Objectives: Airway epithelial cells (AEC) from CF lungs respond to viral infection with poor innate immune responses. The contribution of defective CF transmembrane conductance regulator (CFTR) channel function in these responses is not known. Here, we investigated whether CFTR correcting drugs could improve responses by CF AEC to human rhinovirus (HRV).
Methods: Primary AEC were obtained from 11 children with CF (five Class II, six Class III mutations) and six healthy, non-CF controls by airway brushing. Primary cultures were expanded by conditional reprogramming before infection with HRV1b (multiplicity of infection: 12.5) for 24 hrs. To compare the effect of CFTR modulation on anti-viral responses, AEC pre-treated for 72 hrs with lumacaftor and/or ivacaftor and then infected with virus were compared with AEC infected with virus alone. Supernatant was assessed for interferons (IFNβ, IFNλ_1 and IFNλ_2) and inflammatory cytokines (IL-6, IL-8, RANTES) by ELISA.
Results: In non-CF AEC, HRV1b infection resulted in significantly increased IL-8 and RANTES ($p < 0.05$), but not IL-6, IFNβ, IFNλ_1 or IFNλ_2. In addition, responses did not significantly change in the presence of CFTR modulators. In contrast, AEC of both CF genotypes generated IL-8 and IL-6 responses to HRV1b ($p < 0.05$). With CFTR modulator treatment however, IL-6 production post infection was not significantly elevated. Combined CFTR therapy for Class II CF was also found to reduce RANTES production ($p < 0.05$). IFN responses by CF AEC to HRV1b infection were mixed, with minimal levels detected following infection except for IFNλ_1, which was significantly elevated ($p < 0.05$). However, this was no longer significant after CFTR modulator therapy for both Class II and Class III ($p < 0.05$).
Conclusion: CFTR therapies were found to reduce some inflammatory responses by CF AEC to HRV1b, but also modulate IFNλ_1. We are currently analysing RNAseq data to compare innate immune gene networks between treatments and phenotypes.

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Analysis of the airway surface liquid pH in pediatric patients. Pilot study

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Objectives: CFTR is a complex ABC protein that acts as a chloride channel and regulates the function of other membrane channels. The mutation of the gene produces a missing or dysfunctioning CFTR protein, which leads to an electrolytic disbalance in the apical membrane of the epithelial cells, giving an ionic alteration: hyposecretion of chloride and bicarbonate with sodium hyperabsorption.
There is experimental evidence that CFTR, in addition to being a cAMP-dependent chloride channel, may have other functions, for example the transport of bicarbonate and the regulation of other endogenous channels of chloride and calcium.
Some studies have proposed that if the acidic pH of the airway liquid surface (ASL) decreases its bactericidal capacity and increases the viscosity of mucus, increasing this pH, bacterial infections of the airway could be diminished.
The aim of this study was to analyze the pH of the ASL of 6 pediatric patients with a cystic fibrosis (CF) diagnosis and 2 non-CF pediatric patients.
Methods: Prospective, cross-sectional, descriptive study developed in 8 pediatric patients attended at the Pediatric Allergy and Pulmonology Unit of the Sabadell's hospital.
Induced sputum samples were collected in 8 patients (6 CF and 2 non-CF) between 6 and 16 years. The pH of the ASL was analyzed by pHmetry. Patients were randomly selected according to the clinical therapeutic needs of performing the induced sputum in each of them.
Results: From the CF population, a sample of 6 patients was obtained; the mean pH in the ASL was 6,746. From the non-CF, a sample of 2 patients (Dx ciliary dyskinesia) was obtained, whose average pH in ASL was 6.83.
Conclusion: In CF pediatric patients, the pH of the ASL could be acidic due to the defect in CFTR activity.
We recommend extending the study to a more significant sample.

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Nasal potential difference in βENaC-overexpressing mouse reveals pH-sensitive channel hyperactivity and shift of subunits stoichiometry

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Objectives: To characterize transepithelial nasal potential difference in β-ENaC overexpressing mouse model engineered to mimic CF airway disease.
Methods: We used transepithelial potential difference (PD) to measure ion transport across the nasal mucosa of wild-type and *scnn1b-Tg* heterozygous (Tg/+) mice. The following parameters were recorded: (1) the most negative basal PD value; (2) change in PD after nasal perfusion with buffered Ringer's solution at pH 7.4 but no change with pH 6.5; (3) decrease in PD after perfusion with Ringer's solution containing 10^{-4} M amiloride; (4) subsequent changes in PD after perfusion with Ringer's solution in which Cl^- was replaced by gluconate, and amiloride and 10^{-5} M forskolin were added. To test the hypothesis that elevated Na^+ transport in Tg/+ mouse airways is due to an increased number of αβγENaC channels or a possible change in subunits stoichiometry, we analyzed ENaC subunit transcripts in mouse nasal epithelium and in nasal epithelial cells in 3D primary cultures.
Results: More polarized basal PD values were recorded in Tg/+ than in wild-type mice. In Tg/+, but not in wild-type, perfusion of Ringer's-pH 7.4 rapidly induced a small depolarization (+4mV) that was prevented by adjusting the pH to 6.5. The response to amiloride was significantly increased (+50%) in Tg/+ compared to wild-type mice. Perfusion of chloride-free containing forskolin induced a hyperpolarization of similar magnitude in Tg/+ and in wild-type mice. Both α and βENaC transcript levels were elevated in nasal cells derived from Tg/+ mice with no significant change in γ-subunit expression.
Conclusion: Our data indicate that the CFTR-dependent chloride conductance is not affected by ENaC hyperactivity in mice. We showed here a pH-sensitive hyperactivity of ENaC in nasal epithelium of *scnn1b-Tg* mice possibly associated with a switch in subunit stoichiometry of the ENaC channel. However, the possible role of inflammation should also be considered.

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Mitochondrial dysfunction in cystic fibrosis

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Background: Cystic fibrosis (CF) is a chronic, progressive disease, caused by a mutation of the gene encoding for the CF Transmembrane Conductance Regulator (CFTR). Animal and cell studies have identified consequences of CFTR dysfunction, including reduced mitochondrial complex activity independent of chloride channel function. It is likely that mitochondrial complex I and complex IV are defective in cystic fibrosis patients, however this has not been proven in vivo. CFTR dysfunction has been linked to an increase in reactive oxygen species which alters the cellular redox status, may trigger apoptotic events and produce inflammatory responses that may affect innate immunity.
Objectives: To compare oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in CF patients and control subjects. To use OCR is an indicator of mitochondrial respiration and extracellular acidification rate (ECAR) as an indicator of glycolysis.
Methods: Seven control and 6 homozygous F508del CF patients were recruited to donate one blood sample. Using density gradient separation, neutrophils were removed from residual PBMCs, oxygen consumption and glycolysis were measured using the Seahorse XF analyser by the addition of various inhibitors of the oxidative phosphorylation pathway in the PBMCs of each cell population. This provided measures of cellular activity including basal cell respiration, maximal oxygen consumption, non-mitochondrial cellular respiration and extracellular acidification rate.
Results: Results indicate control subjects have higher stimulated maximal oxygen consumption compared with CF subjects by 24% ($p < 0.1$). Basal and