

# Role of Cilia, Mucus, and Airway Surface Liquid in Mucociliary Dysfunction: Lessons from Mouse Models

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## ABSTRACT

Mucociliary clearance is an important primary innate defense mechanism that protects the lungs from deleterious effects of inhaled pollutants, allergens, and pathogens. Mucociliary dysfunction is a common feature of chronic airway diseases in humans. The mucociliary apparatus consists of three functional compartments, that is, the cilia, a protective mucus layer, and an airway surface liquid (ASL) layer, which work in concert to remove inhaled particles from the lung. A synopsis of clinical and pathological observations in patients with cystic fibrosis, primary ciliary dyskinesia, asthma, and chronic bronchitis indicates that abnormalities in each compartment of the mucociliary system can compromise mucus clearance and cause chronic airway disease. However, the mechanisms that lead to deficient mucus clearance are still incompletely understood. Genetically engineered mice with defects in individual elements of the mucociliary apparatus are powerful tools to study the pathogenesis of mucociliary dysfunction *in vivo*. In this concise review, I assess the pulmonary phenotypes of mouse models with genetically defined abnormalities in ciliary structure/function, mucus production, and ASL regulation, and discuss the results of these animal studies in the context of current pathogenetic hypotheses for mucociliary dysfunction. Recent data driven from these animal studies point to a critical role of ASL dehydration in the pathogenesis of mucociliary dysfunction and chronic airway disease. In mice with airway-specific overexpression of epithelial Na<sup>+</sup> channels (ENaC), which constitute a rate limiting pathway for absorption of salt and water from airway surfaces, ASL depletion caused reduced mucus clearance, and a spontaneous chronic airway disease with mucus obstruction, goblet cell metaplasia, chronic inflammation, reduced bacterial clearance, and high pulmonary mortality. This mouse model of mucociliary dysfunction will allow an *in vivo* evaluation of novel therapeutic strategies designed to improve mucociliary clearance, and will aid the preclinical development of novel therapies for chronic airway diseases.

**Key words:** mucociliary clearance (MCC), mucus, cilia, airway surface liquid (ASL), airway ion transport, epithelial Na<sup>+</sup> channels (ENaC), chronic bronchitis, cystic fibrosis (CF), primary ciliary dyskinesia (PCD), asthma, pulmonary defense

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## INTRODUCTION

CHRONIC AIRWAY DISEASES belong to the most common chronic diseases in children and adults worldwide. Mucociliary dysfunction is a characteristic feature of a broad spectrum of genetically determined and acquired forms of chronic airway diseases, including cystic fibrosis (CF), primary ciliary dyskinesia (PCD), asthma, and chronic bronchitis. Because normal mucus clearance constitutes an important protective mechanism of the lung, it was predicted that mucociliary dysfunction plays an important role in the pathogenesis of these diseases. This concept is supported by the clinicopathological sequence of different chronic airway diseases in humans, indicating that diverse abnormalities in ciliary function, airway surface hydration, and mucus production may impair mucus clearance and cause chronic airway disease. Genetically engineered mice, in which the single components of the mucociliary system, that is, cilia, mucus, or airway surface liquid ASL were targeted by gene disruption or overexpression provide powerful tools to dissect the relative roles of these individual elements in the pathogenesis of mucociliary dysfunction and chronic airway disease. Based on a brief summary of current knowledge and pathogenetic concepts of mucociliary dysfunction that were originally derived from human studies in patients with chronic airway diseases, this review focuses on the pulmonary phenotypes of transgenic mice with defined abnormalities in the mucociliary apparatus. The lessons learned from these mouse models are discussed in the context of their usefulness for future studies on the complex *in vivo* pathogenesis of chronic airway diseases, and the development of novel therapeutic interventions that target mucociliary dysfunction.

### *Role of mucociliary clearance in pulmonary defense*

With tidal breathing of several thousand liters of air per day, human airway surfaces are constantly exposed to diverse environmental stimuli including inhaled particles, allergens, and pathogens.<sup>(1)</sup> These agents are potent stimuli for airway inflammation and cause infections, if they are not removed efficiently from the lungs.<sup>(2–5)</sup> Therefore, mucociliary clearance has long been recognized as a primary innate defense mecha-

nism of mammalian airways that works in concert with a chemical shield of antimicrobial substances including lysozyme and lactoferrin, to protect the host from the noxious effects of airborne pathogens, pollutants, allergens.<sup>(1,6,7)</sup> Integrated into the microanatomy of airway surfaces, the mucociliary clearance system consists of several functional elements that work together in a coordinated fashion. Healthy airway surfaces are lined by ciliated epithelial cells and covered with an ASL, which is partitioned into a mucus layer that entraps inhaled particles and pathogens, and a low viscosity periciliary liquid (PCL) layer that lubricates airway surfaces and facilitates ciliary beating and efficient mucus clearance from the lungs to the mouth.<sup>(1,6,8)</sup> Appreciation of the importance of normal mucociliary function as a primary defense mechanism of the lung led to the concept that mucociliary dysfunction may be an important predisposing factor for airway disease, and spurred human studies that compared mucus transport rates in healthy individuals, and patients with chronic airway diseases under baseline conditions and during pulmonary exacerbations.<sup>(1)</sup>

### *Mucociliary dysfunction in chronic airway disease*

In a series of human studies using inhaled radioisotopes and gamma scintigraphy to determine mucus transport rates *in vivo*, it was documented that impaired mucus clearance is a key feature of a broad spectrum of chronic airway diseases including asthma,<sup>(9–11)</sup> cigarette smoke-induced chronic bronchitis,<sup>(12–15)</sup> CF,<sup>(16)</sup> and PCD.<sup>(17,18)</sup> Interestingly, several of these studies demonstrated that reduced mucus clearance is an early functional abnormality that precedes clinical symptoms and airway pathology, and persists during remissions.<sup>(11,13,14,16)</sup> The data from these functional studies, together with the observed commonalities in the clinicopathological sequence of these diseases including airway mucus obstruction, chronic inflammation, and increased susceptibility to pulmonary infections,<sup>(5,19–21)</sup> indicated that mucociliary dysfunction plays an important role in the early pathogenesis of chronic airway diseases of different etiologies<sup>(1)</sup> (Table 1). However, these observations also led to the challenging question how diverse disease etiologies with marked differences in their molecular and cellular pathologies can cause a common defect in mucociliary clearance.

TABLE 1. PULMONARY PHENOTYPES IN PATIENTS WITH PRIMARY CILIARY DYSKINESIA (PCD), ASTHMA, AND CYSTIC FIBROSIS (CF)

	<i>PCD</i>	<i>Asthma</i>	<i>CF</i>
Primary defect	Ciliary dysfunction caused by mutations in dynein genes (e.g., DNAH5 or DNAI1)	Th2-mediated inflammation thought to play key role in complex pathogenesis	Dysregulation of airway ion transport caused by mutations in CFTR gene
Airway mucus plugging	Variable	Severe in fatal asthma Not determined in stable asthma	Severe and early onset
Airway inflammation	Mostly neutrophilia	Mostly eosinophilia	Mostly chronic neutrophilia
Mucociliary clearance	Reduced (cough clearance remains intact)	Reduced (most severe during exacerbation)	Reduced (cough clearance is deficient)
Pulmonary mortality	Normal life expectancy with appropriate therapy	~1.5 deaths per 100,000 population	Reduced life expectancy (mean ~36 years)

*Possible mechanisms causing mucociliary dysfunction: lessons from chronic airway diseases in humans*

Hypotheses on the mechanisms involved in the *in vivo* pathogenesis of mucociliary dysfunction focus on defects in ciliary function, defects in the regulation of ASL volume and abnormalities in mucus secretion and/or composition. These concepts were originally deducted from clinical and pathological observations in patients with PCD, CF, and other chronic airway diseases, and are briefly summarized below.

*PCD: defects in ciliary function*

PCD is a rare genetic disease characterized by abnormal ciliary structure and function.<sup>(22,23)</sup> In the lung, deficient ciliary function causes impaired mucus clearance, and the development of chronic bronchitis with increased susceptibility to bacterial infections, and formation of bronchiectasis.<sup>(17,18,21)</sup> Further, the PCD syndrome is associated with chronic sinusitis, male infertility, situs inversus, and other laterality defects, reflecting the role of cilia in upper airway defense, sperm motility, and determination of the left-right body axis during embryonic development.<sup>(24)</sup> Thus, the pulmonary phenotype of PCD patients validated the concept that abnormal ciliary function leads to the development of chronic airway disease (Table 1). In contrast to the substantial reduction in basal cilia-dependent mucus clearance, cough clearance remains intact in PCD patients, demonstrating that cough is a cilia-independent backup mechanism of mucus clearance in the human lung.<sup>(18)</sup>

*CF: defects in airway ion transport*

CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.<sup>(25)</sup> With a frequency of 1:2000 to 1:3000 affected newborns in Caucasian populations, CF is the most common genetic form of chronic airway disease. Clinically, CF is characterized by early onset airway mucus plugging followed by chronic airway inflammation and infection with *Pseudomonas aeruginosa* and other CF-related pathogens leading to progressive bronchiectasis and damage of lung parenchyma<sup>(4,5,26,27)</sup> (Table 1). The CFTR protein is expressed in the luminal membrane of airway epithelial cells, where it acts as a cAMP-dependent Cl<sup>-</sup> channel and regulator of the epithelial Na<sup>+</sup> channel (ENaC).<sup>(28-31)</sup> Early *in vivo* studies documented that CFTR mutations cause a characteristic defect in cAMP-dependent Cl<sup>-</sup> secretion and a two- to threefold increase in ENaC-mediated Na<sup>+</sup> absorption in CF airway epithelia.<sup>(32-34)</sup> ENaC is rate limiting for active Na<sup>+</sup> absorption across airway epithelia, and sets the driving force for passive absorption of Cl<sup>-</sup> and H<sub>2</sub>O along the paracellular pathway.<sup>(35)</sup> Therefore, increased ENaC activity in combination with deficient CFTR-mediated Cl<sup>-</sup> secretion was predicted to cause accelerated net fluid absorption and depletion of ASL volume, and result in dehydration and surface adhesion of mucus, and impaired ciliary function in CF airways.<sup>(5,34,36)</sup> This “low-volume” hypothesis was further substantiated in studies using primary cultures of normal and CF airway epithelia, which retain active ion transport and mucus transport properties of native airway tissues.<sup>(6,37,38)</sup> These studies demonstrated that ASL volume is actively regu-

lated by normal airway epithelia setting the height of the PCL to approximately 7  $\mu\text{m}$ , that is, the length of extended cilia in human airways. In CF cultures, ASL volume regulation fails, and PCL height is consistently reduced, causing the cilia to collapse on epithelial surfaces, which in turn, leads to a substantial reduction in mucus transport velocity on the surface of CF compared to normal airway cultures.<sup>(6,37,38)</sup> Taken together, these *in vivo* and *in vitro* studies supported the concept that mucociliary dysfunction can be caused by ASL volume depletion in the absence of primary defects in ciliary structure, and that this mechanism forms the basis for deficient airway mucus clearance in CF patients.

#### *Asthma and chronic bronchitis: mucus hypersecretion*

In asthma and chronic bronchitis, the most common chronic airway diseases, the mechanisms that cause reduced mucus clearance and airway mucus plugging are less clear. Both conditions are characterized by chronic airway inflammation commonly triggered and/or perpetuated by inhaled allergens or chronic exposure to cigarette smoke.<sup>(20,39)</sup> Subsequent development of goblet cell metaplasia, mucus hypersecretion, and intraluminal mucus obstruction are key features of both disease entities. In asthma, the most striking evidence that insufficient mucus clearance could contribute substantially to airway obstruction comes from autopsies of patients who died in status asthmaticus. Severe airway mucus plugging has been reported consistently in these autopsy specimen, making it likely that asphyxiation from intraluminal obstruction may indeed be a major contributing cause of death in fatal asthma<sup>(19,40)</sup> (Table 1). Intraluminal mucus obstruction of the small airways has recently also been identified as a key factor determining disease progression in patients with cigarette smoke-induced chronic bronchitis.<sup>(20)</sup> Because primary abnormalities in ciliary structure and/or airway ion transport have not been reported in patients with chronic bronchitis or asthma, it has been proposed that reduced mucus clearance in these diseases is caused by changes in the quantity and/or viscoelastic properties of airway secretions.<sup>(1)</sup> This notion is supported by the observation that mucus transport rates decrease in parallel with increased sputum expectoration during acute exacerbations.<sup>(13)</sup>

Taken together, data from these human studies indicated that defects in each compartment of the mucociliary system, that is, abnormalities in ciliary function, ASL volume regulation, and mucus secretion can compromise mucus clearance and cause chronic airway disease. Given the importance of mucociliary clearance in pulmonary defense, these defects may also serve as targets for novel therapies of chronic airway diseases. However, further elucidation of the relative roles of ciliary dysfunction, ASL volume depletion, and mucus hypersecretion in the pathogenesis of impaired mucus clearance has been hampered by the complex anatomy of human airways, and the inherent difficulties of *in vivo* studies of the microanatomy of airway surfaces in the human lung.

#### *In vivo pathogenesis of mucociliary dysfunction: lessons from transgenic mouse models*

Advances in the generation of genetically engineered mice in the past 2 decades, both by gene targeting and by gene insertion, made it possible to target individual elements of the mucociliary apparatus, either by deletion or by overexpression of candidate genes that were predicted to play important roles in normal mucociliary function.<sup>(41,42)</sup> These efforts resulted in a number of transgenic mouse models with abnormalities in mucus secretion,<sup>(43)</sup> ciliary structure/function,<sup>(44–47)</sup> and ASL homeostasis.<sup>(48)</sup> The comparison of the pulmonary phenotypes of these mice produced some surprising findings and novel insights regarding the relative roles of mucus, cilia, and airway surface hydration in pulmonary defense (Table 2).

#### *Mouse models of asthma*

Mouse models exhibiting airway goblet cell hyperplasia and mucus hypersecretion were predominantly derived from studies that tested the role of various Th2 cytokines in the pathogenesis of asthma. These studies demonstrated that lung specific overexpression of IL-13 and various other Th2 cell-derived cytokines under control of the Clara cell-specific promoter (CCSP) causes a pulmonary phenotype that shares key features of asthma in humans including airway eosinophilia, goblet cell metaplasia, and mucus hypersecretion, and airway hyperresponsiveness (AHR).<sup>(49–52)</sup> Hence, these studies identified a key role of Th2

TABLE 2. PULMONARY PHENOTYPES CAUSED BY CILIARY DYSFUNCTION, MUCUS HYPERSECRETION, AND ASL DEPLETION IN TRANSGENIC MOUSE MODELS

	<i>Ciliary dysfunction</i>	<i>Mucus hypersecretion</i>	<i>ASL depletion</i>
Mouse models	Knockout of Hfh-4 or Mdnah5	Overexpression of Th2 cytokines (IL-4, IL-9, IL-13)	Overexpression of $\beta$ ENaC
Goblet cell metaplasia	—	+++	+++
Airway mucus plugging	—	+/-	+++
Airway inflammation	—	Transient eosinophilia	Chronic neutrophilia
Mucociliary clearance	Not determined	Accelerated bacterial clearance	Reduced mucus transport Slowed bacterial clearance ~50% mortality
Pulmonary mortality	—	—	—

mediators in the *in vivo* pathogenesis of asthma, and produced mouse models of mucus hypersecretion (Table 2). Notably, although mucus producing cells and expression of airway mucins including Muc5ac and Muc5b are significantly increased in asthma mouse models, airway mucus plugging is not a consistent feature of these animals. Although measurements of mucus clearance rates, for example, by gamma scintigraphy, are well established in humans,<sup>(9–11)</sup> application of these techniques is still in its infancy in small animals, and quantitative data on mucus clearance rates are not available for mouse models of mucus hypersecretion. However, the absence of substantial airway mucus plugging in the presence of striking goblet cell metaplasia and mucus hypersecretion indicates that mucociliary clearance remains intact in these mouse models. Interestingly, in bacterial challenge studies, pulmonary clearance of *Pseudomonas aeruginosa* was increased in mice with lung-specific overexpression of IL-4 compared to wild-type animals.<sup>(53)</sup> These data indicated that mucociliary clearance may even be accelerated in the presence of mucus hypersecretion, and thus compensate for excess mucus secretion into the airway lumen, and avoid mucus plugging. Taken together, the data driven from asthma mouse models suggest that increased mucus production alone is not rate limiting for mucociliary clearance in murine airways. Therefore, mouse models of mucus hypersecretion remain useful models for studies on the identification of potential compensatory mechanisms that prevent airway mucus obstruction in the presence of mucus hypersecretion. Further elucidation of such compensatory mechanisms may result in novel therapeutic strategies for the treatment of mucociliary dysfunction and mucus plugging in human asthma.

#### *Mouse models of PCD*

Targeting of several genes that are critical for ciliogenesis or ciliary structure, including genes encoding for the winged helix/forkhead transcription factor Hfh4, and the mouse axonemal dynein heavy chain 5 (Mdnah5) resulted in mouse models, in which cilia are either absent or dysfunctional<sup>(44–47)</sup> (Table 2). Similar to PCD patients, systemic loss of ciliary function caused situs inversus in these mouse models, supporting a central role of cilia in left-right axis formation. Additionally, PCD mice also develop hydrocephalus internus during early brain development with high mortality due to neurological complications.<sup>(44–47)</sup> Recent studies indicated that hydrocephalus formation in PCD mice is likely caused by deficient cilia-mediated flow of central spinal fluid through the ventricular system of the murine brain.<sup>(54)</sup> Due to the high and early mortality related to hydrocephalus formation, a detailed analysis of the pulmonary phenotype has been difficult in PCD mice, and remains limited to a small number of animals that were studied at relatively young ages.<sup>(45)</sup> In these studies, the most salient health problem in PCD patients, that is, a chronic airway disease with increased susceptibility to bacterial infections has not been described in PCD mice (Table 2). Hence, the absence of a spontaneous pulmonary disease phenotype in the absence of functional cilia indicates that murine airways are equipped with cilia-independent backup mechanisms for mucus clearance. Cough-mediated mucus clearance is an important backup system in humans that remains intact in PCD patients.<sup>(18)</sup> However, given the high respiratory frequency of more than 100 breaths per minute, efficient cough clearance is an unlikely backup mechanism in mice. Alternatively,

mucus transport by two-phase gas–liquid flow (gas liquid pumping), a mechanism by which mucus is propelled longitudinally by airflow along a tube with a lubricated surface, may compensate for deficient cilia-dependent mucus transport in PCD mice.<sup>(55)</sup>

As noted above, the high and early mortality caused by hydrocephalus formation has been a major obstacle for studies aiming to identify more subtle pulmonary phenotypes that may be caused by ciliary dysfunction in PCD mice. For example, it is possible that the onset of chronic airway disease in the absence of cilia-mediated mucus transport requires longer time periods and/or exposure to environmental stimuli (e.g., airborne pollutants or pathogens) that are not present in the pathogen-free environments, in which the animals were reared. With recent progress in tissue-specific ablation of gene function, for example, by combining the Cre/lox system with a promoter that drives Cre-mediated gene excision exclusively in ciliated airway cells,<sup>(42,56–58)</sup> it is now possible to develop mouse models, in which ablation of ciliary function is limited to the lung. These novel mouse models with airway-specific knockout of ciliary function will be invaluable tools to further define the role of cilia in mucus clearance, and in the *in vivo* pathogenesis of chronic airway disease.

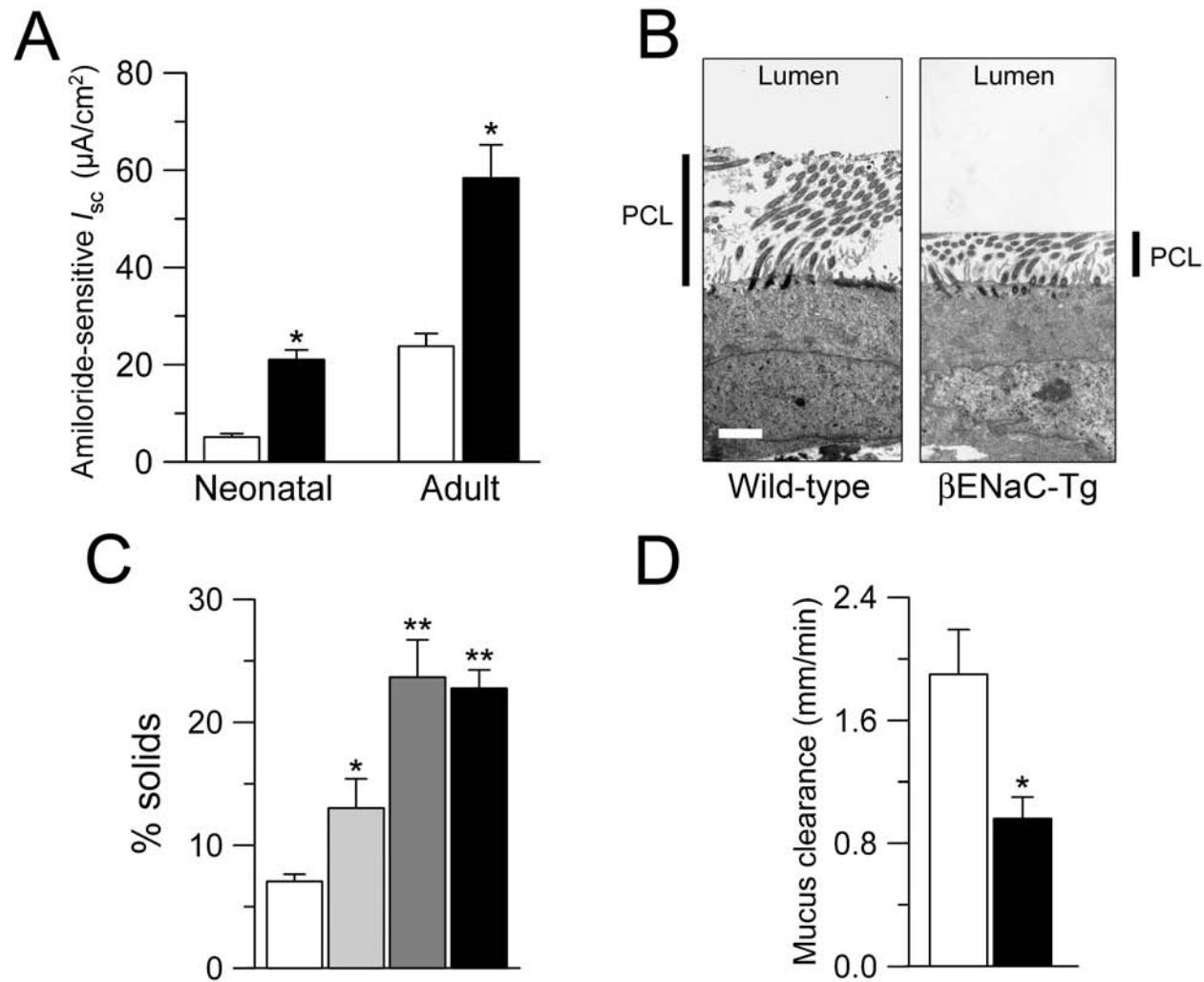
#### *Mouse models of CF*

The cloning of the CFTR gene made it possible to generate mice, in which gene function was deleted either by homologous recombination, or by introduction of specific mutations in the murine CFTR gene.<sup>(59–61)</sup> This strategy was the first attempt to generate an animal model that recapitulates the basic ion transport defects (i.e., deficient cAMP-dependent Cl<sup>-</sup> secretion and increased Na<sup>+</sup> absorption) of human CF airways, to study their role in the *in vivo* pathogenesis of chronic airway disease. In several independent studies it was shown that CF mice exhibit characteristic defects in intestinal ion transport producing a severe CF-like gastrointestinal phenotype similar to meconium ileus in CF infants.<sup>(59,60)</sup> Surprisingly, even when CF mice were rescued from neonatal mortality due to intestinal blockage by treatment with an osmotic laxative, lack of functional CFTR did not cause CF-like airway disease.<sup>(61)</sup> In contrast to CF patients, in whom chronic obstructive lung disease remains the ma-

jor cause of morbidity and mortality, CF mice do not exhibit airway mucus plugging, goblet cell metaplasia or chronic inflammation and bacterial infection of the lower airways. Subsequently, detailed functional analyses of epithelial ion transport in different regions of the airways demonstrated that CF mice exhibit Na<sup>+</sup> hyperabsorption and deficient cAMP-dependant Cl<sup>-</sup> secretion, and an associated phenotype of ASL depletion and goblet cell metaplasia in the nasal epithelium.<sup>(38,61,62)</sup> In contrast, Na<sup>+</sup> transport is not elevated, and deficient CFTR-mediated Cl<sup>-</sup> secretion is compensated by CFTR-independent Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels (CaCC) in lower airways of CF mice.<sup>(61,63,64)</sup> More recently, it became possible to measure mucociliary transport in small animals by a technique that determines the clearance of a fluorescent dye deposited into the lower airways with microdialysis.<sup>(65)</sup> Consistent with normal ion transport properties and lack of pathology in lower airways, it was demonstrated that *in vivo* mucus clearance rates are normal in CFTR-deficient mice.<sup>(65)</sup>

#### *A mouse model with airway surface dehydration*

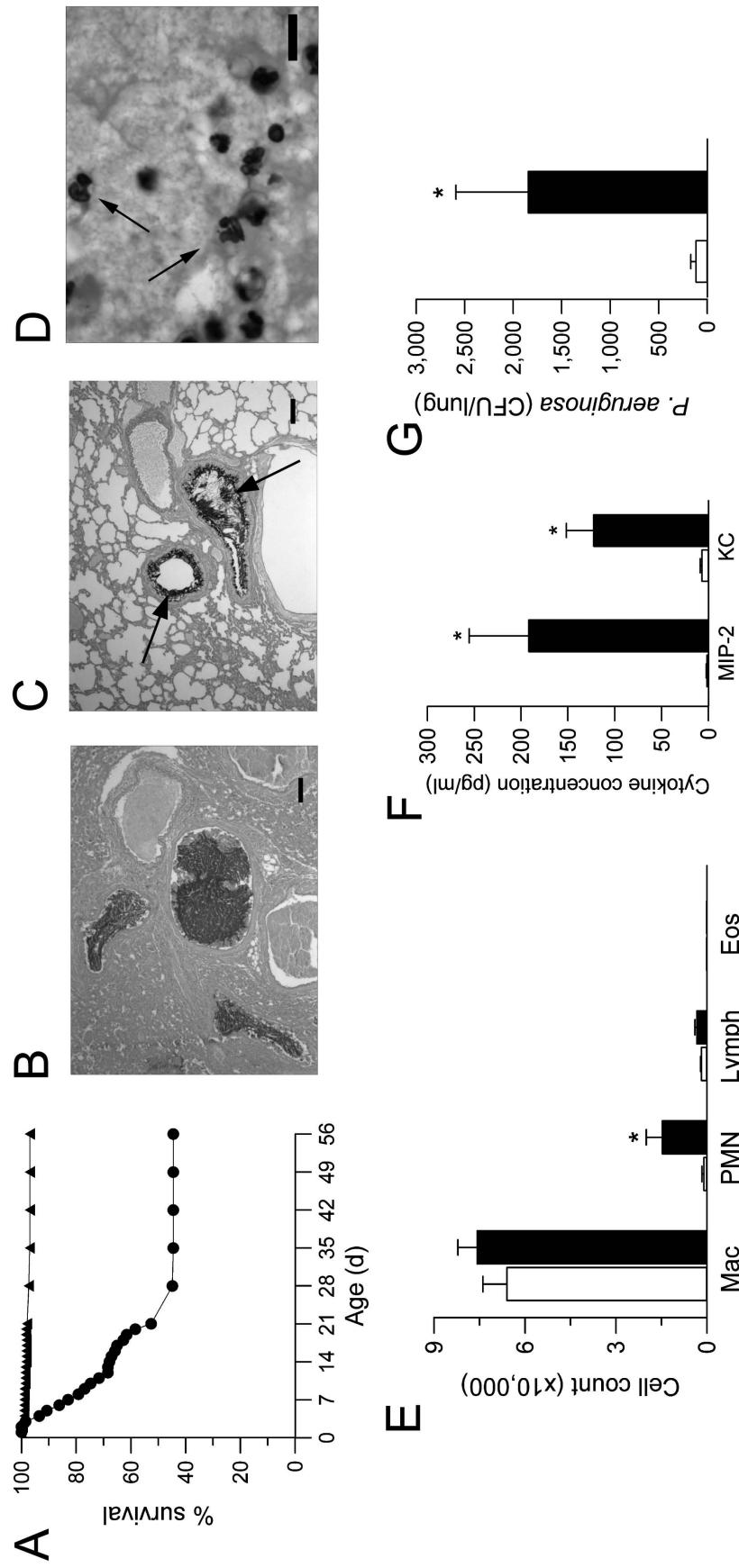
Based on the results driven from CFTR-deficient mice, we sought alternative strategies to further elucidate the role of deficient ASL volume regulation in the *in vivo* pathogenesis of mucociliary dysfunction and chronic airway disease. Because the molecular identity of CaCC remains uncertain, we generated transgenic mice with airway-specific overexpression of ENaC to mimic increased Na<sup>+</sup> absorption in human CF airways.<sup>(48,66)</sup> ENaC is a heteromultimeric protein composed of three subunits ( $\alpha$ ,  $\beta$ , and  $\gamma$ ), and tissue-specific overexpression of each individual subunit was achieved by using the CCSP promoter, which targets transgene expression to lower airway epithelia.<sup>(67)</sup> Interestingly, although coexpression of all three subunits ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) is required to obtain maximal ENaC-mediated Na<sup>+</sup> currents in heterologous cells,<sup>(29)</sup> overexpression of  $\beta$ ENaC (also known as Scnn1b) alone was sufficient to produce a ~3-fold increase in amiloride-sensitive Na<sup>+</sup> absorption in airways from neonatal to adult  $\beta$ ENaC transgenic mice (Fig. 1A). Taken together with the observation that endogenous  $\beta$ ENaC is expressed at relatively low levels in mouse lower airways, these data suggest that  $\beta$ ENaC expression levels are rate limiting for Na<sup>+</sup> absorption in the airways. Similar to primary



**FIG. 1.** Increased airway  $\text{Na}^+$  transport causes ASL volume depletion and reduced mucus transport in  $\beta\text{ENaC}$ -transgenic mice. (A) Increased airway  $\text{Na}^+$  absorption, as determined from measurements of the amiloride-sensitive short circuit current ( $I_{sc}$ ) in neonatal and adult  $\beta\text{ENaC}$ -transgenic mice (black bars) compared to wild-type littermates (white bars). \* $p < 0.0001$ . (B) Reduced PCL height, as visualized by transmission electron microscopy of airway surfaces fixed with osmium tetroxide in perfluorocarbon (73) in  $\beta\text{ENaC}$ -transgenic compared to wild-type mice (scale bar, 2.5  $\mu\text{m}$ ). (C) Airway mucus dehydration in  $\beta\text{ENaC}$ -transgenic mice, as evidenced by an increased percentage of solids in sampled mucus (light gray), and in mucus plugs extracted from the airways of adult (dark gray) and neonatal (black)  $\beta\text{ENaC}$ -transgenic mice compared to wild-type littermates (white). \* $p < 0.05$  compared to wild-type. \*\* $p < 0.05$  compared to sampled mucus from  $\beta\text{ENaC}$ -transgenic mice. (D) Reduced tracheal mucus clearance rates in  $\beta\text{ENaC}$ -transgenic mice (black bars) compared to wild-type littermates (white bars). \* $p < 0.01$ . Adopted from Ref. (48).

cultures from human CF airway epithelia, increased  $\text{Na}^+$  absorption in the airways of  $\beta\text{ENaC}$ -transgenic mice caused volume depletion of both compartments of the ASL. As shown in Figure 1, the height of the PCL (Fig. 1B), and the water content of airway mucus specimens (Fig. 1C) are significantly reduced in  $\beta\text{ENaC}$ -transgenic mice compared to wild-type littermates. Measurement of mucociliary transport using the same technique as for CFTR deficient mice<sup>(65)</sup> demonstrated that ASL depletion results in a significant

reduction of mucus clearance rates *in vivo* (Fig. 1D). Further phenotypic characterization of  $\beta\text{ENaC}$ -transgenic mice demonstrated that mucociliary dysfunction produced a spontaneous chronic airway diseases with high pulmonary mortality due to mucus plugging (Fig. 2A and B), and chronic bronchitis with chronic neutrophilic inflammation, elevated concentrations of the potent proinflammatory cytokines KC and Mip-2 (murine homologues of human IL-8), goblet cell metaplasia, and mucus hypersecretion



**FIG. 2.** ASL volume depletion and muociliary dysfunction cause a CF-like chronic obstructive airway disease in  $\beta$ ENaC-transgenic mice. (A) Survival curves of  $\beta$ ENaC-transgenic mice (circles,  $n = 399$ ) and wild-type littermates (triangles,  $n = 391$ ) depicting significant spontaneous mortality of  $\beta$ ENaC-transgenic mice (~50% in the first 3 weeks of life). (B) Histopathological evaluation of the lung of a  $\beta$ ENaC-transgenic mouse that died at 3 weeks of age showing extensive mucus plugging of large and small airways (representative of  $n = 28$  deceased  $\beta$ ENaC-transgenic mice, stained with AB-PAS; scale bar, 100  $\mu$ m). (C) Pulmonary disease phenotype in a surviving adult  $\beta$ ENaC-transgenic mouse characterized by intraluminal mucus obstruction and goblet cell metaplasia (representative of  $n = 15$  adult  $\beta$ ENaC-transgenic mice, stained with AB-PAS; scale bar, 100  $\mu$ m). (D) High-power magnification of lung section identifying inflammatory cell infiltrates (arrow) in intraluminal mucus in the airway of a  $\beta$ ENaC-transgenic mouse (stained with H&E; scale bar, 10  $\mu$ m). (E, F) Bronchoalveolar lavage analyses demonstrating chronic neutrophilic inflammation (E), and elevated concentrations of neutrophil-attracting chemokines KC and Mip-2 (F) in adult  $\beta$ ENaC-transgenic mice (black bars) compared to wild-type littermates (white bars). \* $p < 0.0001$ . (G) Slowed pulmonary clearance of *Pseudomonas aeruginosa*, as evidenced by increased bacterial burden in lungs from  $\beta$ ENaC-transgenic mice (black bars) compared to wild-type controls (white bars) 3 days after tracheal instillation of the pathogen. \* $p < 0.0001$ . Adopted from Ref. (48).

(Fig. 2C–F), and slowed clearance of bacterial pathogens that are frequently encountered in chronic airway diseases in humans including *Pseudomonas aeruginosa* and *Haemophilus influenzae*<sup>(5,48,66)</sup> (Fig. 2G) (Table 2).

Taken together, the phenotype of the  $\beta$ ENaC-transgenic mouse demonstrates that mucociliary dysfunction induced by ASL volume depletion initiates chronic airway disease, and indicates how this disease may be perpetuated *in vivo*. Because  $\beta$ ENaC-transgenic mice do not develop spontaneous pulmonary infections when reared under pathogen free conditions, chronic airway inflammation is likely caused by a failure to clear inhaled environmental particles and irritants, which in turn, trigger the release of pro-inflammatory chemokines (e.g., KC and Mip-2) from airway epithelia and/or macrophages on airway surfaces.<sup>(2)</sup> When these cytokines become concentrated on dehydrated airway surfaces, neutrophils are recruited into the airway lumen, and trigger airway inflammation in the absence of bacterial infection. Subsequently, several mechanisms may cause the goblet cell metaplasia and mucus hypersecretion in the airways of  $\beta$ ENaC-transgenic mice and contribute to airway mucus obstruction. Reduced mucus clearance likely causes a concentration of neutrophil products including neutrophil elastase on airway surfaces. It is well documented that neutrophil elastase is a potent stimulus for goblet cell metaplasia and mucin hypersecretion.<sup>(68–70)</sup> Further, neutrophil elastase can itself induce chemokine secretion by airway epithelia,<sup>(71)</sup> and thus produce a self-perpetuating inflammatory cycle. As a result, mucostasis, chronic inflammation, airway epithelial remodeling, and mucus hypersecretion cause intraluminal accumulation of thickened mucus, which forms a nidus for bacterial infection.

## SUMMARY AND CONCLUSION

Advances in the generation of genetically engineered mice have resulted in mouse models with defined abnormalities in the individual components of the mucociliary clearance system. Recent *in vivo* studies on these mouse models revealed some surprising findings regarding the relative roles of mucus hypersecretion, primary ciliary dysfunction, and ASL volume depletion in the *in vivo* pathogenesis of mucociliary dysfunction. Neither transgenic induction of goblet cell

metaplasia and mucus hypersecretion, nor ablation of ciliary function alone were sufficient to cause airway mucus obstruction and/or increased susceptibility to bacterial infection, indicating that these abnormalities are not rate limiting for mucociliary clearance in mouse airways. Therefore, these mouse models remain useful for studies on the identification of potential compensatory mechanisms that prevent mucociliary dysfunction in the context of mucus hypersecretion and ciliary defects, and may thus lead to novel therapeutic targets.

On the other hand, emerging evidence from *in vivo* studies on the  $\beta$ ENaC-transgenic mouse suggests that ASL volume depletion plays a critical role in the *in vivo* pathogenesis of mucociliary dysfunction. The phenotype of the  $\beta$ ENaC-transgenic mouse demonstrates that ASL depletion caused by a dysbalance of  $\text{Na}^+$  absorption and  $\text{Cl}^-$  secretion impairs mucus clearance and produces a spontaneous chronic airway disease. Interestingly, while abnormalities in ion transport are well documented in CF airways, recent data from a human study suggest that cigarette smoke can cause an acquired deficiency of CFTR-mediated  $\text{Cl}^-$  secretion in the airways indicating that ASL depletion may also contribute to the pathogenesis of chronic bronchitis in smokers.<sup>(72)</sup> As a mouse model of mucociliary dysfunction, the  $\beta$ ENaC-transgenic mouse will be a useful tool for studies on the relationship between deficient mucus clearance, the impact of environmental stimuli (i.e., airborne irritants, allergens, and pathogens), and the evolution/progression of chronic airway disease *in vivo*. Further, this mouse model will allow an *in vivo* evaluation of novel therapeutic strategies that target mucociliary dysfunction, and will thus aid the preclinical development of efficient therapies for chronic airway diseases. From the lessons learned from the comparison of the pulmonary phenotypes of these transgenic mouse models so far, it is predicted that restoration of adequate ASL volume to airway surfaces may be a successful strategy for the treatment of CF and possibly other chronic airway diseases.

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