Vaping: Cell Damage at the Receiving ENDS

Electronic nicotine delivery systems (ENDS), better known as vaping devices or e-cigarettes, are increasingly popular and promoted by tobacco companies and others alike. Advertised as a less harmful alternative to conventional cigarettes, ENDS use developed into a new mainstream habit among U.S. youth over the past few years (1). A recent study of nearly 18,000 U.S. youths and adults confirmed the general suspicion that vaping is associated with a higher likelihood of smoking in youths (12–17) and young adults (18–24) (2). However, this report also found higher rates of smoking cessation in adults who had smoked and switched to e-cigarettes (2).

The general perception of safety of ENDS is not supported by any substantial scientific data. An editorial in *The Lancet* responding to a positive testimonial for e-cigarette use by the UK's House of Commons Science and Technology Committee report (in 2018) rightly stated, "It is naïve and premature of the committee to confuse an absence of evidence with an absence of harm" (3). Indeed, just last year we experienced a dramatic rise in acute lung injury rates in people who used ENDS (4), ringing alarm bells for the first time and alerting the public to the potential harm of e-cigarettes (5).

In this issue of the *Journal*, Serpa and colleagues (pp. 306–316) describe their intriguing work in which they thoroughly studied the effects of ENDS on the viability of cells of the respiratory tract (6). Using an elegant system of *in vitro* ENDS-aerosol delivery of the main e-cigarette constituent propylene glycol and glycerol, the authors studied the effects of exposure to different types of human and murine respiratory epithelial cells. The main finding was that exposure to ENDS aerosol led to substantial cell death from apoptosis or secondary necrosis. Interestingly, the addition of nicotine aggravated cell death rates, whereas nicotine alone had no effect on epithelial cell viability. These data confirm and extend earlier reports that documented the danger of individual components of vaping solvents on primary bronchial cells or bronchial cell lines, showing enhanced oxidative stress and cell damage after prolonged exposure (7, 8).

To more closely mimic the physiological situation of barrier tissues, Serpa and colleagues went on to investigate the transepithelial resistance of a human respiratory epithelial cell monolayer and found a pronounced decrease after only 4 minutes of daily ENDS aerosol exposure over 1 week, suggesting quite dramatic effects on the epithelial barrier function. Whereas earlier reports described disruptive effects of e-cigarette vapor on endothelial cells (9), this report specifically tested effects on transepithelial barrier function. Moving on to pulmonary immune cells, this report examined the impact of ENDS aerosol on macrophages, using bone marrow–derived macrophages as a substitute for alveolar macrophages. Doing so, they found that macrophages responded to ENDS aerosol by undergoing pyroptosis, an inflammatory death pathway resulting in cell leakage and release of intracellular contents, further aggravating local inflammation. Interestingly, this event occurred independently of nicotine. Furthermore, when studying immune effector functions of macrophages, the authors noted a decreased capacity of macrophages to perform phagocytosis, which was again aggravated by the addition of nicotine. Arguing that the high number of apoptotic epithelial cells arising from ENDS aerosol exposure would need to be cleared by lung macrophages, the authors finally tested the capacity of macrophages to perform this task. Like the inhibitory effects exerted by ENDS aerosol on phagocytosis, efferocytosis was impaired as well, although, importantly so, only in the presence of nicotine. Although the impact of vaping solvents on macrophage function was not understood so far, another recent study tested the effect of vaping on efferocytosis, using human macrophage-like cells, and detected no effect of the solvents, whereas e-cigarette extract supplemented by apple flavors or nicotine inhibited efferocytosis (10). Together, these data are reassuring, especially because these authors used different cell types and e-cigarette extract delivery systems; they confirm that additives in conjunction with solvents can cause separate effects, in addition to cellular toxicity.

These data provide important information about the cellular cytotoxicity of ENDS aerosol *in vitro*, addressing important aspects of the ongoing confusion about the potentially harmful effects of e-cigarettes (11). The current lack of consistent data, combined with the large variety of ENDS compositions, makes it difficult to fully understand the biological impact. More data from *in vivo* experiments are urgently needed, which are currently limited (12). In that study, the authors used a combination of the solvent most frequently used by ENDS companies and supplemented this with nicotine. Testing the resulting aerosols disclosed harmful effects, leading to cell death. Although *in vitro* cell systems certainly cannot recapitulate the *in vivo* situation, in which apoptotic cells are cleared by neighboring phagocytes, the authors addressed this issue by studying the ability of macrophages to eliminate apoptotic cells in the presence of ENDS aerosols. Unfortunately, ENDS aerosols in combination with nicotine even impaired the clearance of apoptotic cells, suggesting another layer of toxicity by nicotine-containing e-cigarettes. These data highlight the potential danger of vaping and truly call for large-scale studies to investigate the potentially harmful effects in humans. ■

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