Peer Review File

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Review comments:

1. *Exposure to e-cigarettes can damage specific cell types of the innate immune system, including airway epithelial cells, pulmonary macrophages and neutrophils. What is the mechanism of innate immune system damage? Are there any preventive measures?*

   Response: Highlighted throughout the review are potential mechanisms of direct cellular toxicity secondary to e-cigarette aerosol or liquid exposure that are cell-type specific to the innate immune system. Related to airway surface liquid (ASL) and airway epithelial cells, e-cig exposure activated TRPA1, reducing ASL volume and increasing ASL viscosity (Page 7 Lines 5-17). In different experiments, e-cig exposure inhibited CFTR function, resulting in dehydration of the ASL. In airway epithelial cells, e-cig exposure reduced tight junction expression allowing for increased airway permeability (Page 9 Lines 9-21). Additionally, exposure resulted in increased adherence of certain bacteria such as S. pneumonia through platelet-activating factor receptor (Page 10 Lines 22-26). In macrophages, e-cig exposure impaired phagocytosis through reduced scavenger receptor A1 (SR-A1) (Page 14 Lines 22-26) while other studies identified immune system modulation by promoting pro-inflammatory cytokine release (Page 13 Lines 13-19). Lastly, in neutrophils, the most prominent mechanism of e-cig exposure impairment is through suppression of neutrophil extracellular trap formation (NET) that allow bacteria to persist and replicate in the lung (Page 16 Lines 5-14). Hence, there are multiple proposed mechanisms of how e-cigarette exposures impair innate immune function. Targeting one or multiple pathways in combination may provide future therapeutic development (addition to Summary on Page 19 Lines 7-9).

   With regards to prevention, the most effective measure is abstinence from all e-cigarette inhalation. Considering e-cigarettes, like combustible cigarettes, are highly addictive, abstinence is often much more difficult than described (addition to summary on Page 20 Lines 13-15).

2. *Recently, with the popularity of e-cigarettes or ventilators, product-related lung injury highlights the importance of additional chemicals that may be added to the e-cigarettes to dilute the main inhaled drugs. How to improve these chemicals to reduce evali?*

   Response: The chemical most commonly associated with EVALI is vitamin E acetate, or VEA (Page 5 Lines 17-19). VEA is a highly viscous chemical used to dilute THC concentrations and provide greater viscosity to an e-liquid. Direct exposure to VEA via inhalation in mice has been shown to cause acute lung injury (NEJM 2020; 382: 1175-1177). Thus, reducing or eliminating VEA in e-liquids, most likely, contributed to the reduced incidence of EVALI cases requiring hospitalization over the past year. However, the authors of this review postulate that other viscous chemicals with similar properties and composition to that of VEA may have similar effects on the respiratory tract (addition to Page 5 Lines 19-24). Hence, reducing the risk from EVALI by modifying e-liquids or associated devices may be nearly impossible. Additionally, ‘mod’ based devices and cartridges are intentionally modified by the user and therefore not subject to any degree of regulation for safety or quality. Otherwise, for the small proportion of patients with EVALI from legal retail sources, improved regulations for heating components and associated e-liquid may provide some protection.

3. *MUC5AC concentration and MUC5AC / MUC5B ratio in sputum of smokers were significantly higher than*

those of non-smokers, and similar results were found in smokers. Is there any drug that can reduce MUC5AC in clinical application?

Response: One of the most commonly used treatments for elevated levels of MUC5AC (at least, pre-clinically) is the anti-oxidant N-acetylcysteine (NAC). NAC's primary mechanism of action is through repletion of glutathione providing additional cysteine, an essential precursor in glutathione production. NAC also binds toxic metabolites and scavenges free radicals. In COPD, cystic fibrosis and other lung conditions where MUC5AC levels can be elevated, nebulized NAC has been used as a mucolytic, anti-inflammatory and antioxidant. NAC is FDA-approved for acetaminophen toxicity, and thus, could be re-purposed for treatment of e-cigarette users (addition included in Page 9 Lines 17-20).

4. *Before future clinical trials appear, suppliers and parents should continue to advocate for stricter e-cigarette regulations, legislative actions and counter suggestions to protect children and young people from starting to use e-cigarettes. How to set the legal age limit for prohibiting smoking electronic cigarettes?*

   Response: This is an important question that regulatory agencies and government officials will have to decide. We hope our review will help inform this decision-making process but we do not have any specific insight into the challenges of policymaking. Tobacco 21 legislation has set a minimum age (21 years old) for tobacco product use, including e-cigarette use. Professional societies, such as the American Medical Association and California Thoracic Society, have formally endorsed a ban of all e-cigarette flavorings, while many cities and states have introduced legislation to ban flavored e-cigarettes. Other national societies, such as the American Academy of Pediatrics and American Academy of Pediatric Dentistry, support routine screening for e-cigarette use and preventing e-cigarette use and/or initiation in children and adolescents. However, the intent of this review was to provide education to healthcare providers and researchers on the potential mechanisms of innate immune impairment secondary to e-cigarette exposure rather than direct advocacy of e-cigarette policy (addition to Page 20 Lines 16-18).

5. *When MRSA itself is exposed to EV, some of its virulence factors are amplified, including its biofilm formation, hydrophobicity and adhesion to epithelial cells, and increased resistance to LL-37 (antimicrobial peptide released by airway epithelial cells). Are there any measures to inhibit the amplification of these virulence factors?*

   Response: Multiple mechanisms targeting biofilm formation, epithelial adhesion and bacterial replication could be postulated and targeted for reducing the virulence of MRSA in the presence of e-cigarette vapor exposure. Assuming a true bacterial infection occurs with both MRSA and e-cigarette exposure, antibiotics that are bactericidal to MRSA may reduce virulence associated with e-cig exposures (addition to Page 12 Lines 9 -13).

6. *The exact mechanism of how e-cigarette exposure increases the virulence of airway epithelial cells is still unclear. How to study the related mechanisms in the next step?*

   Response: First, additional research is required to identify individual chemicals and other constituents within e-cigarette liquids and associated e-devices that lead to increased virulence and impaired innate immunity. Second, research should also evaluate the combination of chemicals found in e-cigarette liquids to assess not only whether the individual components but also the mixture contributes to impaired innate immunity. Third, additional clinical and translational studies are required to validate the identified

pathways seen in those preclinical studies highlighted within the review. More specifically, additional clinical evidence is required to assess whether e-cigarette users, or dual e-cigarette and tobacco users, are at higher risk for respiratory tract infections compared to the general population (additional paragraph added highlighting this construct on Page 19 Lines 10-18).