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Origins of Cystic Fibrosis Lung Disease

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At the basic level, we know the genetic cause of cystic fibrosis: it is an autosomal recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR).[1,2] At the clinical level, we know that chronic bacterial airway infection, prominent neutrophilic inflammation and mucus in airways, and progressive bronchiectasis characterize advanced cystic fibrosis lung disease, which causes most morbidity and death in people with cystic fibrosis.[2] Between those two extremes, the way in which loss of CFTR-mediated chloride and bicarbonate transport leads to chronic airway infection has remained uncertain.

Over the past two decades, investigators have conducted studies involving people with cystic fibrosis (defined as persons who carry known disease-causing *CFTR* mutations) at progressively earlier time points. We have learned that bronchiectasis is present in nearly one in three children with cystic fibrosis by 3 years of age,[3] although the host-defense defects that trigger infection continue to be debated.[4-10] Even before the onset of symptoms, pulmonary inflammation and infection are often present, although which condition comes first has been uncertain.[11,12] Findings on chest computed tomography (CT) are abnormal in most babies with cystic fibrosis as early as 3 months of age,[13] although the relative contribution of inflammation, airway remodeling, and other factors remains undefined. Studies involving children at even earlier ages might reveal the origins of cystic fibrosis lung disease and thereby change clinical practice.

Indeed, simply knowing that disease begins before symptoms develop has been a factor driving cystic fibrosis centers to intervene early, and the outcomes have been encouraging.[14] Understanding the initial host-defense defects in the airways of people with cystic fibrosis could suggest new preventions and treatments, as well as the means to assess disease status and the efficacy of therapeutic agents. Additional reasons to elucidate the origins of this disease are the implementation of universal screening to detect cystic fibrosis in newborns and potential new therapeutic agents that target CFTR.[15-17] However, access to organs and tissue in newborns is extremely limited, and the invasive in vivo and ex vivo experimental interventions required to elucidate the pathogenesis most often cannot be performed in humans.

The lack of an animal model that mirrors cystic fibrosis in humans has hindered progress in discovering the origins of the lung disease.[18] Respiratory disease such as that in humans does not develop in mice with *cftr* mutations. However, lung disease that mimics that in humans with cystic fibrosis occurs in other recently generated animal models. In this review, we focus primarily on the newborn period, because this time window is key to discovering the origins of cystic fibrosis airway disease.

NEW ANIMAL MODELS THAT MIRROR CYSTIC FIBROSIS IN HUMANS

To circumvent the limitations of studying cystic fibrosis in mice and humans, investigators have developed new animal models of cystic fibrosis in pigs, ferrets,

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and rats.[19-21] We focused on pigs because, as compared with mice, their anatomical, physiological, biochemical, and genetic characteristics, as well as their size and life span, are more similar to those in humans.[22] Because embryonic stem cells that can contribute to the germ line had been developed only for mice, a different approach was required. We and our colleagues modified the *cftr* gene in porcine fetal fibroblasts and then used them for somatic-cell nuclear transfer (the procedure that was used in cloning Dolly the sheep) to produce pigs with cystic fibrosis.[19] With the exception of mice, these pigs were the first mammalian disease models generated by targeted gene modification.

Pigs that lack CFTR have a phenotype like that which is typically observed in people with cystic fibrosis, including meconium ileus, exocrine pancreatic destruction, focal biliary cirrhosis, atresia of the vas deferens, an abnormally small gallbladder, and abnormal glucose homeostasis (early cystic fibrosis–related diabetes mellitus).[19,23-25] Within weeks to months after birth, airway and nasal sinus disease with hallmark features of cystic fibrosis (infection, inflammation, tissue remodeling, mucus accumulation, and obstruction) develops spontaneously in pigs with cystic fibrosis (Fig. 1).[26-28] As is the case in humans, the appearance of airway disease is heterogeneous, both within and among pigs.[26-29] Pigs bearing the common cystic fibrosis–associated mutation, ΔF508-*cftr*, also have characteristic features that mirror those of cystic fibrosis in humans; these include intestinal, pancreatic, and airway disease.[27]

A similar gene-targeting strategy was used to produce ferrets lacking CFTR.[20] Intestinal, airway, and reproductive features consistent with human disease develop in ferrets with cystic fibrosis; these animals may be particularly valuable for studying cystic fibrosis–related diabetes mellitus.[20,30-33] Rats with a disrupted *cftr* gene were recently produced with the use of zinc-finger endonuclease techniques.[21] Intestinal, airway, and reproductive features consistent with human disease also develop in them.

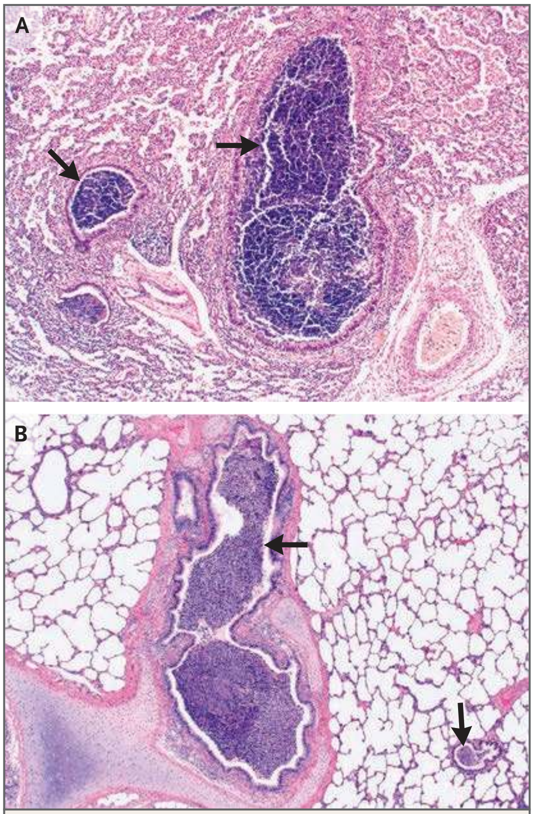


Figure 1. Pathologic Features of Airway Disease in Humans and Pigs with Cystic Fibrosis.

Histologic images (hematoxylin and eosin) of the lungs of a 3-month-old infant with cystic fibrosis (Panel A) and a 2-month-old pig with cystic fibrosis (Panel B) are shown. Neutrophilic inflammation (arrows) obstructs the airway lumens.

LOSS OF CFTR FUNCTION AND CONGENITAL AIRWAY ABNORMALITIES

Since airway obstruction occurs early in the lives of babies with cystic fibrosis,[13,34,35] the question has been raised regarding whether obstruction might, in part, be congenital. A similar question has been asked regarding hypoplasia of the nasal sinuses, which has been well described in people with cystic fibrosis.[36] CFTR is expressed early during development,[37] so in utero alterations are plausible. Indeed, studies in mice, pigs, and rats with cystic fibrosis shortly after birth reveal structural tracheal abnormalities, including narrowed proximal airways with assorted alterations in airway cartilage, hypoplastic submucosal glands, and prominent airway smooth-muscle bundles[21,27,38,39] (Fig. 2). The presence of this congenital defect in mice with cystic fibrosis, which lack other respiratory abnormalities associated with this disease, suggests a distinct mechanism for this defect. Hypoplastic nasal sinuses are also present at birth in piglets with cystic fibrosis (Fig. 2)[28]; this suggests that a primary cystic fibrosis defect contributes to these congenital changes. These abnormalities may have physiological significance, because newborn piglets with cystic fibrosis have airflow obstruction and air trapping in the absence of inflammation or mucus obstruction (Fig. 2).[40]

Congenital abnormalities in three species suggest that humans might also have altered airway development. A reappraisal of reported autopsy findings[41] from the tracheas of infants who were younger than 2 weeks of age showed that babies with cystic fibrosis had narrowed tracheas, a finding that was similar to that seen in newborn animal models.[38] Furthermore, a recent study showed that 15% of young children with cystic fibrosis (median age, 16 months) had bronchoscopic evidence of tracheomalacia, which was associated with more severe airway disease.[42] Thus, the airway obstruction and air trapping observed in infants with cystic fibrosis as early as 2 to 3 months of age[34,35] might, at least in part, be congenital in nature.

REDUCED CHLORIDE SECRETION, NOT SODIUM HYPERABSORPTION

Cystic fibrosis alters the electrophysiological properties across airway epithelia, and measures of nasal voltage have been used to aid in the diagnosis and assessment of the effectiveness of interventions.[2,15] Two processes determine the bulk of the electrophysiological characteristics — CFTR-mediated anion (chloride and bicarbonate) secretion and epithelial sodium channel–mediated sodium absorption.[2] Alterations in either process might change electrophysiological properties.

The airway epithelia in newborn pigs with cystic fibrosis, which extend from the nose to the bronchi, lack cyclic AMP–stimulated chloride secretion.[43] This is expected because CFTR is an apical membrane anion channel that is regulated by phosphorylation with cyclic AMP–dependent protein kinase. These findings are consistent with those in studies of the airway epithelia of ferrets,[20,31] rats,[21] and humans with cystic fibrosis[2]; these epithelia consistently have a loss of anion permeability. In addition, the salt concentration in airway-surface liquid is similar in wild-type newborn pigs and in those with cystic fibrosis.[44]

A widely held hypothesis is that CFTR inhibits the epithelial sodium channel and that loss of that effect causes amiloride-inhibitable sodium hyperabsorption, which dehydrates airways, reduces the height of the periciliary liquid layer, and disrupts mucociliary clearance.[9,45-47] Studies of cultured human airway epithelia, as well as of mouse fibroblasts and dog kidney-cell lines (both of which were expressing recombinant CFTR and epithelial sodium channels), suggest that without CFTR, epithelial sodium channel–mediated sodium absorption increases.[9,45-47] In addition, mice with overexpression of the epithelial sodium channel have decreased height of the periciliary liquid layer and reduced mucociliary clearance,[9,45-47] suggesting that increased activity of epithelial sodium channels can alter airway-surface liquid.

Airway epithelia in newborn pigs with cystic fibrosis do not hyperabsorb sodium, a finding that contrasts with the hypothesis that sodium hyperabsorption initiates disease.[43] Studies involving neonatal ferrets with cystic fibrosis[31,32] and 3-to-6-week-old rats with cystic fibrosis,[21] as well as some studies of airway epithelia in humans with cystic fibrosis,[48] also showed no evidence of increased sodium absorption. In addition, two other human tissues that express both CFTR and the epithelial sodium channel — sweat-gland ducts and submucosal glands — do not hyperabsorb sodium in cystic fibrosis.[4,49,50] Secondary changes in airways might increase sodium absorption as the disease progresses, but data suggest that loss of CFTR does not directly increase activity of the epithelial sodium channel at the genesis of disease. Nevertheless, in the nasal epithelia of both humans and pigs with cystic fibrosis, as compared with controls, amiloride inhibits a greater fraction of the transepithelial voltage and short-circuit current, which is sometimes taken to indicate increased sodium absorption. Sweat-gland ducts show similar changes without hyperabsorbing sodium. How is this apparent paradox explained? The CFTR chloride conductance and the epithelial sodium channel conductance sit in parallel in the apical membrane, and elimination of the chloride conductance (a shunt pathway, in part) magnifies sodium-dependent electrophysiological properties without increasing sodium absorption.[43]

LOSS OF CFTR AND REDUCED PH OF AIRWAY-SURFACE LIQUID

CFTR conducts bicarbonate,[51] and loss of CFTR eliminates bicarbonate secretion by airway epi-

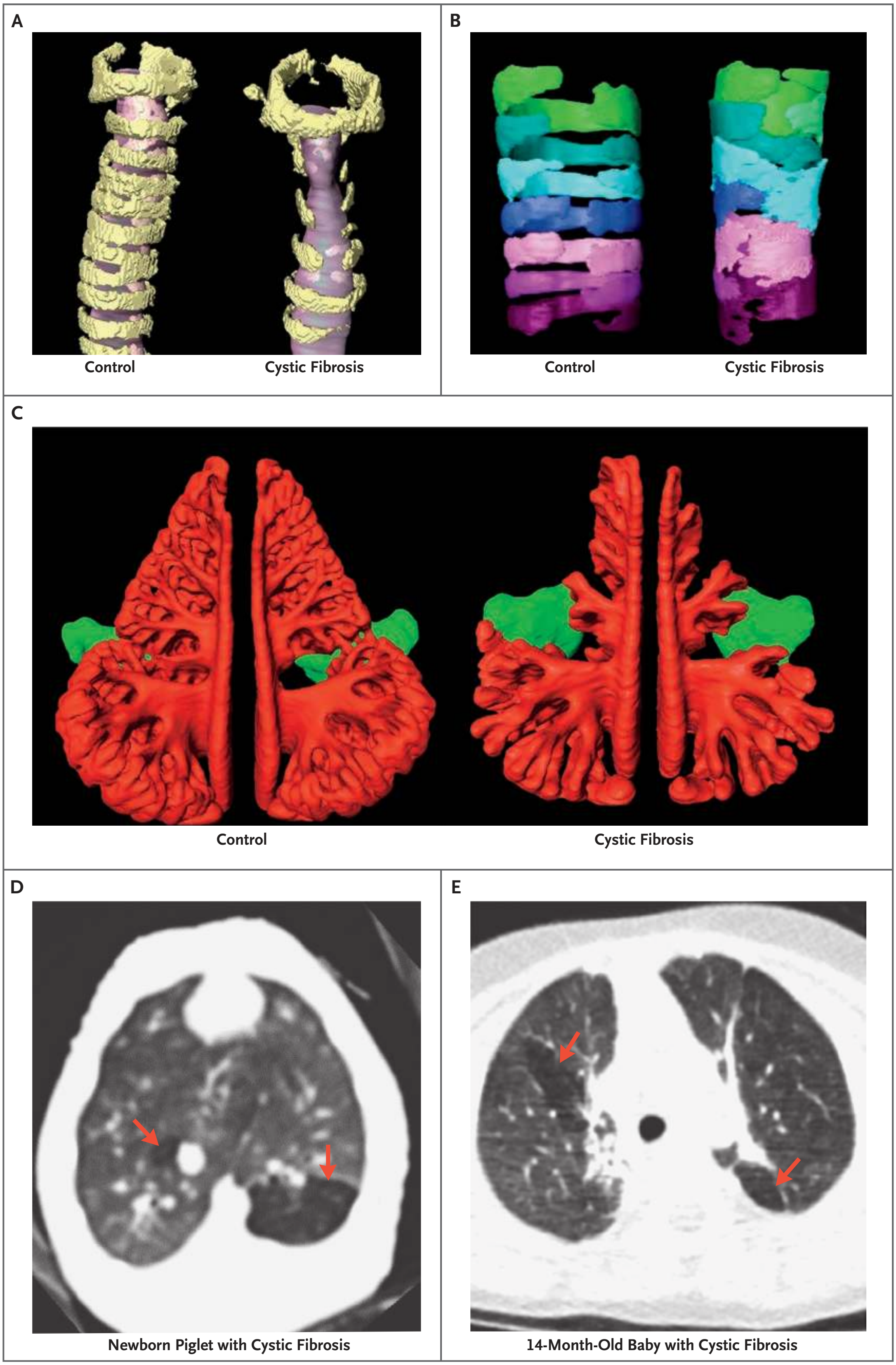

A
Control
Cystic Fibrosis
B
Control
Cystic Fibrosis
C
Control
Cystic Fibrosis
D
Newborn Piglet with Cystic Fibrosis
E
14-Month-Old Baby with Cystic Fibrosis

Figure 2 (facing page). Structural Airway Abnormalities in Cystic Fibrosis.

Panel A shows three-dimensional reconstructions from microcomputed tomographic images of the laryngeal and upper tracheal region of mice with cystic fibrosis and wild-type controls at 6 to 8 weeks of age. The structure of the cartilage rings (yellow) is disrupted, and the tracheal lumen (purple) is narrowed in mice with cystic fibrosis. Panel B shows three-dimensional reconstructions based on optical coherence tomographic images of tracheal cartilage rings in newborn pigs. Different colors indicate the individual cartilage rings. Images courtesy of Drs. Melissa Suter (Massachusetts General Hospital) and Eman Namati (University of Iowa). Panel C shows three-dimensional reconstructions based on computed tomographic (CT) images of ethmoid (red) and maxillary (green) sinuses in newborn pigs. Hypoplastic ethmoid sinuses are shown in pigs with cystic fibrosis. Panel D shows a chest CT image of a piglet with cystic fibrosis on the day of birth and before the development of airway infection, inflammation, and mucus obstruction. Air trapping (arrows), a sign of airway obstruction, is already present. Panel E shows air trapping (arrows) on a CT image of the chest of a 14-month-old baby with cystic fibrosis. Murine tracheas were provided by Drs. Craig Hodges and Mitchell Drumm (Case Western Reserve University) and analyzed by Ryan Adam (University of Iowa). Sinus image analysis was performed by Dr. Eugene Chang and Tanner Wallen (University of Iowa).

thelia in pigs.[43] As a result, the airway-surface liquid of newborn piglets with cystic fibrosis has a reduced pH when measured in vivo, ex vivo, and in cultured epithelia (Fig. 3A).[44] These findings are in accord with earlier reports that cultured human airway epithelia from patients with cystic fibrosis lack bicarbonate secretion,[53] that the pH of the airway-surface liquid of these epithelia is acidic,[54] and that secretions from their submucosal glands are abnormally acidic.[55] A small study involving infants with cystic fibrosis also showed that the pH in the nasal airway-surface liquid was lower in those infants than it was in infants without cystic fibrosis.[56] However, in older children and adults, genotype-dependent differences in the pH of nasal airway-surface liquid may be more variable.[56-58] The reasons for this variability remain to be determined.

AIRWAY INFECTION PRECEDING LUNG INFLAMMATION

The chicken-and-egg conundrum about infection and inflammation has long vexed researchers and clinicians in the field.[6,10-12,59] During the first hours after birth, piglets with cystic fibrosis show no evidence of inflammation in their airways on histopathological analysis, measurement of cell counts and cytokines, or transcript analysis.[19,26,27,38] Yet, after a pulmonary challenge with *Staphylococcus aureus*, they fail to eradicate bacteria as well as do the airways of controls.[26] Moreover, newborn piglets and neonatal ferrets with cystic fibrosis harbor more bacteria than do littermates without this disease.[26,32]

The species that are isolated include a wide variety of gram-positive and gram-negative organisms, including *S. aureus*. Although *Pseudomonas aeruginosa* is rare in young pigs with cystic fibrosis, it infects older pigs that have clinical disease.[28,29] A similar pattern occurs in cystic fibrosis in humans; during the initial months to years of life, a wide variety of bacteria are recovered from the lungs.[14,60] With time, the lungs become chronically colonized with a more restricted number of species, most notably *P. aeruginosa*.[14]

These findings indicate that within hours after birth, infants with cystic fibrosis have an "equal opportunity" host-defense defect in their lungs that impairs eradication of many different types of bacteria. That abnormality can initiate a cascade of airway inflammation and airway remodeling. Later in life, the types of infection narrow to a few predominant species, probably because of an interplay between a changing host and bacterial genetic adaptations. In addition, although infection precedes inflammation, subsequent inflammatory responses, resolution of inflammation, adaptive immune responses, or all of these might be abnormal.[10]

ACIDIC AIRWAY-SURFACE LIQUID THAT IMPAIRS BACTERIAL KILLING

Airways use multiple mechanisms to protect lungs against infection. One important defense is the complex soup of antimicrobial peptides, proteins, and lipids in airway-surface liquid. Alexander Fleming was the first to identify one of these — lysozyme — after he noticed that sneeze droplets killed bacteria on his culture dish.[61] Since then, more factors have been identified, including lactoferrin, defensins, cathelicidins, and secretory leukocyte peptidase inhibitor.[62] Many of these factors have individual as well as synergistic effects that rapidly kill bacteria.[63]

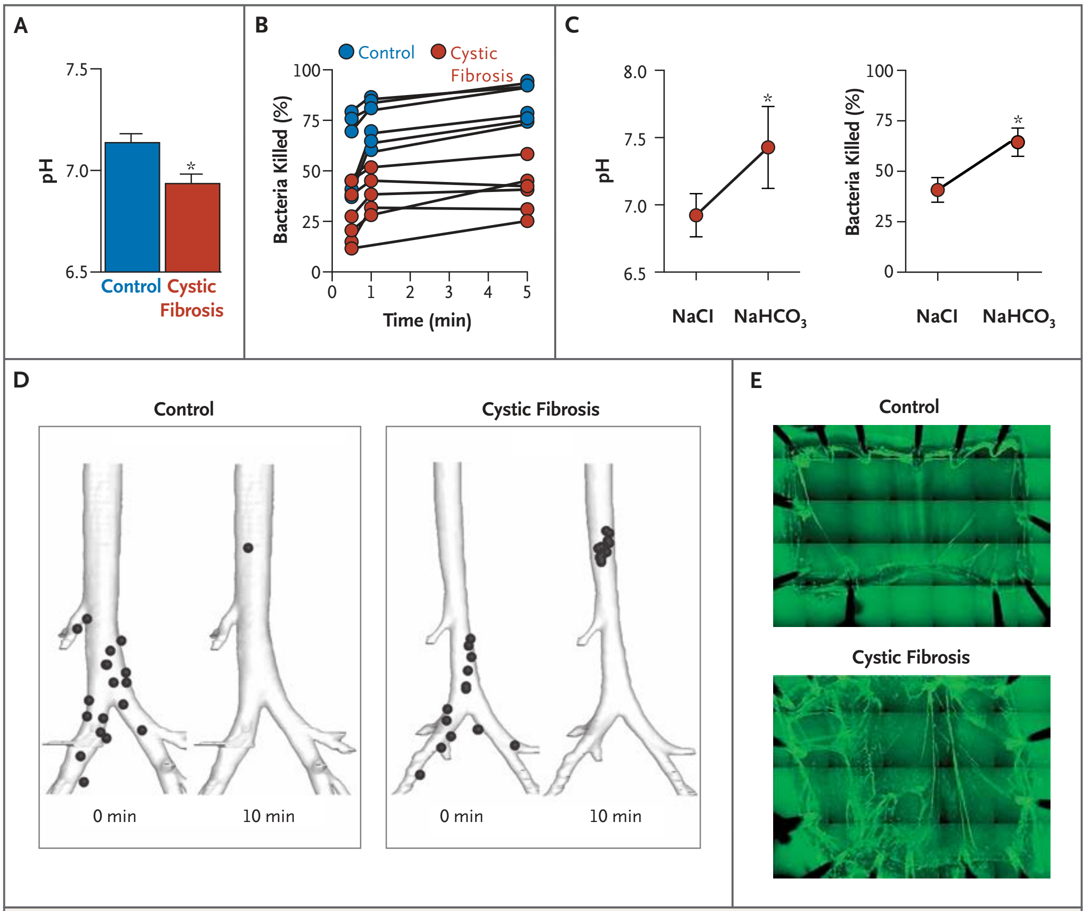


Figure 3. **Host-Defense Defects in Newborn Pigs with Cystic Fibrosis.**

As shown in Panel A, the pH of the airway-surface liquid is more acidic in newborn pigs with cystic fibrosis than in their wild-type littermates. I bars represent the standard error of the mean. Data are from Pezzulo et al.[44] As shown in Panel B, the airway-surface liquid in newborn wild-type piglets quickly killed most *Staphylococcus aureus* applied to the airway surface. In contrast, the percentage of bacteria killed was reduced by about half in piglets with cystic fibrosis. Each set of connected points represents data from one piglet. Data are from Pezzulo et al.[44] As shown in Panel C, increasing the pH of the airway-surface liquid by aerosolizing sodium bicarbonate ($NaHCO_3$) onto the airways of piglets with cystic fibrosis, as compared with treatment with saline (NaCl) alone, corrected the bacterial killing defect of cystic fibrosis. I bars represent standard errors, and asterisks indicate a P value less than 0.05. Data are from Pezzulo et al.[44] As shown in Panel D, images from a computed tomography–based assay of mucociliary transport that tracked radiopaque microdisks were used to create three-dimensional reconstructions of the airway tree in newborn wild-type piglets and piglets with cystic fibrosis. Images were obtained at the beginning and end of the 10-minute tracking period. Black circles represent the positions of the microdisks; the circles shown are larger than actual microdisks. After in vivo cholinergic stimulation of mucus secretion from the submucosal glands, most microdisks cleared the airway tree in the wild-type piglets. In piglets with cystic fibrosis, some microdisks moved normally, but some became stuck and failed to clear the viewing field. Data are from Hoegger et al.[52] As shown in Panel E, mucus strands were visualized with fluorescent nanospheres that bound to mucus in excised tracheas submerged in saline. Tracheas were studied ex vivo after in vivo cholinergic stimulation. In the tracheas of wild-type piglets, most of the mucus (green) accumulated around the tracheal edges, and very few mucus strands were observed. In contrast, in the tracheas of piglets with cystic fibrosis, numerous mucus strands did not detach from submucosal gland ducts. These anchored mucus strands contribute to impaired mucociliary transport.

In wild-type piglets, airway-surface liquid very quickly kills most *S. aureus* (Fig. 3B).[44] In contrast, loss of CFTR reduces rapid bacterial killing by about half. This is not due to a decreased abundance of antimicrobials in airway-surface liquid. Rather, the reduced pH of airway-surface liquid in piglets with cystic fibrosis inhibits its antimicrobial activity. Increasing the pH of air-

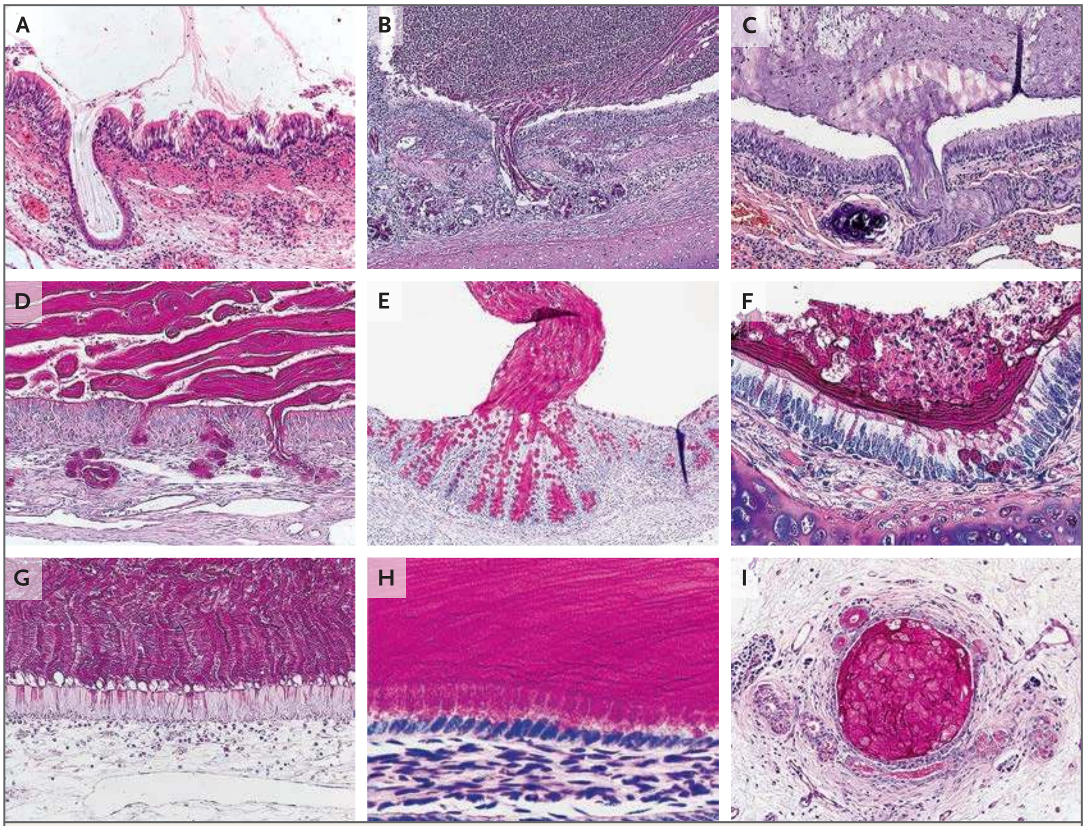


Figure 4. **Accumulation of Mucus in Humans and Animals with Cystic Fibrosis.**

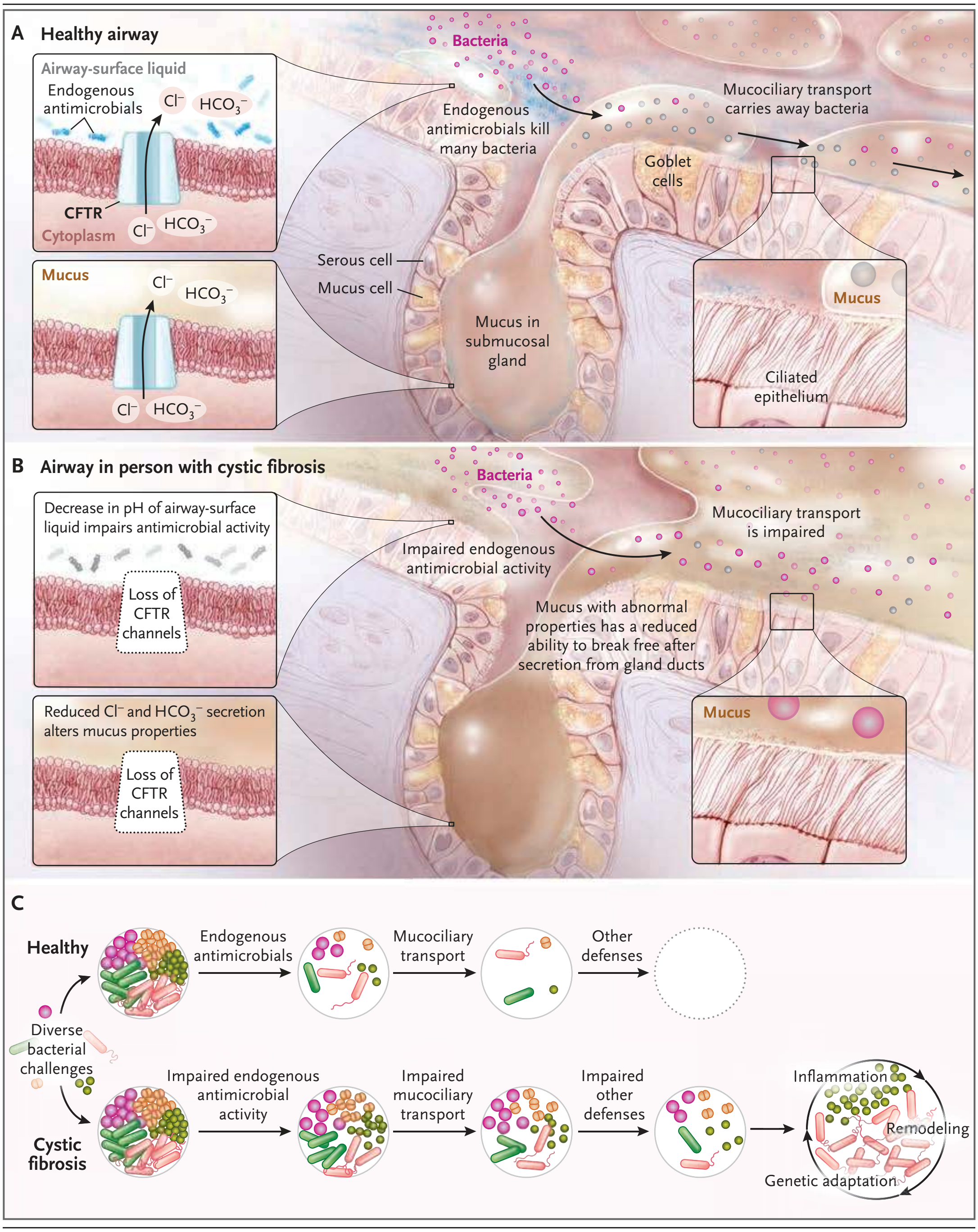
Panels A through E show the “stringy” appearance of mucus arising from glands. Mucus secreted from submucosal glands in pulmonary airways remained in the gland duct in a 7-month-old baby with cystic fibrosis (Panel A), a 2-month-old pig with cystic fibrosis (Panel B), and an 8-month-old ferret with cystic fibrosis (Panel C, reproduced from Sun et al.[32] with permission from the publisher). Mucus also emerged from submucosal glands in ethmoid sinus olfactory epithelium that did not contain goblet cells in a 1-month-old pig with cystic fibrosis (Panel D). Similar to mucus from submucosal glands, mucus arising from colonic crypts of newborn pigs with cystic fibrosis can have a stringy appearance and adherence to the site of origin (Panel E). Panels F through I show the lamellar appearance of mucus along epithelia. In affected intrapulmonary airways in a 2-month-old pig with cystic fibrosis, mucus has a lamellar appearance lying along airway walls (Panel F). A similar pattern of mucus arising from goblet cells is shown in the ethmoid sinuses of a 1-month-old pig with cystic fibrosis. The respiratory epithelium of the ethmoid sinuses lacks submucosal glands, and mucus can sometimes be traced back to the cells of origin (Panel G). Likewise, mucus can have a lamellar appearance and be traced back to the cell of origin in the gallbladder of a newborn pig with cystic fibrosis (Panel H). Pancreatic ducts are obstructed by mucus in a 6-month-old pig with cystic fibrosis (Panel I).

way-surface liquid by aerosolizing sodium bicarbonate onto the airways of piglets with cystic fibrosis corrects the bacterial-killing defect (Fig. 3C). Conversely, increasing the acidity of airway-surface liquid diminishes bacterial killing in wild-type piglets.

These findings directly link loss of CFTR function to a host-defense defect; without CFTR-dependent bicarbonate secretion, the pH of airway-surface liquid decreases and antibacterial activity is impaired. The reduced degree of bacterial killing may be one of the critical first steps in a downward spiral from a sterile newborn lung to one that is chronically colonized.

FAILURE OF MUCUS TO DETACH FROM SUBMUCOSAL GLAND DUCTS

Another important airway defense is mucociliary transport, which guards the lung by trapping invading pathogens and particulates in mucus that is then propelled up the airways by cilia.[64,65] Although people with advanced cystic fibrosis can have slowed mucociliary transport,[65] wheth-



er mucociliary transport is impaired at the origin of the disease has been unknown.[65,66]

Mucociliary transport, assayed with the use of a CT-based approach to track discrete airway particles, appears to be similar in wild-type newborn piglets and in newborn piglets with cystic fibrosis under basal conditions.[52,67] However, after cholinergic stimulation, which elicits copious mucus secretion from submucosal glands, many particles move normally in piglets with cystic fibrosis, but some become stuck and fail to move up the airways (Fig. 3D). Mechanistic investigations of excised airways reveal that submucosal glands in piglets with cystic fibrosis secrete strands and blobs of mucus that sometimes do not break free after emerging and remain anchored to the

Figure 5 (facing page). Model of Host-Defense Defects in the Airway of a Person with Cystic Fibrosis.

As shown in Panel A, healthy airways are protected from inhaled and aspirated bacteria that enter the lung. The airway-surface liquid (left) contains endogenous antimicrobial agents that kill bacteria. Mucociliary transport (right), consisting of motile cilia and mucus produced by submucosal glands and goblet cells, sweeps bacteria out of the lung. CFTR and possibly CFTR-associated transporters provide a pathway for the exit of chloride and bicarbonate across the apical membrane of cells in the surface epithelium and in the submucosal glands. As shown in Panel B, the airway of a person with cystic fibrosis has at least two host-defense defects at the genesis of the disease. Loss of CFTR channels that conduct chloride (Cl^-) and bicarbonate (HCO_3^-) onto the airway surface causes the pH of the airway-surface liquid to decrease, and the acidic airway-surface liquid inhibits antimicrobial activity. Loss of CFTR channels in submucosal glands causes mucus to develop abnormal properties so that it does not break free after emerging and remains anchored to the gland ducts. As shown in Panel C, when bacteria enter healthy airways (top), they are killed by the antimicrobial activity of airway-surface liquid, mucociliary transport sweeps them out of the lung, and other defenses, including phagocytic cells, eradicate them to maintain sterile lungs. In persons with cystic fibrosis (bottom), antimicrobial activity and mucociliary transport are less effective than in healthy persons, and other defenses may also be impaired. Eventually, the host defenses are overwhelmed, and bacteria proliferate, with inflammation, remodeling, immunity, and genetic adaptation in the bacteria influencing the species that will dominate. In addition, the resulting inflammation and airway remodeling may further enhance or impair host-defense mechanisms.

gland ducts, hindering mucociliary transport (Fig. 3E). The defect in mucociliary transport is not attributable to depletion of periciliary liquid, because the defect persists when the airway surface is submerged in saline. Inhibition of anion secretion in the airways of wild-type pigs replicates the abnormalities associated with cystic fibrosis. These results were predicted by earlier analyses[5] from the laboratory of Wine, as well as by studies,[68] performed in the laboratory of Ballard, of the airways of wild-type pigs treated with agents that inhibit anion secretion. These data are consistent with findings of slowed mucociliary clearance in the excised trachea of 3-to-8-month-old ferrets.[32] They are also in concert with the findings of a study that showed that slowed tracheal mucociliary transport in the excised trachea of piglets with cystic fibrosis was not related to reduced depth of the periciliary liquid.[69]

These findings directly link impaired mucociliary transport to loss of CFTR anion transport, indicating that defective mucociliary transport is a primary abnormality that is not dependent on infection, inflammation, or remodeling. Nevertheless, advancing infection and bronchiectasis might further disrupt mucociliary transport and fuel disease progression. Data also suggest that the environment of the submucosal gland lumen into which mucus is initially secreted probably alters its properties, causing abnormal detachment. It remains uncertain whether defective bicarbonate secretion, liquid secretion, or a combination of these factors is the key requirement for abnormal mucociliary transport.[52,68,70-72] Abnormal airway-surface liquid might also alter the properties of mucus secreted from goblet cells.

The finding that mucus abnormalities are a problem in the lungs of persons with cystic fibrosis has parallels with findings in other organs.[71,72] In emphasizing the contributions of defective bicarbonate secretion and abnormal mucus, Quinton referred back to one of the early names for the disease — mucoviscidosis.[71] Indeed, there is a rogues' gallery of mucus abnormalities in multiple organs, including the lungs, intestine, pancreas, and gallbladder of pigs, ferrets, and humans with cystic fibrosis (Fig. 4).

ADDITIONAL IMPLICATIONS AND SPECULATIONS

Discoveries from new animal models raise additional questions for future research and have implications for the care of people with cystic fibrosis, although any therapeutic implications will require assessment in humans. In addition, whether defects that are key at the origins of cystic fibrosis retain pathophysiological importance later in the disease course remains uncertain. The following paragraphs review some of the take-home points of this article.

First, the consensus based on the data reviewed here and clinical experience is that people with cystic fibrosis should be treated early. We suspect that host-defense defects begin on the day babies with cystic fibrosis are born, as they do in piglets with cystic fibrosis. That timing suggests that preventive measures should be initiated immediately. Cystic fibrosis clinics already have substantial momentum toward earlier intervention, and data provide support for that trend.

Second, the loss of CFTR delivers multiple "hits." This loss does not completely eliminate any single defense; instead, it reduces the effectiveness of at least two defenses — mucociliary transport and antimicrobial activity[44,52] — and other defenses may also be degraded[6,10] (Fig. 5). Compromising one host-defense mechanism places a greater burden on other defenses. If those are also impaired, problems may ensue. For example, without robust antimicrobial activity to rapidly kill bacteria, increased numbers of viable organisms might prompt submucosal-gland secretion, leading to impairment of mucociliary transport. Likewise, failure of mucus detachment might allow bacteria to grow under conditions that promote resistance to antibacterial defenses that are already diminished by cystic fibrosis.[44,73] Thus, partially disrupting two or more defenses may elicit a vicious cycle of disease.

Third, cystic fibrosis initially causes an "equal opportunity" host-defense defect that may serve as a gateway for infection with typical cystic fibrosis–associated bacteria. The mix of many different bacterial species in the airways so early in the disease could elicit inflammatory, remodeling, and structural changes that become irreversible and that confer a predisposition to more intractable infections with typical cystic fibrosis pathogens. We speculate that preventive interventions and antibacterial treatments should not wait for the appearance of *P. aeruginosa* or "typical" pathogens that are associated with cystic fibrosis.

Fourth, correcting even one host-defense defect might be beneficial. For example, treating cystic fibrosis with antibiotics may improve a person's clinical status, even though it does not address mucus abnormalities. Another example is primary ciliary dyskinesia, which completely obliterates one defense — mucociliary transport — yet causes less severe lung disease than cystic fibrosis.[74] Lung disease might be less severe in primary ciliary dyskinesia because other defenses (e.g., antimicrobial activity) are intact, although differences in the way these diseases impair mucociliary transport might also explain the differing severity.

Fifth, environmental insults may trigger airway disease in the lungs of people with cystic fibrosis. Another "hit" to airways in persons with this disease might come from infections, environmental injuries, or both. Such insults trigger protective responses, including mucus secretion from the submucosal glands. But in cystic fibrosis, what would normally be a protective reflex might further cripple mucociliary transport.

Sixth, cystic fibrosis lung disease may begin in large and small airways. On the basis of histopathological findings in infants who die within weeks to months after birth, it is often thought that this disease begins in small airways. However, rapidly advancing inflammation and remodeling confound interpretation about the initiating location. Histopathological studies of older pigs with cystic fibrosis have detected disease in both large and small airways.[26,27,29] Large airways have antibacterial and mucociliary transport defects at birth, which suggests that they are a susceptible site for the onset of disease. However, small airways also express CFTR,[75] they probably have defective antibacterial activity, and mucociliary transport in these airways might be impaired by goblet cell–derived mucus. Thus, small airways may also be an initial site of clinical abnormalities. Another consideration is that the total area of small airways is much greater than that of large airways, and thus if physiological defects in both airways were equal on a per-square-meter basis, small airways would be overrepresented.

Seventh, infants with cystic fibrosis may have congenital airway defects. The airway and nasal sinus defects might affect disease progression and complicate assessments. For example, if air trapping is due in part to a congenital defect, rather than to inflammation and abnormal mucus alone, attempting to "treat" on the basis of the appearance of air trapping might not be entirely appropriate.

Eighth, we need better assays of early cystic fibrosis airway disease in humans. Sensitive assays could potentially identify and quantify early host-defense defects and track disease progression and therapeutic interventions. Studies in animal models suggest that assays of the pH of airway-surface liquid, antimicrobial activity, or mucociliary transport could be informative, especially if they are sensitive. For example, the development of methods that assay mucociliary transport in humans with the data granularity achieved in pigs could transform pulmonary imaging of mucociliary transport in cystic fibrosis and possibly other airway diseases.

Finally, these discoveries in cystic fibrosis may also have implications for other diseases. First, they emphasize the value of an animal model that replicates human disease. Second, they highlight the importance of investigating disease at its genesis and before the onset of secondary manifestations. Manifestations of advanced disease may not reflect the initiating events, and without such knowledge, treatments and preventions may not be as effective as they could be. Pulmonary fibrosis is perhaps another respiratory disease in which investigation before clinical manifestations could be revealing. Third, multiple, partial, perhaps even subtle impairments, or "hits," can have a profound effect. That concept may be relevant to more common pulmonary diseases such as asthma and chronic obstructive pulmonary disease, as well as to nonrespiratory diseases.

The origins and initiating factors in cystic fibrosis lung disease probably determine the progression, severity, and disease burden later in life. Understanding the origins, quantifying the initial defects, and intervening early could make a big difference for people with this disease.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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