exacerbations, *Pseudomonas*-positive sputum cultures, and mild bronchiectasis. There are multiple mechanistic possibilities that could link CA12 to CF-like phenotypes, such as extracellular pH regulation. In this study, it is hypothesized that CA12 deficiency will mimic cellular CF phenotypes such as intracellular transport. We have recently shown that CA12 deficiency in mouse cells contributes to the CF phenotypes, supporting the role of bicarbonate regulation as a key modulator of cellular function [2].

**Methods:** We created a CA12 knockout cell line (KO) and its control cell line (Ctrl) based on s H9TEo- human tracheal epithelial cell line using CRISPR-Cas9 technique. Intracellular transport was monitored using filipin staining to follow cholesterol trafficking and rab7 immunostaining to monitor endosomal movement directly.

**Results:** Our previous studies have demonstrated that CF cells have high perinuclear cholesterol accumulation, low microtubule (MT) reformation rate, and limited endosomal distribution. We examined the above phenotypes of KO and Ctrl cells. More than 100 cells were quantified for each group in each experiment method. We first examined cholesterol distribution using filipin staining. The percentage of cells with cholesterol accumulation was much higher in KO (73.6 ± 7.3%) than Ctrl (21.4 ± 5.9%) (p < 0.0001) cells. Next, we examined MT reformation by depolymerizing MTs in the cells and observing the reformation of MTs at different time points. At 2 and 4 minutes, respectively, percentages of cells with MT aster were 17.4 ± 5.3% and 49.0 ± 16.9% for KO and 38.1 ± 8% and 66.3 ± 11.9% for Ctrl. MT reformation of KO was significantly slower than that of Ctrl at 2 minutes (p < 0.0001) and 4 minutes (p < 0.05). Then, we examined the endosomal distribution in these cells using Rab7 immunocytochemistry. Mean percentage of Rab7-covered nuclear circumsferences was 53.2% for KO and 89.6% for Ctrl. All these data demonstrated that the characteristics of Ctrl were similar to those of WT H9TEo-, and KO showed CF-like properties. Last, we tested treatment efficacies of three CF-effective drugs: 8-CPT-2Me—cyclic adenosine monophosphate, 5-aminomimidazole-4-carboxa-
mide ribonucleotide, and tubastatin. After 24 hours of treatment, all three drugs slightly decreased cholesterol accumulation and increased endosomal distribution of KO cells, although they were still significantly different from Ctrl cells, which suggested that these drugs improved intracellular transport in KO but could not fully recover the damage caused by CA12 KO.

**Conclusions:** These findings show that depletion of CA12 expression mimics CF cellular phenotypes without affecting CTRF function. These data point to mechanisms key to CF cellular phenotypes and could explain some of the CF clinical similarities in patients with CA12 mutations.

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**References**


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**Mechanisms of cytokine-mediated polymerization and secretion of Muc5ac and Muc5b**

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**Background:** Mucociliary clearance (MCC) is required for respiratory defense. In healthy people, mucus is thin and easily transported by MCC. In people with cystic fibrosis (CF), mucus is thick and poorly transported. Key pulmonary manifestations of CF are MCC impairment, mucus aggregation, and airway inflammation. Mucus dysfunction is not adequately treated with existing therapies. It is thus essential to improve our mechanistic understanding of mucin biology. Two polymeric mucins, MUC5AC and MUC5B, form the matrix of airway mucus and are critical determinants of understanding of mucin biology. Two polymeric mucins, MUC5AC and MUC5B, form the matrix of airway mucus and are critical determinants of understanding of mucin biology.

**Methods:** We created a CA12 knockout cell line (KO) and its control cell line (Ctrl) based on s H9TEo- human tracheal epithelial cell line using CRISPR-Cas9 technique. Intracellular transport was monitored using filipin staining to follow cholesterol trafficking and rab7 immunostaining to monitor endosomal movement directly.

**Results:** Our previous studies have demonstrated that CF cells have high perinuclear cholesterol accumulation, low microtubule (MT) reformation rate, and limited endosomal distribution. We examined the above phenotypes of KO and Ctrl cells. More than 100 cells were quantified for each group in each experiment method. We first examined cholesterol distribution using filipin staining. The percentage of cells with cholesterol accumulation was much higher in KO (73.6 ± 7.3%) than Ctrl (21.4 ± 5.9%) (p < 0.0001) cells. Next, we examined MT reformation by depolymerizing MTs in the cells and observing the reformation of MTs at different time points. At 2 and 4 minutes, respectively, percentages of cells with MT aster were 17.4 ± 5.3% and 49.0 ± 16.9% for KO and 38.1 ± 8% and 66.3 ± 11.9% for Ctrl. MT reformation of KO was significantly slower than that of Ctrl at 2 minutes (p < 0.0001) and 4 minutes (p < 0.05). Then, we examined the endosomal distribution in these cells using Rab7 immunocytochemistry. Mean percentage of Rab7-covered nuclear circumsferences was 53.2% for KO and 89.6% for Ctrl. All these data demonstrated that the characteristics of Ctrl were similar to those of WT H9TEo-, and KO showed CF-like properties. Last, we tested treatment efficacies of three CF-effective drugs: 8-CPT-2Me—cyclic adenosine monophosphate, 5-aminomimidazole-4-carboxa-
mide ribonucleotide, and tubastatin. After 24 hours of treatment, all three drugs slightly decreased cholesterol accumulation and increased endosomal distribution of KO cells, although they were still significantly different from Ctrl cells, which suggested that these drugs improved intracellular transport in KO but could not fully recover the damage caused by CA12 KO.

**Conclusions:** These findings show that depletion of CA12 expression mimics CF cellular phenotypes without affecting CTRF function. These data point to mechanisms key to CF cellular phenotypes and could explain some of the CF clinical similarities in patients with CA12 mutations.

**Acknowledgements:** Supported by the Cystic Fibrosis Foundation.

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**ELD607 inhibits excessive proinflammatory Ca2+ signaling in cystic fibrosis neutrophils**

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**Background:** The cystic fibrosis (CF) lung is subject to chronic neutrophilic inflammation that is disproportionate to bacterial load and is poorly resolving. Exuberant inflammatory responses contribute significantly to the pathogenesis of CF lung disease by promoting mucus hypersecretion and tissue remodeling, as well as causing collateral damage to host tissues. Many anti-inflammatory drugs are unsuitable for management of CF airway inflammation because of their immunosuppressive effects. Thus, there is an urgent and unmet need to develop anti-inflammatory therapeutics. Store-operated Ca2+ entry (SOCE) is mediated by the plasma membrane Ca2+ channel, Orai1. SOCE plays a central role in neutrophil activation and regulates degranulation, reactive oxygen species production, and release of neutrophil extracellular traps. Ca2+ signaling has been reported to be dysregulated in CF neutrophils, which may contribute to the hyperinflammatory phenotype of the CF airway. We have developed a novel, fully optimized, peptide-based Orai1 inhibitor called ELD607 that we hypothesize can be used to reduce neutrophilic inflammation in CF lungs without immunosuppressive effects.

**Methods:** To investigate the effects of ELD607 on neutrophil Ca2+ signaling, neutrophils were isolated from the blood of people with and without CF using immunomagnetic negative selection and treated for 3 hours with 30 mM ELD607, scrambled peptide control, or vehicle. To measure Ca2+...
signaling, neutrophils were loaded with the Ca\(^{2+}\)-sensitive fluorescent dye fluo-4, and fluorescence was measured in response to stimulation with 1 mM thapsigargin using a Tecan plate reader.

**Results:** Neutrophils were collected from four people without CF and 12 people with CF with a range of CF transmembrane conductance regulator mutations including F508del, L467P, 4209TGTT >A, G551D, and N1303K. Eleven of the 12 were undergoing elexacaftor/tezacaftor/ivacaftor treatment at the time of collection. In non-CF neutrophils, thapsigargin induced a 39 ± 5% increase in cytosolic Ca\(^{2+}\) concentration over 10 minutes. CF neutrophils displayed a 53 ± 6% increase in cytosolic Ca\(^{2+}\) concentration. The rate of increase in cytosolic Ca\(^{2+}\) concentration and area under the curve (AUC) over 10 minutes were significantly greater in CF neutrophils than non-CF controls (\(p < 0.01\) and \(p = 0.04\), respectively), suggesting dysregulated Ca\(^{2+}\) signaling. ELD607, but not scrambled peptide exposure, reduced AUC by 30% (\(p < 0.01\)) in CF neutrophils. Non-CF neutrophils displayed a similar (29%) reduction in AUC that was not statistically significant, probably because of the small sample size.

**Conclusions:** Collectively, these data indicate that CF neutrophils display exaggerated Ca\(^{2+}\) signaling in response to thapsigargin that can be inhibited by ELD607. We predict that ELD607 treatment will benefit people with CF by rebalancing Ca\(^{2+}\) signaling in CF neutrophils and reducing inflammation.

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**384 Impact of elexacaftor/tezacaftor/ivacaftor therapy on pathological reprogramming of lung-recruited neutrophils conditioned by cystic fibrosis airway fluid**

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**Background:** Cystic fibrosis (CF) is caused by mutations of the CF transmembrane conductance regulator (CFTR) on COVID-19 severity, independent sets of

**Methods:** We previously showed that GRIM neutrophils can be mass produced in an in vitro model [1] in which human blood neutrophils are differentiated into a differentiated epithelial layer into CF airway supernatant (CFASN) purified from sputum of treatment-naive patients. Here, we generated and tested the effect of airway supernatant from sputum of patients on ELX/TEZ/IVA modulator therapy (CFMOD) for comparison based on phenotyping (“flow cytometry”), functional (“P. aeruginosa killing”), and metabolic (“\(^{13}\)C-glucose tracing”) analyses of transmigrated neutrophils.

**Results:** CFMOD-recruited neutrophils contained IVA, demonstrating exposure to the modulator (Figure 1). CFASN/CFMOD-recruited neutrophils showed greater primary granule exocytosis; less P. aeruginosa killing; and greater adenosine monophosphate, guanosine monophosphate, and metabolites related to citric acid cycle activity than neutrophils recruited to a control chemoattractant (leukotriene B4). \(^{13}\)C-glucose flux data showed similar rates of extracellular glucose use in all transmigration conditions but higher total glycolysis to lactate in CFASN/CFMOD-transmigrated neutrophils.

**Figure 1.** Ivacaftor, one of the modulator drugs, is measured in the cystic fibrosis modulator therapy (CFMOD)-recruited neutrophils but not in the CF airway supernatant (CFASN), LTB4 (chemoattractant control), or blood neutrophils. Excerpt from our pilot metabolomics data on lung-recruited neutrophils showing intracellular metabolites. One-way repeated-measures analysis of variance (<0.05).

**Conclusions:** Pathological reprogramming of neutrophils upon transmigration into CF airway fluid is not substantially altered by use of ELX/TEZ/IVA modulator therapy. Ongoing transcriptional (ribonucleic acid-Seq) and epigenetic (deoxyribonucleic acid methylation profiling) studies will bring further understanding of this process and suggest potential targets for immunomodulatory therapy to be used as potential adjunct treatment to modulator therapy.

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**References**


**385 Mechanisms by which cystic fibrosis transmembrane conductance regulator may influence SARS-CoV-2 infection**

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**Background:** People with cystic fibrosis (PwCF) have chronic, pronounced respiratory damage and have been considered among those at highest risk for serious harm from SARS-CoV-2. Numerous clinical studies have reported that individuals with CF in North America and Europe, although highly susceptible to COVID-19, do not have mortality levels that exceed those of the general population.

**Methods:** To understand features that might influence lethality of COVID-19 in PwCF, we tested potential relationships between CFTR and viral pathogenesis. As one approach to evaluate impact of CF transmembrane conductance regulator (CFTR) on COVID-19 severity, independent sets of