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Original Article

Early pulmonary disease manifestations in cystic fibrosis mice

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Abstract

Background: Altered pulmonary function is present early in the course of cystic fibrosis (CF), independent of documented infections or onset of pulmonary symptoms. New initiatives in clinical care are focusing on detection and characterization of preclinical disease. Thus, animal models are needed which recapitulate the pulmonary phenotype characteristic of early stage CF.
Methods: We investigated young CF mice to determine if they exhibit pulmonary pathophysiology consistent with the early CF lung phenotype. Lung histology and pulmonary mechanics were examined in 12- to 16-week-old congenic C57bl/6 F508del and R117H CF mice using a forced oscillation technique (flexiVent).
Results: There were no significant differences in the resistance of the large airways. However, in both CF mouse models, prominent differences in the mechanical properties of the peripheral lung compartment were identified including decreased static lung compliance, increased elastance and increased tissue damping. CF mice also had distal airspace enlargement with significantly increased mean linear intercept distances.
Conclusions: An impaired ability to stretch and expand the peripheral lung compartment, as well as increased distances between gas exchange surfaces, were present in young CF mice carrying two independent *Cftr* mutations. This altered pulmonary histopathophysiology in the peripheral lung compartment, which develops in the absence of infection, is similar to the early lung phenotype of CF patients.

Keywords: Cystic fibrosis; Lung mechanics; Mouse models

1. Introduction

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CF pulmonary phenotype worsens over time; progressing from early, pre-symptomatic pathophysiologic changes in the lung and airways to overt disease with mucus obstruction and bronchiectasis. Progressive lung disease characterized by recurrent infections is the major cause of morbidity and mortality. However, growing evidence suggests that the CF pulmonary phenotype develops prior to, and partially independent of, the recurrent cycle of infection, inflammation and obstruction [1–5]. Early manifestations of the CF pulmonary phenotype include increased airway resistance, evidence of gas trapping and diminished expiratory flow rates and volumes. These abnormalities have been reported in young patients with mild CF [6] and in clinically stable individuals without detectable infection [7–14]. Using the single frequency forced oscillation technique, decreases in reactance (Xrs) and

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increases in resistance (Rrs) of the respiratory system in CF patients has been reported, although the magnitude of these differences was not consistent across studies [7,15–17], likely due to variability of clinical status in these cohorts. Taken together, these findings indicate that a phenotype of altered pulmonary function is present early in the course of CF, independent of documented infections, or onset of pulmonary symptoms.

The advent of newborn screening has enabled clinical studies of lung function parameters in pre-symptomatic patients with CF, and has extended the time between diagnosis and onset of overt symptoms. This silent period provides a window of opportunity where therapeutic interventions may be most effective [18,19]. Thus, a recent focus has been on the early detection [18,20–24] and treatment [25,26] of pre-symptomatic CF patients. However, these efforts are challenged by the fact that there is an incomplete understanding of the underlying pathobiology of lung function in early CF. As clinical strategies shift from treatment of symptoms to prevention of progressive lung disease, animal models are needed which manifest the pulmonary pathology characteristic of the early CF phenotype. In the present work, we demonstrate that two different mouse models of human CFTR mutations (F508del and R117H) exhibit significant histological and lung mechanical differences, which are localized to the peripheral lung compartment. CF mouse lungs exhibited a greater mean free distance between gas exchange surfaces and had an impaired ability to stretch and expand. These alterations in respiratory mechanics and lung structure were identified in the absence of infection, detectable airway mucus or inflammatory cell infiltration, and are similar to the early pulmonary phenotype described in CF patients [20,27–29].

2. Materials and methods

2.1. Mice

Two congenic CF mouse models were utilized: homozygous F508del Cftr mutation (*Cftr*^{tm1kth}) [30], and homozygous R117H mutation [31]. These mutations have been backcrossed >10 generations to the C57Bl/6J strain to make the strains congenic. Non-CF mice were wild type (WT) littermates of the CF mice. All CF and non-CF mice used in the study were young adults (8–16 weeks old). Mice were allowed unrestricted access to chow (Harlan Teklad 7960; Harlan Teklad Global Diets, Madison, WI) and sterile water with an osmotic laxative, Colyte (Schwarz Pharma, Milwaukee, WI), and were maintained on a 12 h light/dark cycle at a mean ambient temperature of 22 °C. WT mice (n = 7; 4 males and 3 females) weighed 25.6 ± 3.6 g. F508del CF mice (n = 6, 4 males and 2 females) weighed 22.4 ± 2.7 g ($p < 0.04$ compared to WT mice), and R117H CF mice (n = 6, 3 males and 3 females) weighed 19.1 ± 2.4 g ($p < 0.01$ compared to WT mice). While the body weights of both CF groups were less than WT, there were no statistically significant differences in lung weight, height or width (Table 1).

Table 1
Lung measurements.

	Body weight (g)	Lung weight (g)	Lung height (mm)	Lung width (mm)
WT	25.6 ± 3.6	0.16 ± 0.04	10.6 ± 0.7	5.8 ± 0.5
F508del	22.4 ± 2.7 *	0.15 ± 0.04	10.5 ± 0.7	5.7 ± 0.6
R117H	19.1 ± 2.4 *	0.13 ± 0.01	10.2 ± 0.3	5.3 ± 0.5

* $p < 0.05$ compared to WT.

2.2. Screening for infectious organisms and specific pathogen testing

Sentinel mice from the colony were screened on a monthly basis via culture of bronchoalveolar lavage fluid. During the course of this study, no active infections were detected in any of the sentinel animals housed alongside the experimental animals. In addition, all the experimental animals were tested using serum ELISA for *Bordetella hinzii*, which is the only known pathogen to be identified in this mouse colony. All experimental mice tested negative for any current or previous *B. hinzii* infection.

2.3. Respiratory mechanics

Forced oscillation measurements were obtained in intact, intubated, anesthetized mice. Following induction of anesthesia via intraperitoneal injection of ketamine (150 mg/kg) and xylazine (15 mg/kg), tracheotomy and cannulation (Becton Dickinson Angiocath, 20GA, trimmed to 20 mm length) were performed. Animals were mechanically ventilated using a computer-controlled mechanical ventilator (flexiVent system, SCIREQ) at a rate of 150 breaths/minute with tidal volumes of 10 mL/kg and positive end-expiratory pressure of 3 cmH_2O. Lung mechanics measurements were made using automated maneuvers and analysis algorithms as described previously [32]. Briefly, inspiratory capacity was measured by slow lung inflations to 30 cmH_2O over 3 s. Data obtained from single-frequency forced oscillation maneuvers were used to calculate overall respiratory system resistance (Rrs), compliance (Crs) and elastance (Ers), using operating software and assuming a single compartment model of the respiratory system. Respiratory input impedance was calculated from automated measurements made during a broadband low-frequency forced oscillation maneuver with a mixed frequency forcing function comprised of multiple prime frequencies ranging from 0.25 to 20 Hz. Operating software fit a constant phase model to the respiratory input impedance to determine Newtonian resistance (Rn), tissue damping (G) and tissue elastance (H). Parameters from individual data sets were included in the final analysis, provided that the coefficient of determination assessing the fit of the model to the experimental data was ≥0.95. Pressure–volume curves were produced by stepped increases and subsequent decreases in airway pressure. At each step, a pause of 1 s was conducted during which the plateau pressure and change in lung volume was measured. Static lung compliance (Cst) was calculated from the slope of each curve. The area (hysteresis) of the pressure–volume curve and a