Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

blood samples from virally infected individuals were genotyped. Blood was obtained from 424 U.S. patients hospitalized with severe COVID-19 and a much larger European cohort of 7147 healthy individuals and 2587 individuals with severe COVID-19. Deoxyribonucleic acid in both studies was probed for the F508del variant. In other experiments, we investigated the possibility that lack of CFTR might alter viral binding and propagation. We used human bronchial epithelial cell (HBEC) monolayers from individuals without functional CFTR for this purpose. Finally, we examined effects of CF airway secretions and features such as viscosity, pH, and protease/anti-protease imbalance during SARS-CoV-2 infection.

Results: We found no evidence of a relationship between deficient CFTR function (based on carrier status for the severe F508del defect) and clinical outcomes from COVID-19. In addition, viral propagation studies using airway epithelial monolayers (a model that reproduces many aspects of in vivo tissue biology) were not influenced by homozygous absence of CFTR. We show that levels of angiotensin converting enzyme-2 receptor messenger ribonucleic acid (mRNA) appear normal in CF primary epithelium, whereas transmembrane serine protease 2 mRNA is variable but lower ($p < 0.001$) in a manner that correlates with viral infectivity ($R^2 = 0.76$). Dependence of viral proliferation on features of CF mucosal fluid—including pH (viral replication optimum at pH 7–7.5), viscosity (diminished propagation in highly viscous apical media), and protease/anti-protease imbalance were identified as likely contributors to efficiency of SARS-CoV-2 replication and pathogenesis.

Conclusions: These findings using patient data, CF and non-CF primary airway epithelia, and CF airway secretions fail to demonstrate a causal relationship between loss of CFTR and susceptibility to severe COVID-19. Notwithstanding the caveat that addition of virus in small buffer volumes disrupts airway surface liquid depth and composition, our findings also argue against a role for CFTR during acute infection of airway cells in vitro. On the other hand, chronic disruption of periciliary liquid, diminished pH, altered protease/anti-protease homeostasis, and increased fluid viscosity (sequelae that occur in CF lungs) were implicated as contributors to impaired SARS-CoV-2 propagation. Such studies provide a basis for future work to test relationships between CFTR and severity of COVID-19.

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Association between myeloperoxidase activity and methionine oxidation products in bronchoalveolar lavage and risk of bronchiectasis in infants and toddlers with cystic fibrosis

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Background: Lung inflammation characterized by exuberant neutrophil influx and high concentrations of myeloperoxidase (MPO) occurs shortly after birth in cystic fibrosis (CF). MPO is the only enzyme in humans that produces hypochlorous acid (HOCl), a strong and promiscuous oxidant, under physiological conditions. We previously associated methionine sulfoxide (MetO), a HOCl byproduct, with MPO, neutrophils, and bronchiectasis using bronchoalveolar lavage fluid (BALF) in a cross-sectional study of early CF. Here, we sought to determine the relationship between MPO and oxidized metabolites and risk of bronchiectasis development in people with CF across a range of ages.

Methods: BALF was prospectively collected from people with CF younger than 6 who developed bronchiectasis at 9 years ($n = 14$) and matched people with CF who did not have evidence of bronchiectasis at 9 years ($n = 14$). MPO enzymatic activity was measured using immunocapture and Amplex Red oxidation assay. Methionine oxidation products were detected using high-resolution mass spectrometry via Q Exactive High Field operated in positive and negative modes after separation using a HILICON iHILIC-(P) Classic high-performance liquid chromatography column. We analyzed Spearman correlations of metabolites with MPO, subsetting for aged 0 to 2 and 3 to 6 and averaging repeated measures.

Results: MPO activity was significantly greater in CF children who developed bronchiectasis than in those who did not (mixed effects $p < 0.001$). MetO and dehydromethionine (dhMet), another methionine oxidation product of HOCl, were significantly higher in BALF of CF children who developed bronchiectasis than in those who did not (mixed effects $p < 0.001$). dhMet, but not MetO, was also significantly correlated with MPO activity in children younger than 2 (Spearman $r = 0.6031$, $p = 0.001$) and aged 3 to –6 (Spearman $r = 0.7351$, $p < 0.001$), without subsetting for future bronchiectasis development. Subsetting for bronchiectasis development revealed correlation between MPO and dhMet in 3- to 6-year-olds who developed bronchiectasis ($r = 0.600$, $p = 0.02$).

Conclusions: MPO is active in the airways of CF infants and toddlers and is higher in patients who develop bronchiectasis later in life. Methionine oxidation products MetO and dhMet, which are linked to MPO activity via HOCl, are associated with future bronchiectasis development. These data extend prior observations with MetO to dhMet and reveal its association with interindividual variations in MPO activity. Further study of these pathways may reveal strategies for treating, preventing, and monitoring inflammation in CF.

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Preinfection knockdown of Muc5b reduces severity of chronic *Pseudomonas aeruginosa* infection in the cystic fibrosis transmembrane conductance regulator knockout rat

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Background: One of the major gel-forming mucins in mucus is Muc5b, which is secreted from submucosal glands in strands and bundles to clear bacteria and other debris from the airway. In cystic fibrosis (CF), these mucins are overexpressed by hypersecreting cells and glands, contributing to mucus plugging and preventing normal clearance of pathogens such as *Pseudomonas aeruginosa*. Our *Cftr*$^{-/-}$ (CFTR knockout [KO]) rat model exhibits submucosal glands that, when mature, lead to development of a mucus defect that recapitulates the human CF lung environment. Data from our lab show that this mucus defect, apparent at 6 months of age in the KO rat model, is correlated with chronic infection with *P. aeruginosa*. Using our novel rat model, we want to assess the effect of altering mucin secretion into the airways on acute and chronic *P. aeruginosa* infection outcomes.

Methods: CFTR KO rats aged 6 months and older received two doses of 20 µg/300 µL Muc5b small interfering ribonucleic acid (siRNA) or scramble siRNA via intratracheal inoculation with 48 hours between treatments. Rats were then intratracheally inoculated with 10^6 colony forming units (CFUs) of the *P. aeruginosa* mucoid clinical isolate PAM57-15 48 hours after last treatment and euthanized 3 or 14 days after infection. Muc5b concentration in the bronchoalveolar lavage fluid (BALF) was determined using dot blot. Inflammatory cells in the BALF were quantified using Diff-Quik staining. Enzyme-linked immunosorbent assays were used to assess levels of important cytokines in BALF. Bacterial burden was assessed by homogenizing and plating lung tissue. Lung tissue was prepared for routine histopathology.

Results: There was no difference in infection outcomes between the treatment groups 3 days after infection. At 14 days after infection, Muc5b concentrations were significantly lower after Muc5b siRNA treatment than in the control. Although there was no difference in macrophage percentages in the BALF between the Muc5b siRNA and control at 14 days after infection, the percentage of neutrophils was significantly lower in the Muc5b siRNA treatment group, indicating less inflammatory cell influx. By 14 days after infection, CFUs in the lung were significantly lower in the Muc5b siRNA–treated group. Histopathological studies of lung and tracheal tissue using Alcian blue periodic acid-Schiff staining at 14 days after infection show reduced plugging of submucosal glands in the

tracheae and less mucus accumulation in the small airways of Muc5b siRNA–treated rats.
Conclusions: siRNA knockdown of Muc5b in CFTR KO rats aged 6 months and older alters development of chronic *P. aeruginosa* infection, reducing bacterial burden, mucus plugs, and inflammation. Additionally, interleukin (IL)-1β levels are lower in the BALF of chronically infected rats treated with Muc5b siRNA, indicating that knockdown of Muc5b before bacterial exposure appears to target the vicious muco-inflammatory cycle between IL-1β and mucin secretion. Muc5b is crucial for development of chronic *P. aeruginosa* infection in the CFTR KO rat.

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Rapid viscoelastic characterization of airway mucus using a benchtop rheometer

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Background: In muco-obstructive lung diseases (e.g., cystic fibrosis (CF), asthma, chronic obstructive pulmonary disease) and other respiratory conditions (e.g., viral/bacterial infections), biophysical properties of mucus are altered by goblet cell hypersecretion, airway dehydration, pH alteration, and inflammation that elicits oxidative stress and release of extracellular deoxyribonucleic acid. Previous studies showed that sputum viscoelasticity correlated with pulmonary function. Treatments affecting sputum rheology (e.g., rhDNase in CF) resulted in remarkable clinical benefits. In general, rheological measurements of non-Newtonian fluids rely on elaborate, time-consuming approaches (e.g., parallel/cone-plate rheometers, microbead particle tracking) that require extensive training to perform the assay and interpret the data. This study tested the reliability, reproducibility, and sensitivity of Rheomuco (Rheonova, Saint-Martin-d'Hères, France), a user-friendly benchtop device that is designed to rapidly measure the viscoelasticity of mucus and sputum in a fast-paced clinical setting. The straightforward protocol and standardized calibration were developed to generate accurate viscoelastic measurements of mucus samples by non-rheology specialists.
Methods: Rheomuco operates by measuring torque and displacement after oscillations with controlled angular displacement to produce a standardized strain sweep curve and establish a linear viscoelastic region. The dynamic oscillation with a shear-strain sweep provides linear viscoelastic moduli (G', G'', G^*, tan δ) and gel point characteristics (γ_c and σ_c) for clinical samples within 5 minutes. Device performance was validated using various concentrations of a mucus simulant, 8 MDa polyethylene oxide (PEO) against a traditional bulk rheometer (DHR-3, TA Instruments). A clinical isolate harvested from an intubated patient with status asthmaticus was assessed in triplicate measurements, as well as the effects of a potent mucus reducing agent, tris (2-carboxylethyl) phosphine hydrochloride (TCEP).
Results: PEO solutions were viscous-dominated ($G'' > G'$) and values were comparable between Rheomuco and the traditional DHR-3 rheometer. Triplicate measurements performed on 1.5% PEO solution and asthmatic mucus confirmed that linear viscoelastic characteristics (G^*, G', and G'') were repeatable, with less than 10% coefficient of variation for the biological sample TCEP treatment (final concentration of 20 μM for 20 minutes at 37°C) of asthmatic mucus resulted in a decrease in elastic modulus from 8.62 Pa to 1.67 Pa and a change toward a more "liquid-like" behavior overall (e.g., higher tan δ).
Conclusions: These results demonstrate that Rheomuco measures the viscoelasticity of mucus samples rapidly and reliably and could therefore be used to explore the effects of approved mucolytic drugs (e.g., rhDNase, N-acetyl cysteine) in clinics to adapt treatment on a case-by-case basis or in laboratory preclinical studies to test the efficacy of novel mucoactive compounds.

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Toxicology of ELD607, a novel immunomodulator of inflammatory responses in cystic fibrosis lungs

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Background: Management of airway inflammation is a vital aspect of cystic fibrosis (CF) treatment, but beyond ibuprofen, there are no approved anti-inflammatory drugs to treat people with CF. Orai1 is a plasma membrane Ca^{2+} channel that regulates inflammation by controlling gene expression and cytokine secretion. We have generated ELD607, a fully optimized Orai1 inhibitory peptide that is CF transmembrane conductance regulator (CFTR)-mutation agnostic and proteolytically stable and has a half maximal inhibitory concentration (IC_{50}) of 9 nM. ELD607 inhibits Orai1 in the lungs of neutrophilic mouse models to reduce pulmonary inflammatory responses. Although we have shown efficacy of ELD607, toxicological effects must be determined before it can be advanced as a therapeutic. We assess the safety and toxicology of ELD607.
Methods: To determine potential cytotoxicity, HEK293T and human primary bronchial epithelial cells were incubated with 100 μM ELD607 (approximately 10 000 times the IC_{50}) for 24 hours, and cell viability was measured using calcein-AM. The human ether-à-go-go-related gene (hERG) K^+ channel plays a vital role in cardiac repolarization, and inhibition of hERG is fatal. hERG activity in response to 100 μM ELD607 was measured using a patch clamp assay; 100 μM ELD607 was also screened against the Eurofins Safety 44 Panel. We next switched to an in vivo model to evaluate the safety of ELD607. C57Bl/6J mice were dosed with 10 mg/kg ELD607 (20 times the predicted therapeutic dose) intratracheally for 7 days. To determine the location of inhaled ELD607, mice were dosed with 1.0 mg/kg of ELD607-labeled Tamra intratracheally, and the lung was excised 1 hour later. Whole lungs were fixed and imaged using light sheet microscopy. To analyze the pharmacokinetics of ELD607, mice were exposed to 0.5 mg/kg of ELD607 intratracheally. Bronchoalveolar lavage fluid (BALF) and blood were collected at timed intervals after inhalation, and samples were measured for ELD607 levels using high-performance liquid chromatography (HPLC). We are currently dosing nonhuman primates with ELD607 because nonhuman primate airways better mimic human airways.
Results: There was no change in cell viability when exposed to ELD607 and no change in the transepithelial electrical resistance, indicating that ELD607 does not change epithelial integrity and permeability. ELD607 did not have any effect on hERG activity and did not bind to any of the targets. After a 7-day dosing, mouse weight did not change, and there were no histopathological changes in the lungs, heart, liver, or kidneys. Light sheet microscopy revealed that ELD607-Tamra was found in the airway lumen (from the main bronchi to the alveoli) but was not detected outside of the airways. HPLC analysis detected low ELD607 levels in the BALF, with 20% of the initial dose found at 10 minutes, declining to 10% 2 hours after dosing. Extremely low ELD607 levels were found in the blood (0.2% of initial dose) throughout the timed-interval collections. Results from the nonhuman primate studies will be discussed at the time of presentation.
Conclusions: ELD607 has no significant toxic effects in vitro or in vivo in small animal models and may serve as a safe, novel therapeutic for anti-inflammatory effects in people with CF.
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