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Modulation of airway epithelial ion transport by allergic airway inflammation in mice

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The airway epithelium is a central effector tissue in allergic inflammation, and Th2-driven epithelial responses such as mucus hypersecretion and airway remodeling contribute to airflow obstruction in allergic airway disease. The Th2 cytokines Interleukin-4 (IL-4) and 13 (IL-13), through the signal transducer and activator of transcription 6 (Stat6), are leading molecules in this allergic response. Previous studies in human bronchial epithelial cells demonstrated that IL-4 and IL-13 also act as potent modulators of epithelial ion transport and airway surface liquid *in vitro*, by inhibiting the ENaC-dependent Na^+ absorption and enhancing the Cl^- secretion, mediated by CFTR and CaCC. However, the *in vivo* link between allergic inflammation and airway ion transport has not been studied. Therefore, the aim of this study was to induce allergic airway disease in mice and determine the effect of allergic airway inflammation on epithelial ion transport along the tracheobronchial tree.

Using two well established asthma models, we generated mice with acute (OVA model) and chronic allergic airway disease [*Aspergillus fumigatus* (Af) model] and determined first the effects of allergic airway inflammation on ion transport in native tracheal tissues in two different mouse strains (BALB/c and C57BL/6). Considering that differences in tracheal ion transport were detected only in the Af model, and were similar between the two strains, we proceeded with this model for further studies in BALB/c mice. The next goal was to study bioelectric properties of different airway regions along the tracheobronchial tree, in the

presence of allergic inflammation. Therefore, we assessed the effects of Af sensitization on bronchial ion transport, as well as on mRNA expression of the ion channels in pulmonary level. In order to elucidate mechanisms entailed in this ion transport regulation, we performed the same experiments using Stat6-deficient BALB/c mice, reported to be protected from the defects caused by allergic airway inflammation. To test whether Th2 cytokines were directly involved in this regulatory procedure, as shown in the *in vitro* studies, we finally induced allergic airway inflammation in mice using Il-13, and assessed ion transport properties in tracheal and bronchial tissues.

We demonstrate that Af-induced chronic allergic inflammation enhanced basal Cl^- secretion in tracheae and bronchi. In bronchi, additionally, it inhibited ENaC-mediated Na^+ absorption and increased Cl^- secretion mediated by Ca^{2+}-activated Cl^- channels (CaCC). These changes in airway ion transport were associated with reduced pulmonary expression of α-, β-, and γENaC, while transcript levels of Cftr and the CaCC candidate Tmem16a remained unchanged. Allergen-induced effects on ENaC function and expression, and on CaCC-mediated, but not basal Cl^- secretion, were completely abrogated in Stat6-deficient mice. Interestingly, different ion transport properties were also detected between tracheae and bronchi of the same mice.

Our studies demonstrate for the first time that Th2-dependent airway inflammation produced a prosecretory ion transport phenotype *in vivo* and that this epithelial response was largely mediated by Stat6. These results suggest that Th2-mediated fluid secretion may improve airway surface hydration and promote clearance of mucus that is hypersecreted in allergic airway diseases such as asthma. Further, epithelial Stat6 signaling is identified as a possible target for therapeutic intervention in airway diseases with reduced mucus hydration.