19. Huang YJ. The respiratory microbiome and innate immunity in asthma. *Curr Opin Pulm Med* 2015;21:27–32.
20. Quinn RA. Integrating microbiome and metabolome data to understand infectious airway disease. *Am J Respir Crit Care Med* 2017;196:806–807.

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Electronic versus Combustible Cigarette Effects on Inflammasome Component Release into Human Lung

To the Editor:

Electronic cigarettes (e-cigarettes) include a wide variety of battery-powered devices that produce an aerosol for inhalation by heating a liquid that contains a solvent (usually composed of propylene glycol and/or glycerin), flavoring, and nicotine. The popularity of e-cigarette use has increased in recent years among both smokers and nonsmokers. Thirteen percent of U.S. adults reported e-cigarette use in 2013, up from 1.8% in 2010, but a third of current users were nonsmokers or former tobacco smokers (1). In this regard, the potential for functional effects of e-cigarette use on the first-pass organ, the human lung, remain understudied.

In this context, lung immunosurveillance is partly dependent on the sensing of danger by macrophage inflammasomes. Inflammasomes are multimeric complexes formed in response to various stimuli and are vital to the clearance of pathogens (2). Most inflammasomes activate caspase-1 via an adaptor molecule, an apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) that promotes pro-IL-1 β cleavage to mature IL-1 β and induces cell pyroptosis. Furthermore, it has been proposed that extracellular ASC may have additional prion-like activity, as ASC characteristically forms polymers that may cause amyloid-like deposits that promote tissue injury (3). Of note, elevated ASC has also been reported in BAL fluid obtained from humans, and in particular patients with chronic obstructive pulmonary disease (3). We therefore elected to determine whether e-cigarette use may promote ASC release into the lung by comparing BAL fluid ASC levels from normal healthy never-smokers, smokers, and e-cigarette users.

Methods

Forty-three normal subjects composed of smokers, never-smokers, and e-cigarette users (Tables 1 and 2) underwent elective bronchoscopy with 100–140 ml saline BAL fluid after Institutional

Review Board (IRB 2015C0088)–approved informed consent, as part of a larger study analyzing the effects of e-cigarettes on lung biomarkers. Smokers were delineated as those who smoked at least 10 cigarettes a day for at least 6 months with no prior e-cigarette use within a year. E-cigarette users were defined as nonsmokers who used at least one nicotine-containing cartridge per day or 1 ml liquid per day for at least 3 months, 12 of whom were former smokers (>5 mo; mean = 8 yr). Never-smokers had smoked fewer than 100 cigarettes in their lifetime, and not for at least 1 year, with no e-cigarette use within that timeframe. Nonsmoking status was confirmed by salivary cotinine (NicAlert; Nymox Pharmaceuticals). All participants were randomly assigned to the right or left lung for bronchoscopy in a 1:1 ratio within each smoking status group (never-smokers, e-cigarette users, and smokers). BAL fluid samples were spun down at 300g at 4°C to separate cells from the supernatant. Cell-free lavage fluid was stored in aliquots and frozen at -80°C until processed. BAL fluid was analyzed by sandwich ELISAs for ASC and caspase-1 combining commercial mouse monoclonal with rabbit polyclonal detection antibodies generated in our laboratory (4) and a bead-based ELISA for IL-1 β (Meso Scale Discovery).

Results

BAL samples were processed for total and differential cell counts. Smokers had the highest total cell counts, which were statistically significant compared with never-smokers ($P = 0.003$; Tables 1 and 2). Smokers had the highest total macrophage counts compared with never-smokers and e-cigarette users, but were only statistically significant compared with never-smokers ($P = 0.006$). Regarding BAL fluid inflammasome components, ASC and caspase-1 were readily detected in unconcentrated fluid, and each correlated with macrophage concentrations ($r^2 = 0.52$ [$P < 0.0001$] and $r^2 = 0.55$ [$P < 0.0001$], respectively).

ASC in BAL fluid was higher in smokers and e-cigarette users when compared with never-smokers ($P = 0.0005$ and $P = 0.04$, respectively), but e-cigarette user ASC levels were not statistically different from smokers (Figure 1). Caspase-1 and IL-1 β levels largely paralleled the ASC data. Caspase-1 levels were higher in smokers than in e-cigarette users and nonsmokers ($P = 0.04$ and $P = 0.0003$, respectively), but e-cigarette user caspase-1 was not different from nonsmokers. Finally, IL-1 β levels in BAL fluid were highest in smokers (median, 4.6 pg/ml; interquartile range [IQR], 2.7–7.3) and statistically significant compared with both e-cigarette users and never-smokers (median, 1.0 pg/ml [IQR, 0.8–1.6; $P = 0.0005$], and median, 1.0 pg/ml [IQR, 0.7–1.2; $P = 0.001$], respectively).

Discussion

This study is the first cross-sectional study of inflammasome protein release in human BAL fluid to compare various smoking categories that include exclusive e-cigarette use; most of the participants were former smokers. Consistent with previous studies, smokers had higher inflammatory total cell counts and macrophage counts compared with never-smokers (5). It is noteworthy that e-cigarette macrophage counts were intermediate between smokers and never-smokers. These findings are consistent with murine (6) and

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Table 1. Baseline Demographics by Smoking Status

Characteristics	Never-Smokers (n = 12)	E-Cig Users (n = 15)	Smokers (n = 16)
Age, yr, mean (range)	26 (21–30)	27 (21–30)	26 (21–30)
Sex			
Female, n (%)	7 (58%)	5 (33%)	4 (25%)
Male, n (%)	5 (42%)	10 (67%)	12 (75%)
Race			
White, n (%)	10 (83%)	12 (80%)	14 (88%)
Black or African American, n (%)	1 (8%)	1 (7%)	1 (6%)
Asian, n (%)	1 (8%)	2 (13%)	1 (6%)
Smoking			
Former, n (%)	0 (0%)	12 (80%)	0 (0%)
Current, n (%)	0 (0%)	0 (0%)	16 (100%)
Never, n (%)	12 (100%)	3 (20%)	0 (0%)
Years of smoking, mean (range)	—	8 (1–15)*	8 (0.6–10)
Cigarettes/d, mean (range)	—	13 (2–20)*	16 (10–20)
Years since last cigarettes, mean (range)	—	2 (0.4–4.1)*	—
Vaping			
Years of vaping, mean (range)	—	3 (0.5–4)	—
Puffs/d, mean (range)	—	127 (20–600)	—
E-liquid, ml/d, mean (range)	—	7 (2–20)	—
Nicotine, mg/ml, mean (range)	—	12 (1.5–36)	—

Definition of abbreviation: E-cig = e-cigarette.

*Prior smoking e-cig users.

human studies (7) that suggest e-cigarette use can affect innate immunity.

In the context of inflammasome activation, the ASC levels in BAL fluids of all subjects were higher than expected. When normalized to estimated epithelial lining fluid (i.e., about 100× diluted in BAL [8]), smoker ASC approximated 4 µg/ml epithelial lining fluid (i.e., about 1,000 times higher than what we have observed for plasma concentrations; unpublished observation). The significance of this lung-centric elevation of ASC remains to be determined, but it has been linked to “prionoid” functions of oligomeric ASC (9, 10). Importantly, ASC levels did reflect smoking status. E-cigarette users had ASC levels intermediate to those of never-smokers and smokers. Both caspase-1 and IL-1β levels mimicked the ASC levels in the three groups, suggesting that

the ASC levels in part reflect inflammasome activation, although there was no notable difference in IL-1β levels between never-smokers and e-cigarette users. Taken as a whole, these data suggest that e-cigarette use contributes less to lung inflammation than does combustible tobacco use. However, in this cross-sectional study, e-cigarette use did provide an inflammatory signal above that of never-smokers. Because we cannot exclude residual influences from prior combustible tobacco use in our e-cigarette cohort, longitudinal studies of e-cigarette use in never-smokers are still needed to better characterize the inflammatory effects of e-cigarette use. Furthermore, future work will need to address the relative effect of nicotine versus other components, including carriers and flavorings, which were not addressed in this study. ■

Table 2. Cross-Sectional Analysis of BAL Fluid Cell Counts

BAL Fluid Cell Count	Never-Smokers (n = 11)*	E-Cig Users (n = 13)*	Smokers (n = 16)	P Value†		
				Never-Smoker vs. E-Cig User	Never-Smoker vs. Smoker	E-Cig User vs. Smoker
Total cell concentration, ×10 ⁶ /L, median (IQR)	240 (168–267)	306 (212–369)	434 (300–699)	0.41	0.003	0.14
Macrophages, ×10 ⁶ /L, median (IQR)	212 (159–264)	265 (184–321)	411 (289–652)	0.53	0.006	0.06
Lymphocytes, ×10 ⁶ /L, median (IQR)	5 (0–16)	13 (6–42)	10 (1–16)	NA‡	NA‡	NA‡
Neutrophils, ×10 ⁶ /L, median (IQR)	0 (0–6)	3 (2–20)	10 (2–21)	0.14	0.05	0.72

Definition of abbreviations: e-cig = e-cigarette; IQR = interquartile range; NA = not applicable.

*Excluded data because of contamination with blood, which altered the total cell count.

†Kruskal-Wallis followed by all-pairs using Steel-Dwass test to control for the multiple comparisons.

‡Pairwise not included because of the Kruskal-Wallis test not being statistically significant.

Figure 1. ASC and caspase-1 levels in BAL fluid by smoking status. Cell-free BAL fluids from never-smokers ($n = 12$), e-cigarette users ($n = 15$), and smokers ($n = 16$) were analyzed by ELISA for ASC and caspase-1. Box plots show medians with interquartile range (IQR), and whiskers show minimum and maximum values. ASC was significantly higher in smokers than never-smokers (median, 37 ng/ml [IQR, 21–64 ng/ml] vs. 11 ng/ml [IQR, 9–15 ng/ml], respectively). E-cigarette user ASC levels (median, 22 ng/ml [IQR, 14–35 ng/ml]) were intermediate to never-smokers and smokers but only statistically significant compared with never-smokers. Caspase-1 levels were significantly lower in never-smokers compared with smokers (median, 12 pg/ml [IQR, 6–14 pg/ml] vs. 42 pg/ml [IQR, 27–68 pg/ml], respectively). The median caspase-1 level was 16 pg/ml [IQR, 9–35 pg/ml] for e-cigarette users, which was statistically different from that of smokers. Kruskal-Wallis was applied comparing inflammasome components among the three smoking status groups. The Steel-Dwass test was used for multiple pairwise comparisons. * $P < 0.05$, ** $P < 0.01$, and NS = no significant difference. ASC = apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; e-cig = electronic cigarette.

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References

1. Dinakar C, O'Connor GT. The health effects of electronic cigarettes. *N Engl J Med* 2016;375:1372–1381.
2. Ravi Kumar S, Paudel S, Ghimire L, Bergeron S, Cai S, Zemans RL, et al. Emerging roles of inflammasomes in acute pneumonia. *Am J Respir Crit Care Med* 2018;197:160–171.
3. Franklin BS, Bossaller L, De Nardo D, Ratter JM, Stutz A, Engels G, et al. The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. *Nat Immunol* 2014;15:727–737.
4. Wewers MD, Dare HA, Winnard AV, Parker JM, Miller DK. IL-1 beta-converting enzyme (ICE) is present and functional in human alveolar macrophages: macrophage IL-1 beta release limitation is ICE independent. *J Immunol* 1997;159:5964–5972.

5. Merchant RK, Schwartz DA, Helmers RA, Dayton CS, Hunninghake GW. Bronchoalveolar lavage cellularity: the distribution in normal volunteers. *Am Rev Respir Dis* 1992;146:448–453.
6. Hwang JH, Lyles M, Sladewski K, Enany S, McEachern E, Mathew DP, et al. Electronic cigarette inhalation alters innate immunity and airway cytokines while increasing the virulence of colonizing bacteria. *J Mol Med (Berl)* 2016;94:667–679.
7. Martin EM, Clapp PW, Rebuli ME, Pawlak EA, Glista-Baker E, Benowitz NL, et al. E-cigarette use results in suppression of immune and inflammatory-response genes in nasal epithelial cells similar to cigarette smoke. *Am J Physiol Lung Cell Mol Physiol* 2016;311:L135–L144.
8. Rennard SI, Basset G, Lecossier D, O'Donnell KM, Pinkston P, Martin PG, et al. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J Appl Physiol (1985)* 1986;60:532–538.
9. Venegas C, Kumar S, Franklin BS, Dierkes T, Brinkschulte R, Tejera D, et al. Microglia-derived ASC specks cross-seed amyloid- β in Alzheimer's disease. *Nature* 2017;552:355–361.
10. Franklin BS, Latz E, Schmidt FL. The intra- and extracellular functions of ASC specks. *Immunol Rev* 2018;281:74–87.

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Comparison of Physiological Performance of Four Adaptive Servo Ventilation Devices in Patients with Complex Sleep Apnea

To the Editor:

Adaptive servo ventilation (ASV) increased the risk for mortality in the SERVE-HF (Treatment of Sleep-Disordered Breathing with Predominant Central Sleep Apnea by Adaptive Servo Ventilation in Patients with Heart Failure) trial, but the underlying mechanisms are unclear (1–3). Conceivably, device algorithms controlling respiratory rate and pressure support may have led to high $\dot{V}_E$ that caused hypocapnia and consequent arrhythmias (2, 4, 5). Whether such findings are a result of a device algorithm-based effect (“device-effect”) or apply to all servo-algorithm devices (“class-effect”) is uncertain (2, 6). We compared the performance of various ASV devices on measures of respiration and electrocardiography. Some of the results of these studies have been previously reported in the form of an abstract (7).

Methods

We performed a randomized controlled crossover physiological experiment of patients with complex sleep apnea with preserved cardiac contractility (left ventricular ejection fraction >45% by echocardiography) who were adherent to ASV therapy. Patients

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with untreated sleep disorders such as insomnia, periodic limb movement syndrome (leg movement index >10/h in prior laboratory-based polysomnography [PSG]), or restless legs syndrome were excluded. Patients were randomly assigned to 4 nights of PSG while receiving the device used in the SERVE-HF trial (ResMed S7 VPAP Adapt [ResMed]; hereafter, “S7 device”), a later version of the S7 device (ResMed S9 VPAP Adapt [ResMed]; hereafter, “S9 device”), a Philips ASV device (System One; Philips-Respironics, Inc.), and a later version of Philips ASV device (Dreamstation; Philips-Respironics, Inc.). For all devices, the expiratory positive airway pressure level was set from 4 to 15 cm H₂O; the minimum pressure support was set at the lowest level possible (3 cm H₂O for the S7 device and 0 cm H₂O for all other devices); and maximum pressure support was 15 cm H₂O, with maximum total pressure of 25 cm H₂O with automatic back-up rate, whereas patients used the same mask interface on all nights. Conventional PSG with two electroencephalography leads each for frontal, occipital, and temporal; right and left electro-oculography; chin electromyography; lead II electrocardiography; finger pulse-oximetry; and respiratory signals derived from the device pneumotachograph output (airflow, $\dot{V}_T$, and their derivatives: instantaneous respiratory rate [respiratory rate = 1/total respiratory cycle time] and $\dot{V}_E$ [product of $\dot{V}_T$ and respiratory rate]) were collected. Electrocardiography signals (200 Hz sampling rate) were analyzed for heart rate and QTc interval (MATLAB software). Patients were blinded to the device, and blinded observers scored PSG, respiratory, and electrocardiographic signals (8). Statistical analysis was performed by individuals blinded to study condition through numerical coding of the device using ANOVA or generalized linear model with repeated measures with adjustment for multiple comparisons (generalized linear model with Holm-Bonferroni correction that adjusts for control of family-wise error rate; IBM SPSS v25.0; IBM Corp.).

Fourteen patients underwent PSG on 4 nights while receiving treatment from four different devices. $\dot{V}_E$ was greater during treatment with the S7 device when compared with all other devices during wakefulness ($P < 0.0001$; Figure 1). The $\dot{V}_E$ for the entire night when receiving therapy with the S7 device was greater than the $\dot{V}_E$ for the entire night when receiving therapy with any of the other devices ($P < 0.02$; right upper panel of figure). Respiratory rate was greater with the S7 device when compared with the S9 device for the entire night ($P < 0.0001$; Table 1). During wakefulness, pressure support level was greater during S7 device therapy when compared with S9 device ($P = 0.002$; Table 1) and tended to be greater than pressure support level administered by the S9 device during sleep ($P = 0.085$). QT interval corrected for heart rate (QTc) during S7 therapy was not different than that during any of the other therapy nights ($P = 0.24$). The tendency for greater frequency of premature ventricular beats during S7 nights when compared with any other night was observed but did not reach statistical significance ($P = 0.20$). There were three episodes of nonsustained ventricular tachycardia during the entire study: two episodes during the S7 nights and one during a System One night. The total apnea-hypopnea index and apnea index were not different across the different devices ($P > 0.6$). The central apnea index tended to be lower in the S7 and S9 devices when compared with the System One and Dreamstation devices (ANOVA; $P = 0.08$).