The Airway Epithelial Response to Air Pollution: It's Not Just Inflammation

Exposure to particulate matter (PM) air pollution is the greatest environmental health risk, ranking above other common factors such as low physical activity, high cholesterol, and drug use (1, 2). Acute thrombotic events account for the majority of PM-associated mortality; however, exposure to air pollution is also a significant cause of morbidity, accounting for 3.1% of global disability-adjusted life years (3, 4). The respiratory effects of PM exposure include increased rates of lung cancer, as well as increased exacerbations of, and deaths from, asthma and chronic obstructive pulmonary disease (5). Although the epidemiological evidence for adverse health outcomes caused by PM exposure is clear, the cellular mechanisms behind these outcomes are poorly understood.

The nasal epithelium is the first point of contact between the respiratory tract and inhaled PM. Studies have shown that exposure of mice to PM leads to increased nasal cytokine levels and inflammatory cell recruitment (6). Experiments using cultured nasal or bronchial epithelial cells have also shown that *in vitro* PM exposure induces inflammatory cytokine expression (7, 8). Although these studies support the idea that PM plays an active role in promoting airway inflammation, most of them focused narrowly on the expression of particular cytokines or other genes of interest. Furthermore, many of these studies relied on the use of airway epithelial cell lines, or primary submerged cultures of airway epithelial cells, which do not faithfully represent the cellular diversity of the *in vivo* epithelium and are known to respond differently to PM exposure compared with air–liquid interface (ALI) cultures (9). There is thus considerable room to better understand the response of the airway epithelium to PM exposure and how this response may promote the poor respiratory outcomes associated with exposure.

In this issue of the *Journal*, Montgomery and colleagues (pp. 172–184) shed new light on this poorly understood area (10). To improve on previous studies, the investigators used whole-transcriptome expression analysis to determine the effect of PM exposure on primary nasal epithelial cell ALI cultures derived from multiple donors. Not surprisingly, the authors found targets of the aryl hydrocarbon receptor and inflammatory cytokines among the highest-induced genes after treatment with PM. Also highly enriched in the PM-treated cultures were genes involved in endoplasmic reticulum protein processing and Golgi transport, as well as genes encoding mucin proteins, including *MUC5AC*, suggesting that PM induces a reprogramming of the epithelial cultures toward a secretory phenotype. Indeed, a comparison of PM-induced differentially expressed genes with a catalog of airway epithelial cell markers obtained from single-cell RNA sequencing demonstrated that PM exposure led to a significant increase in mucus secretory cell markers, accompanied by a loss of ciliated cell markers, suggesting that PM exposure may promote mucous cell metaplasia. This was confirmed histologically, as PM-treated cultures exhibited increased numbers of MUC5AC and Alcian Blue/periodic acid–Schiff–stained cells, as well as increased MUC5AC secretion.

What is the mechanism behind this expansion of secretory cells? The authors provide evidence that PM exposure regulates the expression of epithelial lineage–specific transcription factors. PM exposure increased the expression of the secretory cell regulators SPDEF and FOXA3 in ALI cultures in a dose-dependent manner. By contrast, the ciliated cell regulator FOXJ1 was reduced by PM exposure. Although it was not demonstrated experimentally, the authors suggest that induction of IL-1α and IL-1β by PM may play a role in this reprogramming. *IL1A* and *IL1B* were among the top genes upregulated by PM exposure, and a functional gene network analysis revealed high levels of betweenness in relation to the rest of the transcriptional response to PM. Although IL-1α and IL-1β have been shown to induce SPDEF-dependent mucous cell metaplasia in mice (11), loss-of-function experiments were not included in this study.

Another area where Montgomery and colleagues bring new light to the field is the separation of air pollution particulates into organic and water-soluble fractions. Air pollution particulates consist of carbonaceous particles with adsorbed organic chemicals (such as nitrates, sulfates, and polycyclic aromatic hydrocarbons) and metals (such as iron, copper, nickel, zinc, and vanadium). Although previous studies focused on the relative toxicities of the two fractions, a thorough examination of the gene expression responses to the organic and water-soluble fractions had yet to be completed. Surprisingly, the investigators found that the water-soluble fraction containing various reactive metals had no significant effect on gene expression in ALI cultures. The organic fraction had a dose-dependent effect on gene expression, with both the number of genes regulated and the magnitude of change increasing with the dose of organic extract. Although the authors did not compare the gene-expression changes in response to organic extract with those in response to unfractionated particles, their results suggest that the epithelial response to PM exposure is mostly a consequence of exposure to organic chemicals. It would be of interest to see whether the water-soluble fraction, when associated with the organic phase, affects gene expression more than the water-soluble fraction alone.

Air pollution exposure is a growing global health problem, and although mitigation efforts and production cessations show immediate beneficial health effects, the totality of the evidence suggests that there is no "safe" level of PM exposure (1, 12).

Originally Published in Press as DOI: 10.1165/rcmb.2020-0116ED on April 20, 2020

It is thus important for us to develop a thorough mechanistic understanding of the biological responses to air pollution so that we can better protect at-risk populations. The study by Montgomery and colleagues sheds light on the mechanisms by which PM exposure affects airway cellular composition and mucus production, possibly contributing to disease pathogenesis. It also raises interesting questions for future studies. For instance, how do individual cell types in ALI cultures respond to PM exposure, and what is the etiology of the increased numbers of mucus-producing cells? Time-resolved experiments using single-cell RNA sequencing will prove particularly interesting in this regard. Does inhibition of IL-1 prevent the mucous cell metaplasia seen after exposure to PM? What other gene networks are regulated in response to signaling downstream of IL-1 or other cytokines induced by PM? Finally, Montgomery and colleagues used nasal epithelial cells from an outstanding 12 individual donors, larger studies may be able to shed light on genetic networks that vary based on variables such as race, sex, and age. Our understanding of the health effects of air pollution exposure is far from complete. In-depth studies such as this one are needed to illuminate the mechanistic links between exposure and disease. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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*G.M.M. is Associate Editor of *AJRCMB*. His participation complies with American Thoracic Society requirements for recusal from review and decisions for authored works.

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