Vaping Away Epithelial Integrity

Lung epithelial cells are the first line of defense against pathogens, chemicals, and xenobiotics. With each breath, we inhale a plethora of foreign antigens that may elicit a host immune response depending on the type of antigen or immunogen exposure, genetic predisposition, and structural integrity of lung epithelial cells. The asserted response also depends on the dose and chronicity of the exposure. The cellular complexity of the airways and alveoli is astounding and includes ~50 different cell types, ~12 of which are epithelial (1). Almost half of these epithelial cells are ciliated (pseudostratified columnar and cuboidal), with the remainder being comprised of goblet cells, basal cells, club cells, and, at the terminal alveolus, type I and type II alveolar epithelial cells (2). Together, these cells play a large and complex role in the host immune responses to constant bombardment from foreign insults (inhaled or aspirated).

Epithelial injury initiates a variety of lung diseases, which may occur due to multiple factors. Cigarette smoke is a major risk factor, particularly in chronic obstructive pulmonary disease, asthma, and idiopathic pulmonary fibrosis. It is the principal preventable cause of death and disease in the United States (3) and possibly worldwide. With the growth in the use of e-cigarettes, also known as vaping, a current perception is that these tobacco alternatives are safer than cigarettes. This concept has rapidly led to an explosion in usage, especially among teens, who are attracted by the appealing flavors and ease of use. However, the field lacks understanding of the mechanisms related to the deleterious effects of e-cigarette vapor.

In this issue of the *Journal*, Lin and colleagues (pp. 162–173) report on an investigation of the effects of e-cigarette vapor on CFTR (cystic fibrosis transmembrane conductance regulator) in airway epithelial cells, and they describe a previously unknown dose-dependent inhibitory effect of e-cigarette vapor on chloride anion transport by CFTR (4). Cigarette smoke induces defects in CFTR function (5), but the effects of e-cigarettes were unknown. Acquired dysfunction in CFTR resulting in impaired mucociliary transport and clearance has previously been noted in patients with chronic obstructive pulmonary disease, and especially in patients with chronic bronchitis (6, 7). Lin and colleagues establish that the pyrolysis product of e-cigarettes, acrolein, reduces the short-circuit chloride current without affecting cell survival. Toxic effects of e-cigarettes on airway epithelial cells, including a reduction in their viability, were previously reported (8), although the mechanisms remain elusive.

The authors also found that, unlike cigarette smoke, e-cigarette vapor reduces ion conductance. The precise cause of this exclusive effect is not understood, but vaporization appears to trigger the dysfunction. Lin and colleagues also showed that primary human bronchial epithelial cells from donors were more sensitive to e-cigarette vapor-induced inhibition of CFTR-dependent chloride transport than Calu-3 cells, perhaps due to lower baseline expression of CFTR in human bronchial epithelial cells. This finding is important as we consider different cell types and cell lines to study different diseases. The authors detected a reduction in epithelial sodium channel activity, which, in contrast to CFTR dysfunction, is reported with cigarette smoke as well. Because CFTR also transports bicarbonate anions in addition to chloride anions, the e-cigarette vapor-induced CFTR dysfunction might increase the pH on the apical surface of airway epithelial cells and thus affect their physiology. The authors ruled out any changes in the pH by checking the pH in basolateral media of cells exposed to e-cigarette vapor. With longer exposure (60 min), they observed a reduction in transepithelial electrical resistance with e-cigarette vapor, suggesting compromised barrier integrity. Nonetheless, the precise mechanism and probable junctional proteins involved remain to be investigated (Figure 1).

Because nicotine induces airway epithelial dysfunction by regulating CFTR function through nicotinic acetylcholine receptors (9), it likely has similar effects when inhaled as e-cigarette vapor. Like acrolein, nicotine has been associated with the formation of DNA adducts (10). Apart from DNA damage, it also reduces XPC and 8-oxoguanine DNA glycosylase 1/2 proteins, which are responsible for normal repair (11). The authors alluded to potential effects of nicotine on CFTR, but focused only on acrolein in this study. Likewise, other reactive aldehydes, reactive oxygen species (12), and heavy metals were not investigated in this study, although these agents may have a role in CFTR dysfunction in the airway epithelium (13). Acrolein has been shown to directly modify CFTR and inhibit channel gating (5). Considering that nicotine and acrolein form DNA adducts, it is plausible that they may directly or indirectly affect a variety of ion channels (Figure 1).

Previous studies have shown that e-cigarettes dampen the ability of airway epithelial cells to respond to viral infections, increase inflammation, and enhance pneumococcal adherence (14–16). However, the precise mechanisms are not well understood. E-cigarette products, with or without nicotine, have been shown to inhibit expression of SPLUNC1 (short palate, lung, and nasal epithelial clone 1), a molecule required for host defense against human rhinovirus (15). These studies support the findings of Lin and colleagues, and suggest that e-cigarettes may have far-reaching effects in addition to those of nicotine or acrolein alone. Furthermore, other components present in e-cigarette vapor need to be tested for their effect on airway epithelium. Broadly, the current study by Lin and colleagues focuses attention on the involvement of ion channels in loss of epithelial function and how this may

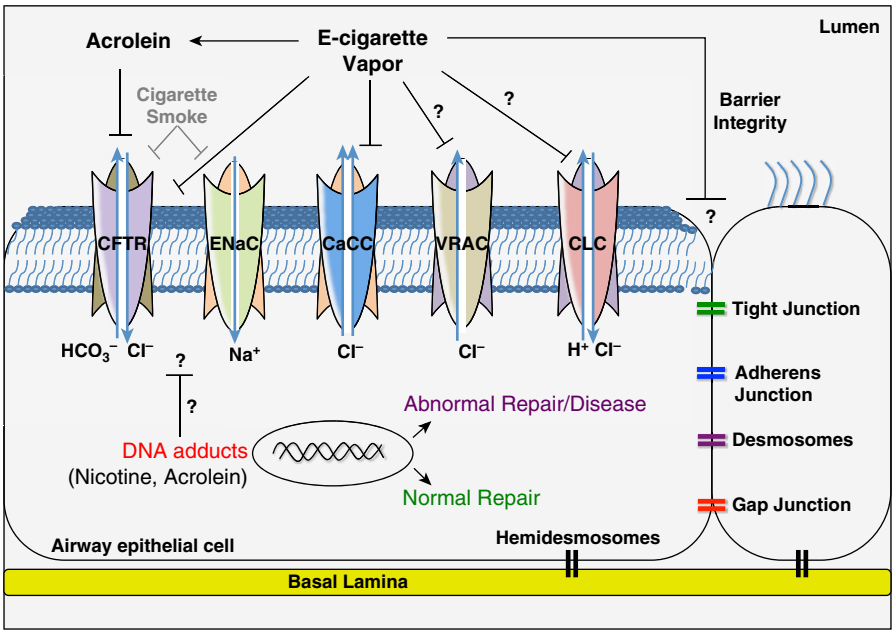

Figure 1. Potential mechanisms of e-cigarette vapor–induced damage to airway epithelium. CaCC = calcium-activated chloride channel; CFTR = cystic fibrosis transmembrane conductance regulator; CLC = chloride channel; ENaC = epithelial sodium channel; VRAC = volume-regulated anion channel.

lead to airway inflammation, infection, and disease predisposition.

Although the authors observed attenuation of chloride current by CFTR dysfunction, the question remains as to whether other chloride channels, such as CLC (17) and volume-regulated chloride channels, are also affected (Figure 1). Lin and colleagues used primary human bronchial epithelial cells from healthy donors to study the effects of e-cigarette vapor. Further studies examining the effects of e-cigarette vapor on epithelial cells derived from healthy versus diseased individuals might also provide information about the functional changes that occur in these cells after exposure to e-cigarette vapor. They may also address questions related to why some individuals do not develop lung disease even after continuous exposure to e-cigarette vapor or cigarette smoke, whereas others do. Moreover, incorporating animal studies will allow a fuller investigation of the *in vivo* effects of e-cigarette vapor on lung cells and facilitate the use of genetic manipulation of ion channels in airway epithelial cells that are affected by e-cigarette vapor, and hence provide comprehensive methods to tease out the precise mechanisms involved.

In summary, the present study provides novel insights into the mechanisms of airway epithelial dysfunction caused by e-cigarette vapor and tobacco smoke through ion channels, particularly CFTR. With evolving information about the chronic effects of e-cigarette vapor on airway epithelium (18), these insights will improve our understanding of how e-cigarettes affect cells and may help us devise targeted therapies against difficult and progressive lung diseases. ■

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