

## Letter to the Editor

# Air pollutant-mediated disruption of sinonasal epithelial cell barrier function is reversed by activation of the Nrf2 pathway

To the Editor:

Particulate matter (PM) is 1 of 6 damaging air pollutants that has been identified under the United States Clean Air Act of 1970 and is regulated because of its harmful effects. PM directly contain redox-active chemicals and transition metals that can generate reactive oxygen species. The harmful effects of outdoor PM are well established and include premature death, and both indoor and outdoor PM have been documented to exacerbate asthma morbidity.<sup>1</sup> PM also has been reported to cause sinonasal inflammation in both mouse and human models. Mice that were challenged intranasally with PM showed nasal epithelial thickening and increased eosinophils in nasal lavage.<sup>2</sup> Furthermore, human studies have also demonstrated that stimulating sinonasal epithelial cells with PM causes increases in proinflammatory cytokines.<sup>3</sup>

At the interface between the environment and exposure to air pollutants is the sinonasal epithelial cell barrier, which acts to limit the transit of noxious materials. Disruption of barrier integrity and function may lead to persistent inflammation through a cycle of inflammation, barrier disruption, and exposure to inflammatory agents.<sup>4</sup> Although Steelant et al<sup>5</sup> have described that house dust mite allergic rhinitic patients have increased epithelial barrier permeability and decreased tight junction proteins, the role of PM in sinonasal epithelial barrier function has not been previously explored.<sup>5</sup>

A key regulator of oxidative and environmental stress is the transcription factor nuclear erythroid 2-related factor 2 (Nrf2). Upon activation, Nrf2 translocates to the nucleus and facilitates expression of genes that enact a cytoprotective response. Although the role of Nrf2 in the sinonasal tract is yet to be investigated, Nrf2 activation through genetic or pharmacologic approaches has been reported to attenuate the asthmatic phenotype in a mouse model of allergic asthma.<sup>6</sup> Nrf2 activation in airway epithelial cells was found to enhance epithelial barrier function and increase localization of the tight junction protein zonula occludens-1 (ZO-1) to the cell surface. Conversely, siRNA knockdown of Nrf2 resulted in removal of epithelial cadherin and ZO-1 from the cell surface as well as decreased expression of tight junction proteins.<sup>7,8</sup> We therefore hypothesized that Nrf2 activation may reverse PM-mediated sinonasal epithelial cell barrier dysfunction.

First, we sought to understand whether PM could destabilize the human sinonasal epithelial cell (HSNEC) barrier. Human sinonasal epithelial cells grown on an air-liquid interface were apically treated with well-characterized outdoor PM<sub>10</sub> (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).<sup>9</sup> Epithelial barrier disruption was noted within 4 hours as assessed by transepithelial electrical resistance (TEER) (Fig 1, A) and paracellular flux quantified by fluorescein isothiocyanate (FITC)-dextran leak (Fig 1, B). Furthermore, the HSNEC barrier-destabilizing effect of PM was found to be dose dependent (Fig 1, B). Next, we tested whether enhancement of Nrf2 using the

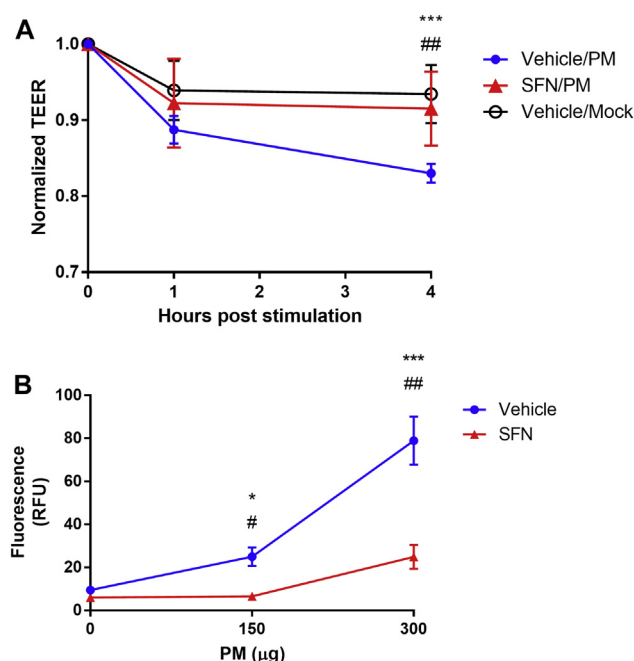


**FIG 1.** HSNEC permeability assessed by TEER (A) and FITC-dextran (B) after PM at 300  $\mu$ g (Fig 1, A) or 150  $\mu$ g and 300  $\mu$ g (Fig 1, B) and SFN stimulation. Comparing PM exposure to no PM exposure, \* $P < .05$  and \*\*\* $P < .001$ . Comparing PM/vehicle to PM/SFN # $P < .05$  and ## $P < .01$ . Error bars represent SEM of at least 3 independent experiments. RFU, Relative fluorescence units.

activator sulforaphane (SFN) was sufficient to reduce PM-induced sinonasal epithelial cell (SNEC) barrier disruption. SNECs were pretreated with 10  $\mu$ M SFN for 72 hours before PM stimulation. SFN pretreatment was found to significantly reduce SNEC barrier instability as measured by both TEER and FITC-dextran leak (Fig 1, A and B). The dose of SFN used was confirmed to induce expression of known target genes of Nrf2 activation (see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

Next, we hypothesized that PM may perturb epithelial barrier function through disruption of intercellular tight junctions. Indeed, we found that 300  $\mu$ g PM significantly reduced cell surface localization of ZO-1, junction adhesion molecule-A (JAM-A), and Claudin-1 (Fig 2, A and B). We tested whether Nrf2 activation could inhibit PM-induced destabilization of SNEC tight junction localization and found that SFN restored cell surface localization of ZO-1, JAM-A, and Claudin-1 (Fig 2, A and B). Last, we confirmed that the barrier-destabilizing effect of PM at 4 hours was not due to cell death as assessed by lactate dehydrogenase release using 500  $\mu$ g PM, a dose even above that at which the barrier-destabilizing effect of PM was observed (see Fig E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

To our knowledge, this is the first study to demonstrate that PM can disrupt SNEC barrier function and tight junction cell surface localization, which may be reversed by Nrf2 activation. These data suggest that activation of the Nrf2 pathway with SFN may represent a potential therapeutic target in chronic sinonasal inflammatory disorders such as allergic and nonallergic rhinitis

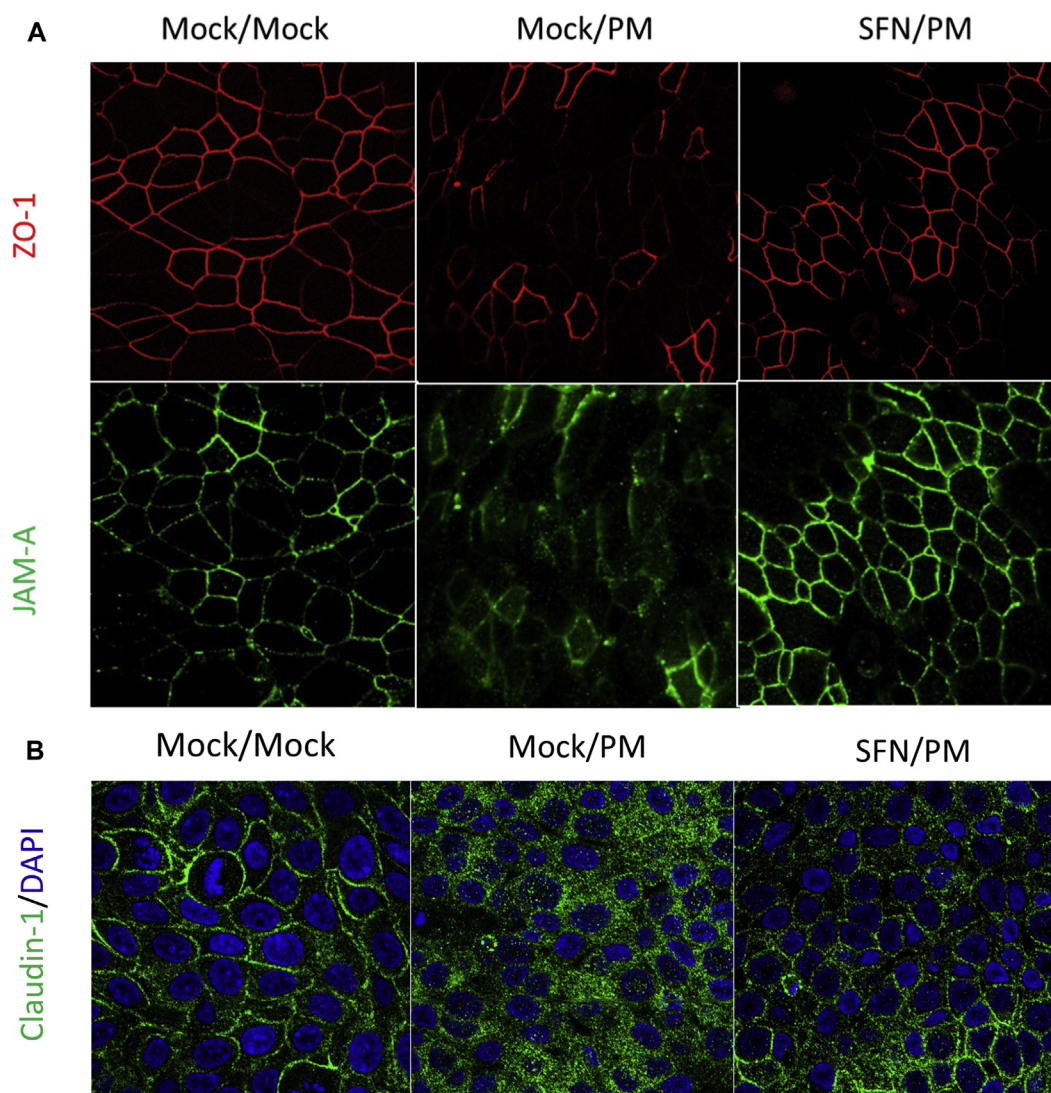


**FIG 2.** HSNEC barrier disruption assessed by immunofluorescence after 300  $\mu$ g PM (middle column) and SFN stimulation (right column). **A**, ZO-1 (upper row, red) and JAM-A (second row, green). **B**, Claudin-1 (green) and DAPI (blue). Images represent at least 3 independent experiments. DAPI, 4'-6-Diamidino-2-phenylindole, dihydrochloride.

and chronic rhinosinusitis (see Fig E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Interestingly, SFN is found naturally in cruciferous vegetables such as broccoli, cabbage, or Brussel sprouts. Thus, additional studies may demonstrate dietary advantages protecting against environmental-induced airway disease. Activation of the Nrf2 pathway may be particularly applicable to settings in which oxidative stress may be contributing to pathophysiology such as air pollution, aeroallergens, and bactericidal antibiotics.

HSNECs were obtained from patients undergoing elective endoscopic sinus surgery for conditions including chronic rhinosinusitis and were grown in culture at the air-liquid interface with the apical surface free of culture medium for at least 21 days before the study. HSNECs were pretreated with 10  $\mu$ M SFN (Toronto Research Chemicals, Toronto, Ontario, Canada) for 72 hours with redosing every 24 hours. HSNECs were then apically stimulated with 300  $\mu$ g of PM in air-liquid interface (ALI) media for 4 hours. TEER was recorded at time points 0, 1,

and 4 hours poststimulation using the EVOM2 Transepithelial Voltmeter (World Precision Instruments, Sarasota, Fla). Four kDa FITC-dextran beads were applied to the apical surface with treatment medium and incubated for 4 hours. FITC-dextran flux was quantified by collecting basolateral media after 4 hours and assessing fluorescence using a plate reader at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. After 4 hours of PM stimulation, HSNECs were washed and fixed with ice-cold 4% paraformaldehyde for 30 minutes at 37°C and permeabilized with 0.3% Triton X-100. SNECs were then blocked with 10% normal donkey serum for 1 hour and incubated with mouse anti-ZO-1 (LifeSpan Biosciences, Seattle, Wash), goat anti-JAM-A (R&D Systems, Minneapolis, Minn), or mouse anti-Claudin-1 (Novus Biologicals, Littleton, Colo). Cells were then incubated with Alexa-Fluor 594 donkey antimouse and Alexa-Fluor 488-conjugated donkey antigoat for 90 minutes. Statistical analysis was performed using ANOVA with *post hoc* Tukey test.

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Disclosure of potential conflict of interest: N. R. London holds patents for composition and methods for treating pathologic angiogenesis and endothelial hyperpermeability and has stock options with Navigen Pharmaceuticals, which are currently of no value. A. M. Rule has received support from the National Institutes of Health (grant no. K23ES020859). The rest of the authors declare that they have no relevant conflicts of interest.

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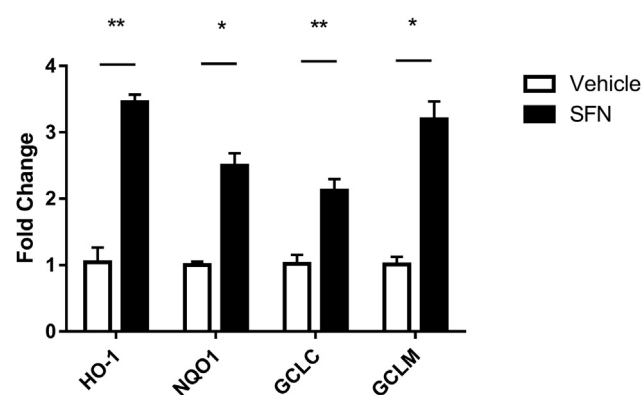


**FIG E1.** SFN increased expression of Nrf2 target genes including heme oxidase (HO-1), NAD(P)H:quinone oxoreductase (NQO-1), glutamate cysteine ligase catalytic subunit (GCLC), and glutamate cysteine ligase modulatory subunit (GCLM) as assessed by quantitative PCR. \* $P < .05$ , \*\* $P < .01$ . Error bars represent SEM of at least 3 independent experiments.

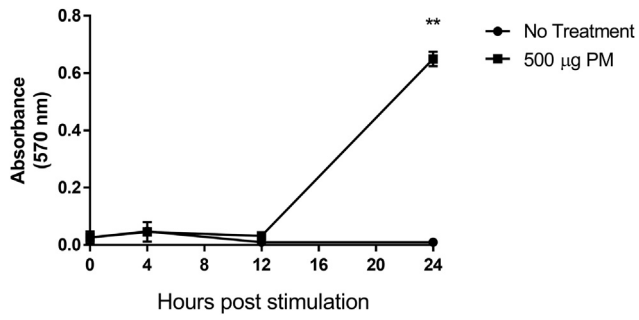


**FIG E2.** HSNEC cell death assessed by lactate dehydrogenase release after 500 µg PM stimulation. \*\* $P < .01$ . Error bars represent SEM of at least 3 independent experiments.

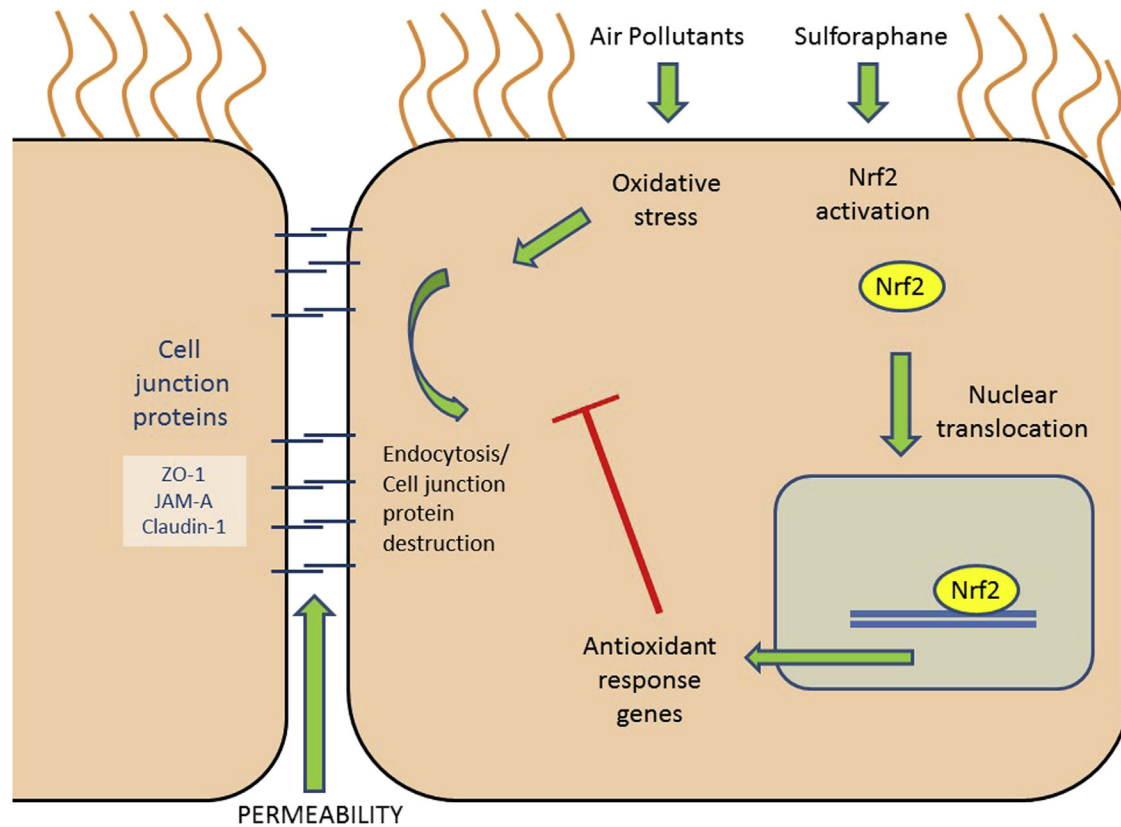


**FIG E3.** Ciliated SNECs form a barrier interface with the environment and are subjected to noxious environmental stimuli. Air pollutants induce oxidative stress in SNECs and reduce tight junction cell surface localization, resulting in increased epithelial barrier permeability. SFN reverses barrier destabilization by activation of the Nrf2 pathway and induction of a cytoprotective response.

**TABLE E1.** Metal content and particle size of the Baltimore PM

| Metal | Metal content (μg/mg) |       |
|-------|-----------------------|-------|
|       | Mean                  | SE    |
| Be    | 0.020                 | 0.028 |
| Ag    | 0.055                 | 0.009 |
| Al    | 3.262                 | 0.572 |
| As    | 0.040                 | 0.058 |
| Ca    | 3.118                 | 0.839 |
| Cd    | 0.013                 | 0.019 |
| Co    | 0.032                 | 0.051 |
| Cr    | 0.057                 | 0.065 |
| Cs    | 0.020                 | 0.032 |
| Cu    | 0.061                 | 0.054 |
| Fe    | 2.721                 | 0.175 |
| K     | 0.946                 | 0.167 |
| Mg    | 2.360                 | 0.721 |
| Mn    | 0.113                 | 0.047 |
| Mo    | 0.030                 | 0.027 |
| Na    | 5.795                 | 1.164 |
| Ni    | 0.042                 | 0.054 |
| Pb    | 0.035                 | 0.029 |
| Sb    | 0.034                 | 0.034 |
| Se    | 0.015                 | 0.009 |
| Sn    | 0.026                 | 0.030 |
| Ti    | 0.215                 | 0.053 |
| Tl    | 0.166                 | 0.291 |
| V     | 0.039                 | 0.050 |
| Zn    | 0.207                 | 0.077 |