF-1), and mice with CF have similar reductions in this serum IGF-1 when they grow older (39). CFTR plays a role in the neuroendocrine system in controlling growth (39).

Transcriptional Regulation
DNA must be properly transcribed into mRNA and then translated into protein (40). Histone acetylation and deacetylation occur within the coding regions of genes, either adding or removing acetyl groups from histones (40). Histone acetylation and deacetylation are two critical processes for proper gene expression and proper cellular function, and they must be balanced for correct protein function (40). A 2011 study by Gunderson et al provides evidence that histone acetylation and deacetylation maintain proper co-transcriptional splicing by facilitating dynamic rearrangements of the spliceosome, which are ATP-dependent (40). There is an increase in histone acetylation due to lack of histone deacetylases (HDACs), resulting in altered spliceosome dynamics (40). Methylation is another type of histone modification that creates binding sites for factors that help with splicing, and therefore improve transcriptional modifications (40). Both methylation and acetylation are stable modificiations, but acetylation could allow more dynamic rearrangements of the spliceosome (40).

Multiple HDACs can remove acetyl groups in a process known as histone deacetylation (40). Deacetylation decreases gene expression, while acetylation increases gene expression (40). Although HDACs work in transcriptional regulation, studies have shown that inhibiting these HDACs could be more productive than allowing them to work (41). A 2011 study by Hutt et al shows that cyclic tetrapeptides restore CFTR activity by

inhibiting HDACs. Cyclic tetrapeptides also correct the most common mutation, F508del, from the ER so that cell surface channel activity can be enriched once again and the smooth influx and efflux of chloride can resume (41). These cyclic tetrapeptides were shown to overcome the trafficking defect associated with the F508del protein, which was monitored on its way to the cell's surface by Western blot analysis (41, 42).[5] Therefore, protein acetylation pathways can actually work in correcting the maturation of the folding and function of this mutation (41). The connection between HDAC inhibition and CF biology can not only help with CF, but could also help to potentially correct other protein-misfolding diseases (41).

Other transcription factors, such as Liver X receptors (LXRs) may contribute to CF. These are transcription factors that regulate lipids and glucose metabolism and belong to a family of nuclear receptors (42). LXRs have been found to regulate the function of several important transport systems in the body such as the epithelial sodium channel, kidney transporters, organic anion transporters, and the sodium-inorganic phosphate co-transporter (42). A 2013 study by Raksaseri et al provides evidence that these LXRs are capable of reducing CFTR-mediated chloride secretion in a cell line in murine primary inner medullary collecting duct cells (42). Results of the Western blot analysis show an inhibition of chloride secretion due to a decrease in CFTR protein, and this is independent of down-regulation of its mRNA expression (42). LXRs, or transcription factors, are able to down-regulate the CFTR-mediated chloride secretion of kidney cells (42).

Another transcription factor, called the glucocorticoid receptor (GR), acts as a repressor of CFTR expression (5). Yigit et al conducted a study in 2013 that tested the binding of GR in nucleosome-depleted regions in a specific cell type (5). If ligand activation of GR down-regulates CFTR expression, then ligand-receptor binding inhibition could cause an increase in CFTR expression in cells, resuming proper conductance of ions into and out of cells (5).

Bacterial infections are more likely to occur in patients with CF due to mucus dehydration and reduction in mucociliary clearance (43). One such infection is gram-negative *P. aeruginosa*, as it is a cause of pulmonary inflammation in lung disease of Cystic Fibrosis patients and correlates with a faster decline in lung function, morbidity, and mortality (43). The innate system responds to the cycle of infection and inflammation through Toll-like receptors (TLRs), which then trigger signaling cascades by transcription factors such as NF-κB (43). Upon *P. aeruginosa* infection, TLRs are stimulated and activate the Extracellular-signal Regulated Kinase (ERK1/ERK2 pathway), which regulates cell growth and differentiation, and is used for inflammatory signal transduction (44).

In order for the inflammatory response to be timely and for regulated termination of signaling to happen, intracellular membrane trafficking and routing must occur in the receptor-ligand complex (43). Once infected with *Pseudomonas aeruginosa* bacteria, the

[5]Western blot analysis-an experimental procedure used to detect histone modifications

detection of the LPS bacterial wall causes TLR4 activation to occur by dimerization (43). The TLR4-LPS bound complex is then internalized, and TLR-induced inflammatory signaling is inhibited once the complex is targeted for degradation (43). This complex is then directed toward endosomes for degradation or toward the Golgi apparatus for recycling (43). The functional CFTR protein works in altering the internal compartmentalization of TLR4 in macrophages, but patients with CF often show signs of ongoing and uncontrolled inflammation (43). Altered TLR4 expression in CF airway cells actually contributes to an increased inflammatory response (43). Patients with CF show chronic uncontrolled activation of NF-κB and an increased amount of released inflammatory mediators than the healthy population does (43).

The airway epithelium is important because it is the first line of defense for the lungs (35). Immune cells act as a mechanical barrier to reduce the risk of infection and to also produce chemokines and cytokines that recruit phagocytic cells to ingest organisms and infected cells (35). Due to the sterility of the lungs, interactions with microorganisms can cause inflammation as a response to infection (35). The immune system essentially gains access to bacterial information by the shedding of surface or intracellular receptors, known as pathogen-associated molecular patterns (PAMPs) (35). When the innate immune system senses these PAMPs, such as the LPS or flagella, inflammation is stimulated (35). In addition to this inflammatory response, the mucosal response is used for clearance of pathogens (35). Excessive inflammation represents an immunocompromised respiratory system, making the regulation of pro-inflammatory signaling so important for the body's cells (35). Components of the innate immune system, such as NF-κB, are activated in order to regulate signaling (35). TLRs are integral membrane glycoproteins that recognize microorganisms, and they sense microbial products or PAMPs, eventually leading to NF-κB expression (35). In CF patients, the lungs are usually in a hyper-inflammatory state, which causes the signaling of NF-κB (35).

Post-Translational Modifications

Success in ER folding and assembly is essential for proteins to exit the ER, and a large fraction of translated proteins fails at the quality control checkpoints that follow (7). Due to this failure, these proteins are retained at ER checkpoints, leading to ubiquitylation and degradation by a proteasome, called 26S, which is a protein complex that breaks down other proteins (7). Ubiquitylation is a post-translational modification, or an addition to a protein after it has been made (7). The CFTR protein does not always get ubiquitylated because it is long and difficult to fold (7). A 2013 study by Ahner et al showed that ER-based machinery containing heat shock proteins facilitated CFTR folding (7). A reduction in the NBD1 aggregation occurred upon interaction with one of the heat shock proteins, and the CFTR folding efficiency improved (7).

Modifier Genes

TGF-β is a genetic modifier of CF, which mediates the pulmonary fibrosis that characterizes respiratory deterioration in patients with CF (12). It has been shown that CF patients with specific polymorphisms in TGF-β_1, a pro-fibrotic cytokine, are at a higher risk for severe lung disease (45). Multi-organ fibrosis is known to occur in cystic fibrosis

of the pancreas with conditions such as liver cirrhosis, pancreatic fibrotic obliteration, and vas deferens obstruction (45). TGF-β mediates fibroblast physiology in the lungs and also contributes to the severity of the disease in patients with CF (46). A myofibroblast is a fibroblast containing smooth muscle, and it has been previously identified as a main mediator of profibrotic conditions (45). Myofibroblast differentiation usually occurs due to tissue injury or mechanical stimulation, and contributes to healing of wound edges, while still promoting the formation of extracellular matrices (45). In a healthy person, when tissue injury is resolved, myofibroblasts undergo apoptosis, or programmed cell death, but in CF patients there has been an increase in myofibroblasts in alveolar tissue (45).

The myofibroblast phenotype depends on TGF-β and occurs due to epithelial injury or inflammation, which are two main characteristics of CF respiratory deterioration (45). TGF-β must be activated to bind the TGF-β receptor complex, which then transmits a signal through Smad protein phosphorylation (pSmad2/pSmad3) (45). Myofibroblast differentiation is induced with the presence of TGF-β and mechanical strain (45). TGF-β signal transduction occurs due to hypoxia, epithelial injury, and an increase in protease activity (45). In addition to the induction of the expression of genes that promote fibrosis, TGF-β1 is secreted by endothelial, hematopoietic, and connective tissue cells, and inhibits epithelial proliferation (45). Myofibroblasts also promote TGF-β activation by contracting, and through mucus-plugging and chronic cough in CF (45). As this modifier signals for the myofibroblast phenotype in the CF lungs, it could provide insight into the necessity for anti-fibrotic therapies, which could decrease tissue scarring and respiratory compromise in CF patients (45).

Pulmonary fibrosis is a common end-stage of CF lung disease, and tissue remodeling of the airways occurs by increased collagen deposition (47). Fibroblasts form cells that produce an extracellular matrix that regulates tissue repair in parenchymal tissue (47). They can secrete strong inflammatory chemoattractants such as chemokines, monocytes, and interleukins, contributing to disease pathogenesis (47). In particular, fibroblast dysfunctions are seen in CF patients and they have a phenotype with increased proliferation and myofibroblast differentiation; dysfunctional fibroblasts are a potential target for treatment of CF patients (47).

In addition to TGF-β, other genes have been found that can be grouped as modifier genes that contribute to phenotypic differences observed in diseases like CF (48). Mannose-binding lectin (MBL) is a serum protein that is produced by the liver and may have an effect on disease severity in CF patients (49). It binds to bacteria, activating the lectin pathway of complement, and causing direct lysis of the target (49). The lectin pathway is antibody-independent and is triggered by MBL binding to carbohydrates on the surface of microorganisms (49). MBL also causes phagocytosis by bridging phagocytes and microorganisms such as *P. aeruginosa* and *Staphylococcus aureus* (49). MBL accumulates in large quantities in the lungs during acute inflammation in patients with CF, thus promoting phagocytosis and complement system activation (49). Lack of MBL has been associated with reduced lung function, earlier infection with *P. aeruginosa*, and a higher rate of the end stages of CF (49).

The mannose-binding lectin (MBL2) gene encodes a protein, which is secreted by the liver and leads to opsonization and activation of the complement system through the classical pathway (48). This serum concentration of the protein and MBL's ability to trigger the complement system depends on single-base mutations in the MBL2 gene (48). These mutations may increase the susceptibility of carriers to colonization by bacteria and viruses. The IL-8 gene codes for a member of the chemokine family and responds in cases of acute inflammatory reactions (48). IL-8 is produced by monocytes, macrophages, and fibroblasts, and helps in activation and movement of neutrophils from the blood to the tissues for an amplification of an inflammatory response (48).

Some genes and gene modifiers play a role in inflammation, such as the tumor necrosis factor alpha (TNFα) gene and Alpha-1-antitrypsin (AAT) (48, 49). TNFα leads to the expression of a pro-inflammatory cytokine that responds to specific triggers; the cytokine stimulates a release of IL-6 and IL-8 cytokines that cause an increase in mucus production (48, 49). AAT, another modifier gene, codes for a serine protease glycoprotein; the glycoprotein limits the amount of tissue self-damage during an inflammatory response. Severe AAT deficiency results in a pulmonary disorder that is similar to emphysema (48).

In contrast to TNFα, peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-controlled transcription factor of the nuclear receptor family that has an anti-inflammatory role in regulating gene expression by transactivation or transrepression (50). In the lungs, PPARγ has been proven to go beyond the regulatory role of anti-inflammation and extends to host defense (50). Results of a 2012 study by Griffin et al provide a strong inverse correlation between PPARγ expression and to a lower extent PON2 gene expression with neutrophil counts; low levels of these two genes are associated with high levels of inflammation (51). This specific study shows the genes in BALF cells associated with *P. aeruginosa* infection only, meaning that this pathogen is so resistant that PPARγ and PON2 could actually be therapeutic if used in an inverse manner (51). If it holds true that less gene expression causes more inflammation, a drug that inhibits PPARγ could protect against the infection and inflammation cycle of *P. aeruginosa* (51). PPARγ signals for the cessation of neutrophils and macrophages and assists in inflammation resolution, thus ending the cycle of infection and inflammation in diseases, and proving to be a therapeutic target (50).

Genome-wide Association Studies

In light of the high cost and lack of feasibility of genome sequencing, genome-wide association studies (GWAS) have become more practical and provide ways to study large populations without bias of pre-existing models (52). Scientific research has more freedom to identify new genes, regulatory sequences, and new pathways (52). Understanding the ways in which genes and their alleles exert their effects can lead to new therapeutic approaches (52). GWAS use genetic profiles geared towards personalized medicine (52). For example, two people with different inflammatory responses could need two different dosages of anti-inflammatory drugs; while these may

be common treatments, each could be more beneficial with the help of modifier genes (52).

Since Cystic Fibrosis is a rare genetic disorder that is caused by several genetic variants within a single gene, and due to the strength of these variants, the disease follows an autosomal recessive inheritance pattern in families who have the disorder (53). In a 2012 study by Bush and Moore, multiple mutations were found in the CFTR gene as the cause of CF (53). Families affected by CF were genotyped using a collection of genetic markers across the genome; markers that diverged in families were examined (53). The technique used to trace these mutations is called linkage analysis, and it is only applied to rare disorders such as CF (55). Linkage analysis does not work well for diseases that have more common disorders such as cancer or heart disease, showing that genetic mechanisms strongly differ among rare diseases (53).

A 2013 study by Weiler and Drumm provides evidence of two new genes associated with CF, called APIP and EHF (52). APIP encodes the Apaf-1-interacting protein, and EHF is an epithelial-specific transcription factor; both act as disease modifiers through different models (52). It is hypothesized that APIP prolongs neutrophilic inflammation and leads to more severe lung disease, while EHF regulates epithelial cell differentiation during times of stress and inflammation (52).

Variability in CF phenotypes is partially due to non-CFTR genetic modifiers. Mucin genes participate in the development of the lung disease and can be seen as genetic modifiers of the CF phenotype (54). Mucins are expressed as glycoproteins that are important in the airway epithelium (54). Mucins contain a tandem repeat (TR) domain that shows variation in repeat number, also known as variable number tandem repeats (VNTRs) (54). Differences in VNTR sizes could cause a change in mucin protein molecular weight by two-fold (54). These secreted mucins are responsible for mucociliary clearance and contribute to glycocalyx barrier functionality (54). Cystic fibrosis, being a respiratory disease, has variations in mucin expression that could contribute to pathophysiology, thus leading to less mucociliary clearance, dehydration of the ASL, and increased risk of chronic lung conditions (54). Since at least 50% of the variability in the severity of the lung disease of CF patients is attributed to heredity, genetic modifiers of CF must explain some of this heritable variation (54). Phenotypic changes due to respiratory mucins could add to genetic modification, especially VNTR length (54).

A 2013 genome-wide association study by Blackman et al was conducted using 3,059 individuals with CF and 644 of them having CF-related diabetes (55). Cystic fibrosis-related diabetes (CFRD) is a common complication of cystic fibrosis associated with severe lung disease, malnutrition, and death (55). CFRD is age-dependent, affecting 19% of adolescents and 40-50% of adults with cystic fibrosis, and is strongly influenced by modifier genes (55). In addition, Single-Nucleotide Polymorphisms were found that were associated with the SLC26A9 gene, an epithelial chloride and bicarbonate channel, which

interacts with the CFTR protein (55). [6] Since diabetes is an extremely prevalent complication of CF, susceptibility can be determined by variants associated with SLC26A9 at four gene loci (55). These gene variants (TCF7L2, CDKAL1, CDKN2A/B, and IGF2BP1) are associated with both Type 2 diabetes and CFRD (55). These loci contribute to both diseases and support the concept that diabetes develops in individuals who may have underlying susceptibility to pancreatic β-cell dysfunction (55). Variants in CDKAL1 have been reported to damage pro-insulin translation and to stimulate the ER stress response that contributes to apoptosis, or programmed cell death (55). CDKAL1 works as a CFRD modifier because ER stress is stimulated by CFTR mis-folding (55). TCF7L2 may affect β-cell mass and pro-insulin processing, and CDKN2A/B suggests a role for growth, apoptosis, and insulin processing in CFRD (55). This study shows that even though CFRD is not the same disease as Type 2 diabetes, they share genetics which cause similar disease pathways, also showing how CF affects the pancreas just as much as other organs (55).

The pancreas has as an endocrine function, which is to synthesize hormones such as insulin from beta cells, glucagon from alpha cells, and somatostatin from delta cells (56). It also has exocrine functions, which are to secrete digestive enzymes into the small intestine and to produce pancreatic polypeptide (PP) producing cells in the pancreas (56). Results of a 2013 study by Zertal-Zidani et al show that a small molecule inhibitor called glibenclamide triggered the endocrine differentiation pathway in the developing pancreas, increasing the number of endocrine cells (56). This study concluded that inhibition of CFTR increases the number of endocrine cells in the developing pancreas, and that these small molecule inhibitors can amplify the development of pancreatic endocrine cells (56). The mechanism in which CFTR inhibition increases the number of pancreatic endocrine cells is unknown; however CFTR functions as a chloride channel activated by cyclic AMP and protein kinase, and facilitates the transport of organic ions (56). There is definitely evidence of its regulation of ion channels, pH, and cell volume (56).

In addition to its effects on the pancreas, Cystic Fibrosis is also linked to bone disease. CF-related bone disease occurs due to low bone mass and increased fracture risk as a result of complications of CF (57). Damage to osteoblast bone formation and increased osteoclast bone resorption both occur, in a process known as “uncoupling bone turnover.” Vitamin D deficiency, inflammatory cytokines, and intestinal malabsorption all contribute to this bone disease (57). A direct link is shown between dysfunctional CFTR and Cystic Fibrosis-related bone disease, especially via the inactivation of the CFTR gene in osteoblasts and its contribution to low bone mass (57). In a 2013 study by Stalvey et al focusing on murine bone, CFTR was expressed in osteoblasts (57). Inactivation resulted in defective differentiation and late new bone formation, and CFTR inactivation also resulted in pancreatic disease (57). As CF patients age, they tend to have age-related complications such as spinal, arm, and hip fractures, and these findings show that CFTR expression in the bone causes reduced osteoblast differentiation and enhances osteoclast bone resorption (57).

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[6]SNP: Single-Nucleotide Polymorphism, or a DNA sequence variation occurring when a single nucleotide (A, T, C, or G) in the genome differs between members of a biological species or paired chromosomes; ex: DNA fragments from different individuals could be AAGC**C**TA to AAGC**T**TA

Not only do genes affect the observed phenotype in CF patients, but environmental factors play a role as well (58). A 2012 twin study by Blackman et al underscores the influence of genetic and non-genetic factors on nutrition in young CF patients who experience the greatest changes in growth rates (58). BMI, or body mass index, (kg/m^2) was used as a marker of nutritional status and more accurately predicts the nutritional failure in CF patients than other conventional measures (58). Nutritional status of young twins and siblings with CF was analyzed, and genes other than CFTR were found to influence the variation in body mass index, such as genetic modifiers located at the loci of chromosomes 1 and 5 (58). CF patients are under nutritional stress due to poor appetite and weight loss; this lack of nutrition could make patients uniquely sensitive to genetic factors, which also affect maintenance of weight (58).

Another twin study from 2011 by Stanke et al suggests that both inherited and environmental factors influence CF disease manifestation (59). The shared prenatal and early postnatal period distinguished twins from siblings, and was associated with low differences within the twin pair itself in weight-for-height percentages (59). In addition, concordance in lung function was dominated by genetic factors, as only monozygous twins shared their entire genetic information, in contrast to dizygous twins, or siblings who only share half of their genetic information (60). Inherited factors have a larger impact on patient-to-patient variability in weight-for-height percentages than for lung function (59).

Current and Potential Therapies for CF

CFTR governs a dominant fluid and electrolyte secretory pathway in the nasal airways, essentially regulating mucociliary clearance (MCC) in airway epithelia (61). This mucociliary clearance is so important for maintaining healthy sinus mucosa because it contains vital elements for function, such as ASL (61). There must be a balance of ion transport across epithelial cell membranes in order to maintain adequate viscosity and depth in this ASL (61). The CFTR channel transports a high amount of chloride and bicarbonate to the lower and upper respiratory epithelium (61). If the chloride transport is disrupted, this could lead to dehydrated ASL and mucus blockage, which is a common occurrence for patients with CF (61). Mucus stasis is shown in people with chronic rhino sinusitis, a condition in which the sinuses surrounding the nasal passages become inflamed or swollen (61). This interferes with drainage and causes mucus to buildup (61). Results from a 2013 study by Zhang et al show that there are certain potentiators, one known as Resveratrol, that cause the CFTR channel to have a higher open probability, thus regulating the mucociliary pathway (61). By changing the CFTR protein itself, the normal governance of the channel could prevent mucus blockage in the sinuses (61).

In people with dysfunctional CFTR or lack of CFTR protein, CFTR regulation can be targeted by drugs that act at a site outside the cell on the CFTR protein; these drugs decrease the function of voltage-dependent blockers or anions, and therefore increase overall CFTR function in CF patients (9). There are currently potentiatiors, or drugs that enhance the function of the defective CFTR protein channel. CF-causing mutations involved in ATP-dependent gating can be corrected by small molecules, which indirectly

change the channel gate via allosteric binding (62). A 2012 study by Eckford et al shows the use of a drug called VX-770, also known as ivacaftor or kalydeco, which causes the defective channel to open via a different mechanism, not involving ATP binding and hydrolysis (62). VX-770 is a small molecule that causes ATP-independent phosphorylation of CFTR to occur (62). VX-770 binds directly to phosphorylated CFTR and opens the channel without ATP (62). VX-770 may help patients with mutations that especially cause disruption to the site of the CFTR protein that ATP would normally bind to during ATP-dependent phosphorylation (62).

Increasing activity of the CFTR protein could potentially treat CF. A 2011 study by Ramsey et al shows a randomized, placebo-controlled trial in which VX-770 was associated with significant improvements in primary and secondary end points in people with CF who had at least one copy of the G551D-CFTR mutation (63). VX-770 is a systemic modulator that could affect CFTR function in GI epithelia, contributing to improved absorption of nutrients in patients with CF; after 48 weeks, patients treated with VX-770 had gained an average of 2.7 kg more weight than those receiving the placebo (63). Patients receiving VX-770 were 55% less likely to have a pulmonary exacerbation than those receiving the placebo over a period of 48 weeks (63). VX-770 was the first agent to show reduced sweat chloride levels, and it also improved CFTR-mediated ion transport (63). Ramsey's study shows that there are drugs that target CFTR dysfunction, leading to the change in phenotype, and that the CFTR protein is a valid therapeutic target for the pathophysiology of CF (63).

Mutant CFTR proteins can be studied in a cell-free system where protein interactions are minimized and ligand concentrations are well controlled (62). The VX-potentiation effect is mediated via specific binding to the CFTR protein (62). Future work could determine whether VX-770 may indirectly change the properties of the phospholipid bilayer (62). There are advantages to allosteric binding rather than directly targeting the active site, such as having a greater potential for selectivity and also conserving ATP-binding sites across members of the ABC family of membrane proteins (62). A compound that targets these active sites, such as VX-770, may have cross-reactivity with different family members (62). VX-770 works by interacting with a region unique to CFTR and away from the catalytic site (62). Since it works without ATP, when VX-770 binds with ATP, an additive effect occurs that stabilizes open conformation and enhances the probability of opening the channel (62). Essentially, the mechanism of action is to target the defect of the mutation in the CFTR channel (62). By using purified and reconstituted CFTR and mutated CFTR proteins, greater precision occurs in targeting the actual pore (62).

VX-770 is also used as a single agent in combination with correctors to target a broad range of mutations (64). The G551D-CFTR mutation is currently targeting CF in its third phase trial in 2-5 year olds (64). Data is expected in approximately 300 children in North America, Europe, and Australia (64). A 2013 Phase 3 study of VX-770 was completed on patients with R117H mutation, which is a residual and mild form of the CF phenotype; results indicate an increase in lung function in patients who took the VX-770 drug as opposed to patients who took the placebo (64, 66). A Phase 2 clinical study of Ivacaftor in people with residual function mutations other than R117H is being conducted by

Vertex Pharmaceuticals (64). Enrollment for this study is complete and data should be expected in the summer of 2014 (64). Vertex is preparing to conduct a 12-week study of a potentiator called VX-661 in combination with Ivacaftor in people with CF who have two copies of the F508del mutation, and enrollment in this trial will occur in the spring of 2014 (64). Ataluren aims to correct the underlying genetic defect in people with Class I nonsense mutations of CF; a phase 3 trial will occur, having proven to work better without tobramycin, a chronic aminoglycoside antibiotic (64). Lynovex is a drug that treats persistent lung infection in people with CF (64). It uses a new peptide-based approach that breaks down excessive mucus, penetrates, and kills bacteria in a way that prevents them from establishing antibiotic-resistant infections. Trials will begin and end with results in 2014 (64). *CF Matters* is a collaboration project that aims to develop personalized antibiotic treatments for CF chest infections, led by Professor Stuart Elborn, Director of Queen's Centre for Infection and Immunity (64). This study will contain 252 patients from 7 countries and will use molecular next generation DNA sequencing methods to detect all the bacteria present in the sputum of CF patients; therefore the study will determine what antibiotics to use in individual patients (64). This precision in determination of bacteria in the sputum will occur in order to avoid antibiotic resistance (64).

In contrast to therapies that open the CFTR channels, there are some therapies that block the opening of this same channel. Open channel blockers are used to block the CFTR channel from influx and efflux of chloride channels in cells of the body, thus inhibiting CFTR from functioning if the current function is overactive. These blockers work by binding to specific sites within the channel pore with high affinity (60). Open channel blockers are anions, and positively charged amino acid side chains in the CFTR channel pore help with electrostatic attraction of chloride ions (60). Mutations that eliminate the positive charge on the lysine side chain significantly reduce the channel blocking affinity of open channel blockers, such as glibenclamide (60). These open channel blockers all have different structures, but they share a common blocking mechanism: they are attracted into the pore by electrostatic attraction between the negative charge on the blocker and the positive charge on the lysine side chain (60). Once inside the pore, they bind tightly enough to block the opening and prevent chloride from leaving (60). The positive charge on the lysine side chain explains the sensitivity of CFTR to anion blockers because a positive charge is necessary to attract chloride ions and maximize the rate of conductance (60). This fixed positive charge also attracts all anions in the cytoplasm, which stay within the vestibule long enough to block the chloride ion passage into the pore region (60). Because the channel usually secretes chloride and bicarbonate at hyperpolarized membrane potentials, the channel contains this fixed positive charge to bind to these anions from the cytoplasm via electrostatic attraction (60). This positive charge ensures efficient attraction of monovalent anions, but beyond this point, anions can pass into a narrow uncharged pore region that acts as a size selective filter to stop larger organic anions from leaving the cell. The pore region probably inhibits anions that are attracted to the positive charge of the lysine side chain but that are too large to pass into the narrow pore region (60). These blocking anions are voltage-dependent; open channel blockers could potentially inhibit CFTR function in people who have unusually elevated levels of CFTR in their body's cells (60).

Sodium channel blockers inhibit the sodium channel and improve hydration of airway surfaces, therefore countering defective CFTR (65). Loop diuretics are used to inhibit the CFTR channel (65). When added to intracellular solution, loop diuretics inhibit CFTR chloride currents with potency approaching that of small molecule inhibitors such as glibenclamide; these inhibitors have structural similarity to loop diuretics (65).

Significance

Even with various phenotypic manifestations depending on the area of the body, the defective CFTR protein itself must be targeted in order to treat the Cystic Fibrosis disease. In order to increase or decrease the balance of ions in and out of the cells of the body, the channel pore can be opened or blocked, which could potentially provide many avenues for future drugs and research. These current drugs are being administered along with therapies in order to find ways to eliminate phenotypic manifestations via the CFTR channel, which is the main defect causing CF. This one channel in the cell affects the whole body, and this realization is a milestone.

Conclusion

In conclusion, cystic fibrosis is a disease that has wide phenotypic variability. It has been proven that mutations in the CFTR gene are the underlying cause of the defect that results in CF. Five classes of mutations were found to be the main causes of the defective CFTR gene that causes CF, some being more common mutations than others. Studies underscore the lack of CFTR protein and its cause of an imbalance of chloride and sodium conductance in the channel. An imbalance of water and salt in regions of the body cause mucus buildup and airway blockage. Animal models show the manifestation of the disease in areas of the body such as the pancreas and gastrointestinal tract, representing an elevated immune response from the blood of these areas of the body. These studies show the complexity of CF in the body's need for both adaptive and innate immune system responses. Transcriptional regulation and post-translational modifications were studied in order to retrace the steps of the CFTR protein processing and to show that the dysfunction of the gene occurs as early as the conversion from gene to protein, and that there are ways to correct for this or cause it by targeting these stages. Gene modifiers have been found to both aid and inhibit the effects of defective CFTR gene function, providing substantial evidence that the CFTR protein is the main regulator of CF, but also showing that there are other factors involved. Finally, therapies that enhance and inhibit the function of normal CFTR protein are currently being employed depending on the extent of the phenotype and its manifestations. Drugs target the defective protein alongside therapies based on the amount of functioning CFTR in the cells in order to maintain homeostasis of the body. By analyzing the defective CFTR protein and its causes, as well as phenotypic manifestations of CF, I have come to understand the importance of the CFTR protein and its role in conductance in the human body.

References:

1. Waugh N, Royle P, Craigie I, Ho V, Pandit L, Ewings P, Adler A, Helms P, Sheldon C. 2012. Screening for Cystic Fibrosis-Related Diabetes: A Systematic Review. Health Technology Assessment, No. 16. 24.
2. Jeune KS, Jeune AL, Jouneau S, Belleguic C, Roux P, Jaguin M, Dimanche-Boitre M, Lecureur V, Leclercq C, Desrues B, Brinchault G, Gangneux J, Corinne M. 2013. Impaired Functions of Macrophage from Cystic Fibrosis Patients: CD11b, TLR-5 Decrease and sCD14, Inflammatory Cytokines Increase. Public Library of Science 8(9):e75667.
3. Derichs N. 2013. Targeting a genetic defect: cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis. European Respiratory Review 33(127):58-65.
4. Tsui L, Dorfman R. 2013. The Cystic Fibrosis Gene: A Molecular Genetic Perspective. Cold Spring Harbor Perspectives in Medicine. doi: 10.1101.
5. Yigit E, Bischof JM, Zhang Z, Ott CJ, Kerschner JL, Leir S, Buitrago-Delgado E, Zhang Q, Wang JZ, Widom J, Harris A. 2013. Nucleosome mapping across the CFTR locus identifies novel regulatory factors. Nucleic Acids Research 41(5):2857-2868.
6. Zhang X, Chen Q, Cai H. 2014. Effects of curcumin on ion channels and transporters. Frontiers in Physiology 5:94.
7. Ahner A, Gong X, Frizzell RA. 2013. Cystic Fibrosis transmembrane conductance regulator degradation: cross-talk between the ubiquitylation and SUMOylation pathways. The FEBS Journal 280(18):4430-4438.
8. Rogan MP, Stoltz DA, Hornick DB. 2011. Cystic Fibrosis Transmembrane Conductance Regulator Intracellular Processing, Trafficking, and Opportunities for Mutation-Specific Treatment. Chest Journal 139(6):1480-1490.
9. Linsdell P 2014. Cystic Fibrosis transmembrane conductance regulator chloride channel blockers: Pharmacological, biophysical and physiological relevance. World Journal of Biological Chemistry 5(1):26-39.
10. Collawn JF, Lazrak A, Bebok Z, Matalon S. 2012. The CFTR and ENaC debate: how important is ENaC in CF lung disease? 302:L1141-L1146.
11. Gianotti A, Melani R, Caci E, Sondo E, Ravazzolo R, Galietta LJ, Zegarra-Moran O. 2013. Epithelial sodium channel silencing as a strategy to correct the airway surface fluid deficit in cystic fibrosis. American Journal of Respiratory Cell and Molecular Biology 49(3):445-52.
12. Hobbs CA, Da Tan C, Tarran R. 2013. Does epithelial sodium channel hyperactivity contribute to cystic fibrosis lung disease? The Journal of Physiology 591 (Pt 18):4377-87.
13. Itani OA, Jeng-Haur C, Karp PH, Ernst S, Keshavjee S, Parekh K, Klesney-Tait J,

Zabner J, Welsh MJ. 2011. Human cystic fibrosis airway epithelia have reduced Cl- conductance but not increased Na+ conductance. Proceedings of the National Academy of Sciences of the United States of America 108(25):10260-10265.
14. Kim SJ, Skach WR. 2012. Mechanisms of CFTR Folding at the Endoplasmic Reticulum. Frontiers in Pharmacology 3:201.
15. Cebotary L, Rapino D, Cebotaru V, Guggino WB. 2014. Correcting the Cystic Fibrosis Disease Mutant, A455E CFTR. Public Library of Science 9(1):e85183.
16. Korbmacher JP, Michel C, Neubauer D, Thompson K, Mizaikoff B, Frick M, Dietl P, Wittekindt OH. 2014. Amiloride-sensitive fluid resorption in NCl-H441 lung epithelia depends on apical Cl- conductance. Physiological Reports 2(1):10.1002.
17. Margarida DA. 2011. Targeting CFTR: How to Treat Cystic Fibrosis by CFTR-Repairing Therapies. Current Drug Targets 12(5): 683-693.
18. Monterisi S, Favia M, Guerra L, Cardone RA, Marzulli D, Reshkin SJ, Casavola V, Zaccolo M. 2012. CFTR regulation in human airway epithelial cells requires integrity of the actin cytoskeleton and compartmentalized cAMP and PKA activity. Journal of Cell Science 125: 1106-1117.
19. Terrin A, Monterisi S, Stangherlin A, Zoccarato A, Koschinski A, Surdo NC, Mongillo M, Sawa A, Jordanides NE, Mountford JC, Zaccolo M. 2012. PKA and PDE4D3 anchoring to AKAP9 provides distinct regulation of cAMP signals at the centrosome. Journal of Cell Biology 198 (4): 607-621.
20. Tomaiuolo G, Rusciano G, Caserta S, Carciati A, Carnovale V, Abete P, Sasso A, Guido S. 2014. A New Method to Improve the Clinical Evaluation of Cystic Fibrosis Patients by Mucus Viscoelastic Properties. Public Library of Science One: 10.1371.
21. Gustafsson JK, Ermund A, Ambort D, Johansson MEV, Nilsson HE, Thorell K, Hebert H, Sjovall H, Hansson GC. 2012. Bicarbonate and functional CFTR channel are required for proper mucin secretion and link cystic fibrosis with its mucus phenotype. The Journal of Experimental Medicine 209(7): 1263-1272.
22. Roux J, McNicholas CM, Carles M, Goolaerts A, Houseman BT, Dickinson DA, Iles KE, Ware LB, Matthay MA, Pittet JF. 2013. IL-8 inhibits cAMP-stimulated alveolar epithelial fluid transport via a GRK2/PI3K-dependent mechanism. The FASEB Journal 27(3):1095-106.
23. Lynch SV, Goldfarb KC, Wild YK, Kong W, De Lisle RC, Brodie EL. 2013. Cystic fibrosis transmembrane conductance regulator knockout mice exhibit aberrant gastrointestinal microbiota. Gut microbes 4(1):41.
24. Reznikov LR, Dong Q, Chen JK, Moninger TO, Park JM, Zhang Y, Du J, Hildebrand MS, Smith RJ, Randak CO, Stoltz DA, Welsh MJ. 2013. CFTR-deficient pigs display peripheral nervous system defects at birth. Proceedings of the National Academy of Sciences 110(8):3083-3088.
25. Innis SM, Davidson GF, Bay BN, Slack PJ, Hasman D. 2011. Plasma choline depletion is associated with decreased peripheral blood leukocyte acetylcholine in children with cystic fibrosis. The American Journal of Clinical Nutrition 93(3):564-568.
26. Meissner A, Yang J, Kroetsch JT, Sauvé M, Dax H, Momen A, Noyan-Ashraf MH, Heximer S, Husain M, Lidington D, Bolz S. 2012. Tumor Necrosis Factor-α-Mediated Downregulation of the Cystic Fibrosis Transmembrane Conductance

Regulator Drives Sphingosine-1-Phosphate Signaling in a Mouse Model of Heart Failure. Circulation 125(22):2739-2750.
27. McIsaac SM, Stadnyk AW, Lin T. 2012. Toll-like receptors in the host defense against Pseudomonas aeruginosa infection and cystic fibrosis. Journal of Leukocyte Biology 92(5):977-985.
28. Findlay EG, Hussell T. 2012. Macrophage-Mediated Inflammation and Disease: A Focus on the Lung. Mediators of Inflammation 2012:140937.
29. Mueller C, Braag SA, Keeler A, Hodges C, Drumm M, Flotte TR. 2011. Lack of Cystic Fibrosis Transmembrane Conductance Regulator in CD3+ Lymphocytes Leads to Aberrant Cytokine Secretion and Hyperinflammatory Adaptive Immune Responses. American Journal of Respiratory Cell and Molecular Biology 44(6):922-929.
30. Young RL, Malcolm KC, Kret JE, Caceres SM, Poch KR, Nichols DP, Taylor-Cousar JL, Saavedra MT, Randell SH, Vasil ML, Burns JL, Moskowitz, Nick JA. 2011. Neutrophil Extracellular Trap (NET)-Mediated Killing of *Pseudomonas aeruginosa*: Evidence of Acquired Resistance within the CF Airway, Independent of CFTR. Public Library of Science 6(9):e23637.
31. Parker D, Prince A. 2011. Innate Immunity in the Respiratory Epithelium. American Journal of Respiratory Cell and Molecular Biology 45(2):189-201.
32. Wu H, Yang J, Su EM, Ling L, Zhao C, Yang X, Gao Z, Pan M, Sun P, Sun W, Jiang Y, Su X. 2014. Lipoxin A4 and Platelet Activating Factor Are Involved in E. coli or LPS-Induced Lung Inflammation in CFTR-Deficient Mice. Public Library of Science 10.1371.
33. Zhou Y, Song K, Painter RG, Aiken M, Reiser J, Stanton BA, Nauseef WM, Wang G. 2013. Cystic Fibrosis Transmembrane Conductance Regulator Recruitment to Phagosomes in Neutrophils. Journal of Innate Immunity 5(3):219-230.
34. Bayes HK, Bicknell S, Macgregor G, Evans TJ. 2014. T Helper Cell Subsets Specific for Pseudomonas aeruginosa in Healthy Individuals and Patients with Cystic Fibrosis. Public Library of Science One 9(2):e90263.
35. Wu D, Ahrens R, Osterfeld H, Noah TK, Groschwitz K, Foster PS, Steinbrecher KA, Rotherberg ME, Shroyer NF, Matthaei KI, Finkelman FD, Hogan SP. 2011. Interleukin-12 (IL-13)/IL-13 Receptor α1 (IL-13Rα1) Signaling Regulates Intestinal Epithelial Cystic Fibrosis Transmembrane Conductance Regulator Channel-dependent Cl- Secretion. The Journal of Biological Chemistry 286(15):13357-13369.
36. Baniak N, Luan X, Grunow A, Machen TE, Ianowski JP. 2012. The cytokines interleukin-1β and tumor necrosis factor-α stimulate CFTR-mediated fluid secretion by swine airway submucosal glands. American Journal of Physiology: Lung Cellular and Molecular Physiology 303(4):L327-L333.
37. Abu-El-Haija M, Sinkora M, Meyerholz DK, Welsh MJ, McCray Jr. PB, Butler J, Uc A. 2011. An Activated Immune and Inflammatory Response Targets the Pancreas of Newborn Pigs with Cystic Fibrosis. Pancreatology 11(5):506-515.
38. Conese M, Donatella P, Carbone A, Castellani S, Di Gioia S. 2014. Hematopoietic and Mesenchymal Stem Cells for the Treatment of Chronic Respiratory Diseases: Role of Plasticity and Heterogeneity. The Scientific World Journal 2014:858917.
39. Keiser NW, Engelhardt JF. 2011. New animal models of cystic fibrosis: what are they

teaching us? Current Opinion in Pulmonary Medicine 17(6):478-483.
40. Gunderson FQ, Merkhofer EC, Johnson TL. 2011. Dynamic histone acetylation is critical for cotranscriptional spliceosome assembly and spliceosomal rearrangements. 108(5) doi:10.1073.
41. Hutt DM, Olsen CA, Vickers CJ, Herman D, Chalfant M, Montero A, Leman LJ, Burkle R, Maryanoff BE, Balch WE, Ghadiri MR. 2011. Potential Agents for Treating Cystic Fibrosis: Cyclic Tetrapeptides that Restore Trafficking and Activity of ΔF508-CFTR. ACS Medicinal Chemistry Letters 2(9): 703-707.
42. Raksaseri P, Chatsudthipong V, Muanprasat C, Soodvilai S. 2013. Activation of liver X receptors reduces CFTR-mediated Cl(-) transport in kidney collecting duct cells. American Journal of Physiology-Renal Physiology 305(4):F583-91.
43. Kelly C, Canning P, Buchanan PJ, Williams MT, Brown V, Gruenert DC, Elborn JS, Ennis M, Schock BC. 2013. Toll-like receptor 4 is not targeted to the lysosome in cystic fibrosis airway epithelial cells. American Journal of Physiology-Lung Cellular and Molecular Physiology 304(5):L371-82.
44. Martel G, Bérubé J, Rousseau S. 2013. The protein kinase TPL2 is essential for ERK1/ERK2 activation and cytokine gene expression in airway epithelial cells exposed to pathogen associated molecular patterns (PAMPs). Public Library of Science 8(3):e59116.
45. Harris WT, Kelly DR, Zhou Y, Wang D, Macewen M, Hagood JS, Clancy JP, Ambalavanan N, Sorscher EJ. 2012. Myofibroblast Differentiation and Enhanced Tgf-B Signaling in Cystic Fibrosis Lung Disease. Public Library of Science One 8(8):e70196.
46. Tuggle KL, Birket SE, Cui X, Hong J, Warren J, Reid L, Chambers A, Ji D, Gamber K, Chu KK, Tearney G, Tang LP, Fortenberry JA, Cadillac JM, Bedwell DM, Rowe SM, Sorscher EJ, Fanucchi MV. 2014. Characterization of Defects in Ion Transport and Tissue Development in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)-Knockout Rats. Public Library of Science 10. 1371.
47. Huaux F, Noel S, Dhooghe B, Panin N, Re SL, Lison D, Wallemacq P, Marbaix E, Scholte BJ, Lebecque P, Leal T. 2013. Dysregulated Proinflammatory and Fibrogenic Phenotype of Fibroblasts in Cystic Fibrosis. Public Library of Science One 10.1371.
48. Sanchez-Dominguez CN, Reyes-Lopez MA, Bustamante A, Cerda-Flores RM, Vollalobos-Torres Mdel C, Gallardo-Blanco HL, Rojas-Martinez A, Martinez-Rodriguez HG, Barrera-Saldaña HA, Ortiz-Lopez R. 2014. The Tumor Necrosis Factor α (-308 A/G) Polymorphism Is Associated with Cystic Fibrosis in Mexican Patients. Public Library of Science One 9(3):e90945.
49. Chalmers JD, Fleming GB, Hill AT, Kilpatrick DC. 2011. Impact of mannose-binding lectin insufficiency on the course of cystic fibrosis: A review and meta-analysis. Glycobiology 21(3): 271-282.
50. Gautier EL, Chow A, Spanbroek R, Genevieve M, Greter M, Jakubzick C, Bogunovic M, Leboeuf, Rooijen N, Habenicht AJ, Merad M, Randolph GJ. 2012. Systemic Analysis of PPARγ in Mouse Macrophage Populations Reveals Marked Diversity in Expression with Critical Roles in Resolution of Inflammation and Airway Immunity. The Journal of Immunology 189(5):2614-2624.

51. Griffin PE, Roddam LF, Belessis YC, Strachan R, Beggs S, Jaffe A, Cooley MA. 2012. Expression of PPARγ and Paraoxonase 2 Correlated with *Pseudomonas aeruginosa* Infection in Cystic Fibrosis 7(7):e42241.
52. Weiler CA, Drumm ML. 2013. Genetic influences on cystic fibrosis lung disease severity. Frontiers in Pharmacology 4:40. doi:10.3389.
53. Bush WS, Moore JH. 2012. Chapter 11: Genome-Wide Association Studies. PLOS Computational Biology: Translational Bioinformatics. 10.1371.
54. Guo X, Pace RG, Stonebraker JR, Commander CW, Dang AT, Drumm ML, Harris A, Zou F, Swallow DM, Wright FA, O'Neal WK, Knowles MR. 2011. Mucin Variable Number Tandem Repeat Polymorphisms and Severity of Cystic Fibrosis Lung Disease: Significant Association with MUC5AC. Public Library of Science 10.1371.
55. Blackman SM, Commander CW, Watson C, Arcara KM, Strug LJ, Stonebraker JR, Wright FA, Rommens JM, Sun L, Rhonda GP, Norris SA, Durie PR, Drumm ML, Knowles MR, Cutting GR. 2013. Genetic Modifiers of Cystic Fibrosis-Related Diabetes. Diabetes 62(10):3627-3635.
56. Zertal-Zidani S, Busiah K, Edelman A, Polak M, Scharfmann R. 2013. Small-molecule inhibitors of the cystic fibrosis transmembrane conductance regulator increase pancreatic endocrine cell development in rat and mouse. Diabetologia 56(2):330-339.
57. Stalvey MS, Clines KL, Havasi V, McKibbin CR, Dunn LK, Chung WJ, Clines GA. 2013. Osteoblast CFTR inactivation reduces differentiation and osteoprotegerin expression in a mouse model of cystic fibrosis-related bone disease. Public Library of Science One 8(11):e80098.
58. Bradley GM, Blackman SM, Watson CP, Doshi VK, Cutting GR. 2012. Genetic modifiers of nutritional status in cystic fibrosis[1,2,3,4]. The American Journal of Clinical Nutrition 96(6):1299-1308.
59. Stanke F, Becker T, Kumar V, Kedtfeld S, Becker C, Cuppens H, Tamm S, Yarden J, Laabs U, Siebert B, Fernandez L, Macek Jr M, Radojkovic D, Ballmann M, Greipel J, Cassiman J, Wienker TF, Tümmler B. 2011. Genes that determine immunology and inflammation modify the basic defect of impaired ion conductance in cystic fibrosis epithelia. Journal of Medical Genetics 48:24-31.
60. Frizzell RA, Hanrahan JW. 2012. Physiology of epithelial chloride and fluid secretion. Cold Spring Harbor Perspectives in Medicine. 2(6):a009563.
61. Zhang S, Blount AC, McNicholas CM, Skinner DF, Chestnut, M, Kappes JC, Sorscher EJ, Woodworth BA. 2013. Resveratrol Enhances Airway Surface Liquid Depth in Sinonasal Epithelium by Increasing Cystic Fibrosis Transmembrane Conductance Regulator Open Probability. Public Library of Science 8(11):e81589.
62. Eckford PD, Canhui L, Ramjeesingh M, Bear C. 2012. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Potentiator VX-770 (Ivacaftor) Opens the Defective Channel Gate of Mutant CFTR in a Phosphorylation-dependent but ATP-independent Manner. The Journal of Biochemistry 287:36639-36649.
63. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, Griese M,

McKone EF, Wainwright CE, Konstan MW, Moss R, Ratjen F, Sermet-Gaudelus I, Rowe SM, Dong Q, Rodriguez S, Yen K, Ordoñez C, Elborn JS. 2011. A CFTR Potentiator in Patients with Cystic Fibrosis and the *G551D* Mutation. The New England Journal of Medicine 365(18):1663-1672.
64. Rayner O. 2013. In the News – October 2013: NACFC, CFTR Modulators, Ataluren, Personalised Medicine and Lynovex. Cystic Fibrosis Unite.
65. Lu M, Scott-Ward TS, Liu J, Khuituan P, Li H, Cai Z, Husbands SM, Sheppard DM. 2014. Loop diuretics are open-channel blockers of the cystic fibrosis transmembrane conductance regulator with distinct kinetics. British Journal of Pharmacology 171:265-278.
66. Lewis K. 2013. Vertex Announces Results of Phase 3 Study of Ivacaftor in People with CF who have the R117H Mutation. Vertex Pharmaceuticals Incorporated.

Table Legend

Table 1: Mutation Classes and their Characteristics

Class	Worldwide Frequency	CFTR Protein Outcome	Severity of Phenotype
I	10%	No CFTR	High
II	70%	Defective Processing	High
III	2-3%	Defective Regulation	High
IV	<2%	Altered Conductance	Reduced
V	<1%	Reduced Synthesis	Reduced

Figure Legend

Figure 1: The Structure of the Cystic Fibrosis Transmembrane Conductance Regulator Protein

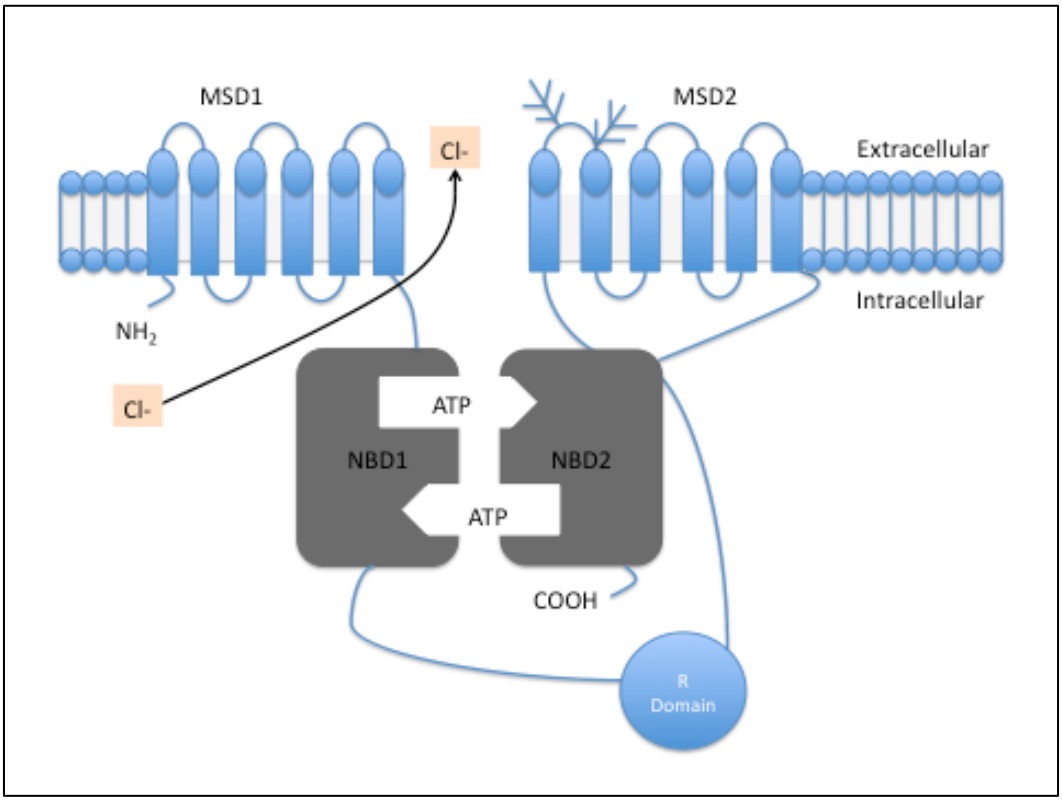

Figure 2: Normal CFTR Conductance

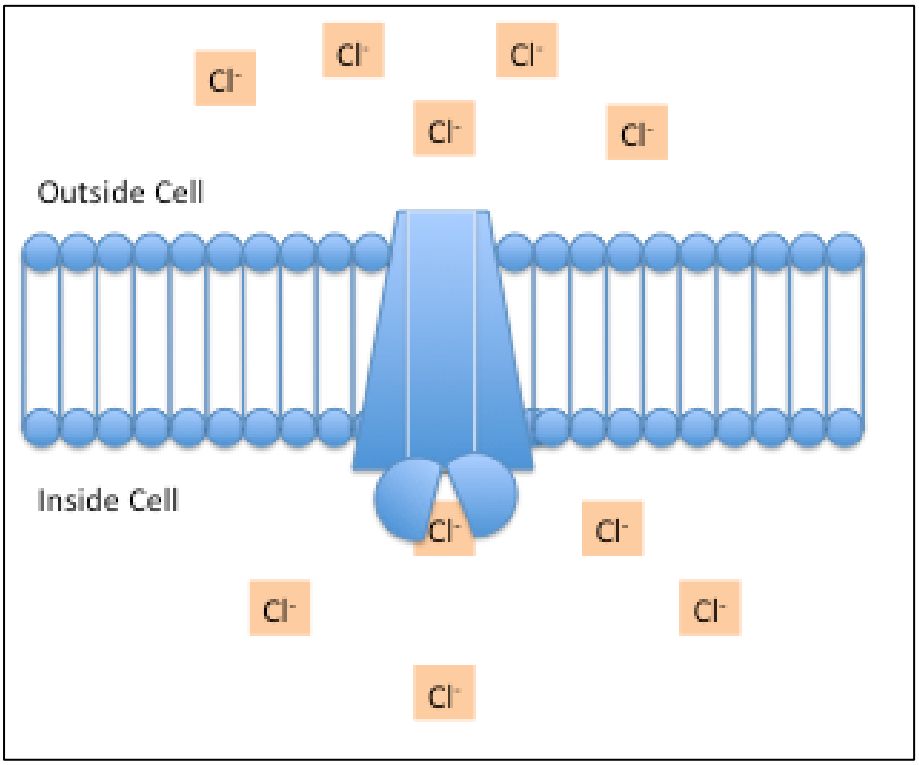

Figure 3: Defective CFTR Conductance

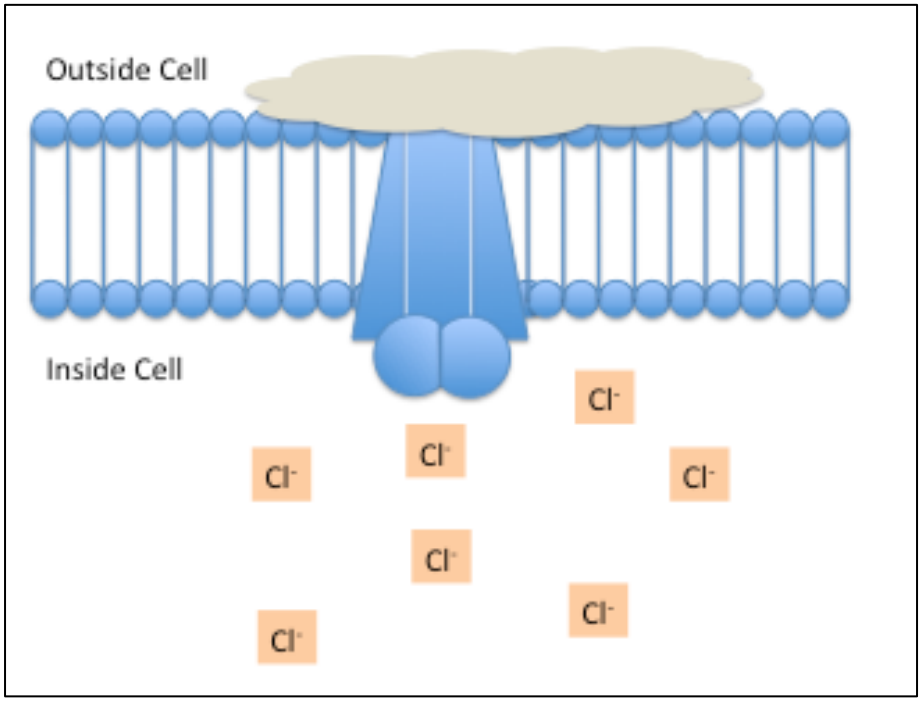

Figure 4: Immunity

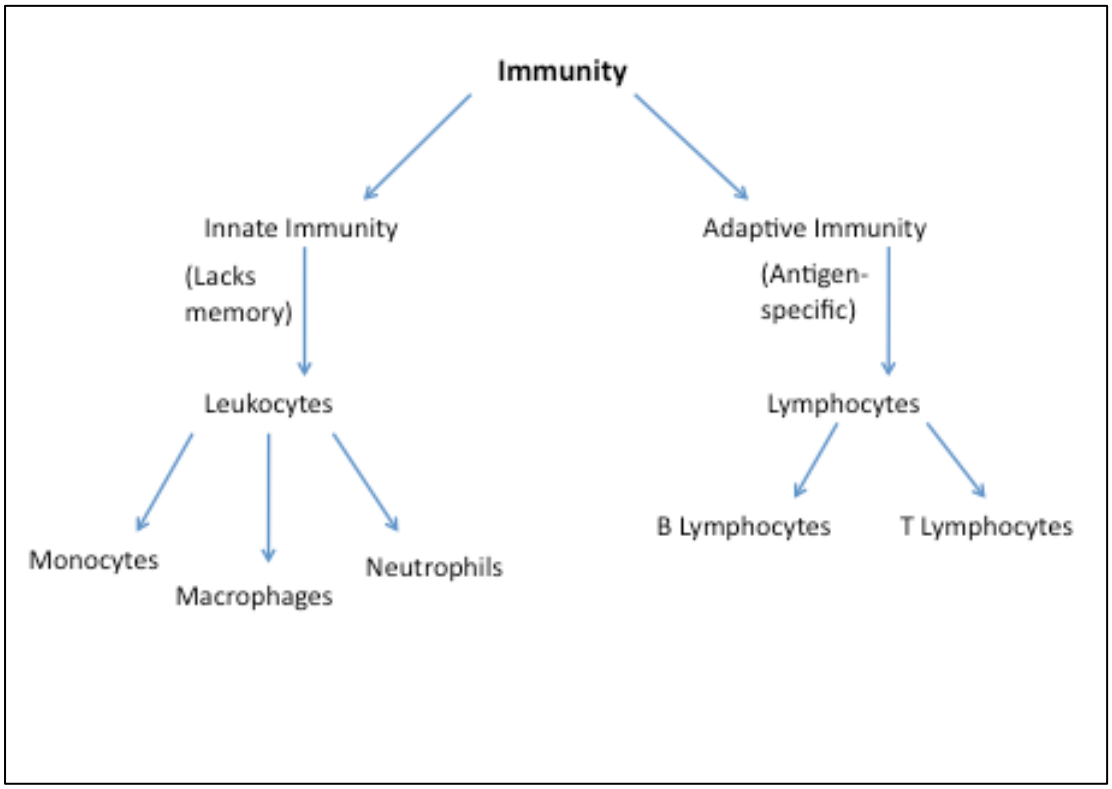

Table Text

Table 1: *Mutation Classes and their Characteristics*
This table shows the four main classes of mutations of the Cystic Fibrosis Transmembrane Conductance Regulator Gene. The first three represent the most severe phenotype or clinical manifestations of the disease, and this is due to no CFTR, or defective CFTR processing or regulation. The second two classes of mutations show a difference in that they cause altered conductance, and less CFTR is made overall; these produce less severe phenotypic manifestations and are also less common in CF patients.

Figure Text

Figure 1: The Structure of the Cystic Fibrosis Transmembrane Conductance Regulator Protein
This figure shows the CFTR protein and its domains. It is a glycoprotein consisting of two membrane-spanning domains, two nucleotide-binding domains (NBD1 and NBD2) that bind and hydrolyze ATP, and a regulatory (R) domain that gates the channel by phosphorylation. It is an ATP-binding cassette (ABC) transporter composed of five domains: two transmembrane domains (TMD11/TMD2) that form the channel pore, one regulatory domain (R), and two nucleotide binding domains (NBD1/NBD2). Phosphorylation of the R domain by protein kinase A and recruitment of ATP to the nucleotide binding domains NBD1 and NBD2 causes opening of the channel. These domains bind and open to the channel pore. In other words when ATP binds at the interface between NBD1 and NBD2, the channel conforms to be open. When ATP hydrolyzes, the NBDs dissociate to close the channel, and ATP activity thus promotes a dissociation of the NBDs, causing the channel to close. This is the overall process of "gating" occurring to open and close the channel, and it allows for the necessary chloride transport and conductance in and out of the cell.
Key:
MSD=membrane spanning domain
NBD=nucleotide-binding domain

Figure 2: Normal CFTR Conductance
This figure shows that when the chloride channel is functional, it causes efflux of chloride ions out of the cell, thus regulating the balance of ions and keeping the mucociliary clearance system of the airways intact.

Figure 3: Defective CFTR Conductance

When the CFTR channel is dysfunctional, chloride ions cannot properly leave the cell, causing a lack of appropriate conductance and a buildup of viscous mucous in the airways. This figure shows a chloride channel that is closed and thus acts as a locked gate for mucociliary clearance.

Figure 4: Immunity
This figure shows the divide between innate and adaptive immune responses in the body and the main cells that are involved in the immune response. The innate immune system includes the release of leukocytes, or white blood cells, which then target areas of the body that are infected and lead to a cycle of infection and inflammation in patients with CF. The adaptive immune system involves B and T cells, which are antigen-specific and require a response from specific antibodies for effective pathogenic clearance, and are seen in higher levels in CF patients.