

An update on immunologic mechanisms in the respiratory mucosa in response to air pollutants



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Every day, we breathe in more than 10,000 L of air that contains a variety of air pollutants that can pose negative consequences to lung health. The respiratory mucosa formed by the airway epithelium is the first point of contact for air pollution in the lung, functioning as a mechanical and immunologic barrier. Under normal circumstances, airway epithelial cells connected by tight junctions secrete mucus, airway surface lining fluid, host defense peptides, and antioxidants and express innate immune pattern recognition receptors to respond to inhaled foreign substances and pathogens. Under conditions of air pollution exposure, the defenses of the airway epithelium are compromised by reductions in barrier function, impaired host defense to pathogens, and exaggerated inflammatory responses. Central to the mechanical and immunologic changes induced by air pollution are activation of redox-sensitive pathways and a role for antioxidants in normalizing these negative effects. Genetic variants in genes important in epithelial cell function and phenotype contribute to a diversity of responses to air pollution in the population at the individual and group levels and suggest a need for personalized approaches to attenuate the respiratory mucosal immune responses to air pollution. (*J Allergy Clin Immunol* 2019;143:1989-2001.)

Key words: Airway epithelial cells, host defense peptides, oxidative stress, antioxidants, gene-pollution interactions

Every day, we breathe in more than 10,000 L of air that contains a variety of air pollutants, including urban particulate matter, diesel exhaust particles, *geogenic dusts*, and *biomass* combustion byproducts, that can pose potential negative

Abbreviations used

AhR:	Aryl hydrocarbon receptor
COPD:	Chronic obstructive pulmonary disease
GSH:	Glutathione
ICAM-1:	Intercellular adhesion molecule 1
NAC:	N-acetyl-cysteine
NADPH:	Nicotinamide adenine dinucleotide phosphate
NF-κB:	Nuclear factor κB
NIST:	National Institutes for Standards and Technology
NO ₂ :	Nitrogen dioxide
Nrf2:	Nuclear factor-like 2
PAH:	Polyaromatic hydrocarbon
RNS:	Reactive nitrogen species
ROS:	Reactive oxygen species
SOD:	Superoxide dismutase
SRM:	Standard reference material
TSLP:	Thymic stromal lymphopoietin

consequences to lung health (Fig 1). The respiratory mucosa is the first line of defense to protect our lungs and is formed from a constellation of mechanical and immunologic impedances.¹⁻³ The coordinated activities of the respiratory mucosa include airway surface lining fluid with host defense peptides, *mucus* production for entrapment of inhaled particulates, *tight junctions* between epithelial cells to restrict transit to deeper lung tissues, and innate immune receptors and signaling pathways that can activate and recruit immune cells (Fig 2). Despite this multipronged defense system, the respiratory mucosa can be overwhelmed by air pollution, which leads to impaired host defense, decreased barrier function, and augmented induction of inflammatory mediators. As a result, excessive respiratory mucosal immune responses to air pollution can contribute to chronic respiratory disease pathologies, including asthma and chronic obstructive pulmonary disease (COPD), and the ability to defend the lung against inhaled pathogens.

The present review will provide an update of recent literature (2017-2019) in the context of seminal articles in the field with a focus on crosscutting host responses and genetic contributions to diverse air pollutant types. The literature reviewed focused on *in vitro* studies of human airway epithelial cells, clinical exposure models, and cohort studies. Host responses to allergen or tobacco smoke exposure are not discussed. Host responses to air pollution will highlight recent advances in the context of airway surface lining fluid, pathogen exposures, immune mediator production, oxidative stress pathways, and how genetic variants in these pathways influence individual-level responses.

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Terms in boldface and italics are detailed in the glossary on page 1990.

AIR POLLUTION COMPOSITION

Observational and experimental data linking air pollution to respiratory mucosal immune responses have been supported by a vast array of different compositions, concentrations, exposure durations, and frequencies both in isolation and in combination.^{4,5} The consistency and overlapping of the experimental findings (described below in greater detail), despite the variety of potential air pollution exposure conditions, suggest that dominant biological pathways are activated by nonspecific triggers contained within each composition. For the scope of the review, we define air pollution as a byproduct of human activities (eg, combustion and urban particulate matter) but not from natural processes (eg, viruses, bacteria, and allergens). In some cases we explore coexposure studies that have interrogated host responses to natural processes in the context of human activities.

Robust experimental designs routinely report detailed characteristics of source material used in experiments, including season and geographic source where the material was collected, definition of any combustion engine characteristics (fuel source, work load, engine model, temperature, humidity, and oxygen content), particulate matter quantities (in units per cubic meter), hydrocarbon combustion byproducts (nitrogen dioxides, and sulfur dioxides), and extraction and dilution parameters, to aid in interpretation of results from diverse exposures. Additionally, routine inclusion of National Institutes for Standards and Technology (NIST) standard reference materials (SRMs) ensures reproducibility of results and provides context for unique source materials generated across the globe in research laboratories.

Despite careful documentation of air pollution composition for observational and experimental studies, a real-world complexity

exists that air pollution exposure is rarely experienced in isolation and likely includes concomitant exposures to geogenic dusts, allergens, bacteria, and viruses.⁶ Furthermore, components of air pollution, including polyaromatic hydrocarbons (PAHs) produced from combustion engines, can undergo photochemical reactions and agglomeration that are influenced by meteorological events that are difficult to reproduce in research environments.⁷ Elemental composition and charge of pollution is also important to consider because various host defense cationic peptide functions can be compromised by introduction of charged components, as observed with residual *coal fly ash*.⁸

The composition of air pollution is dynamic and complex, changing from location to location⁹ and season to season,^{10,11} and influenced by human activities and meteorological events.⁷ Despite variations in compositions, common themes have emerged from air pollution exposure research, clearly defining an effect on respiratory mucosal immune responses important in lung health and disease. We define these in greater detail below and, where possible, highlight the source material.

Airway surface lining fluid, mucus, and junctions

Airway surface lining fluid is composed of periciliary sol with high water content, which is capped by a mucus component that can be shuttled in a directional fashion toward the laryngopharynx by ciliated epithelial cells.^{1,12} Airway and serous epithelial cells contribute to composition of the airway surface lining fluid by regulating ion and fluid transport in addition to secreting host defense peptides, including human **LL-37**, lysozyme, **β -defensins**, lipocalins, and secretory leukocyte protease inhibitor (Fig 2).^{2,3,13}

GLOSSARY

BIOMASS: Plant materials (eg, coal, charcoal, tobacco, wood, and crop waste) and animal waste (dung) used as fuel. Indoor biomass burning generates PAHs and is associated with COPD development in women. Traditional biomass burned in open fires and traditional stoves are used by almost half the world's population and contribute to more than 1 million COPD-related deaths annually.

COAL FLY ASH: A fine-grained, powdery particulate material produced from the burning of pulverized coal in a coal-fired boiler. Fly ash is collected in a coal-fired boiler through several different means, typically before the smoke reaches the smoke stack. Approximately 70% to 75% of fly ash generated is disposed of in landfills or storage lagoons.

CYTOCHROME P450: A protein group comprised of 20 different families involved in xenobiotic metabolism. The enzymes encoded by 6 cytochrome P450 genes (*CYP1A1*, *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4*) are responsible for metabolism of more than 90% of all commonly used drugs.

β -DEFENSINS: A distinct family of antimicrobial peptides produced by epithelial cells of mucosal surfaces, as well as by neutrophils, natural killer cells, and cytotoxic T lymphocytes. Defensins have direct antimicrobial activity, as well as the ability to activate inflammatory responses.

FARM DUST EXTRACT: In this particular study farm dust was collected from the stables of traditional German farms housing cows 50 km around Munich.

GEOGENIC DUST: Naturally derived particles often found in rural arid and agricultural areas; examples include volcanic ash, windborne ash from wildfires, and mineral dusts.

IL-6/SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION

(STAT3) SIGNALING: A signaling pathway operating through Janus kinase 1 tyrosine phosphorylation. Pathway signaling results in acute-phase reactant proliferation, differentiation, and costimulation. *STAT3* mutations result in hyper-IgE syndrome and deficient IL-17 production. IL-6 (important in T_H17 differentiation) deficiencies result in enhanced susceptibility to infection with *Candida* and *Listeria* species.

LL-37: A cathelicidin released from neutrophils and epithelial cells. LL-37 is induced by vitamin D.

MUCUS: A mixture of proteins and liquids, but 95% of its content is water. High-molecular-weight glycoproteins called mucins form a gel with water. Mucins interact with cilia to influence mucociliary clearance. MUC5AC is the predominant secreted mucin in airway surface epithelium.

THYMIC STROMAL LYMPHOPHOETIN (TSLP): A cytokine secreted by gastrointestinal, skin, and lung epithelial cells that promotes T_H2 differentiation through its effect on tissue dendritic cells and innate lymphoid cells. Its name arises from its initial identification in thymic stromal cells.

TIGHT JUNCTIONS: Supramolecular complexes composed of transmembrane proteins (occludin, claudins, and junctional adhesion molecules) and cytoplasmic proteins. Cytoplasmic proteins facilitate cytoskeletal interactions and intercellular signaling. Transmembrane proteins regulate cell adhesion, as well as paracellular permeability and cell migration.

TRANSEPITHELIAL ELECTRICAL RESISTANCE (TEER): A measure of how tightly an epithelium separates one compartment from another. TEER is dependent on how permeant tight junctions are to the ion diffusion.

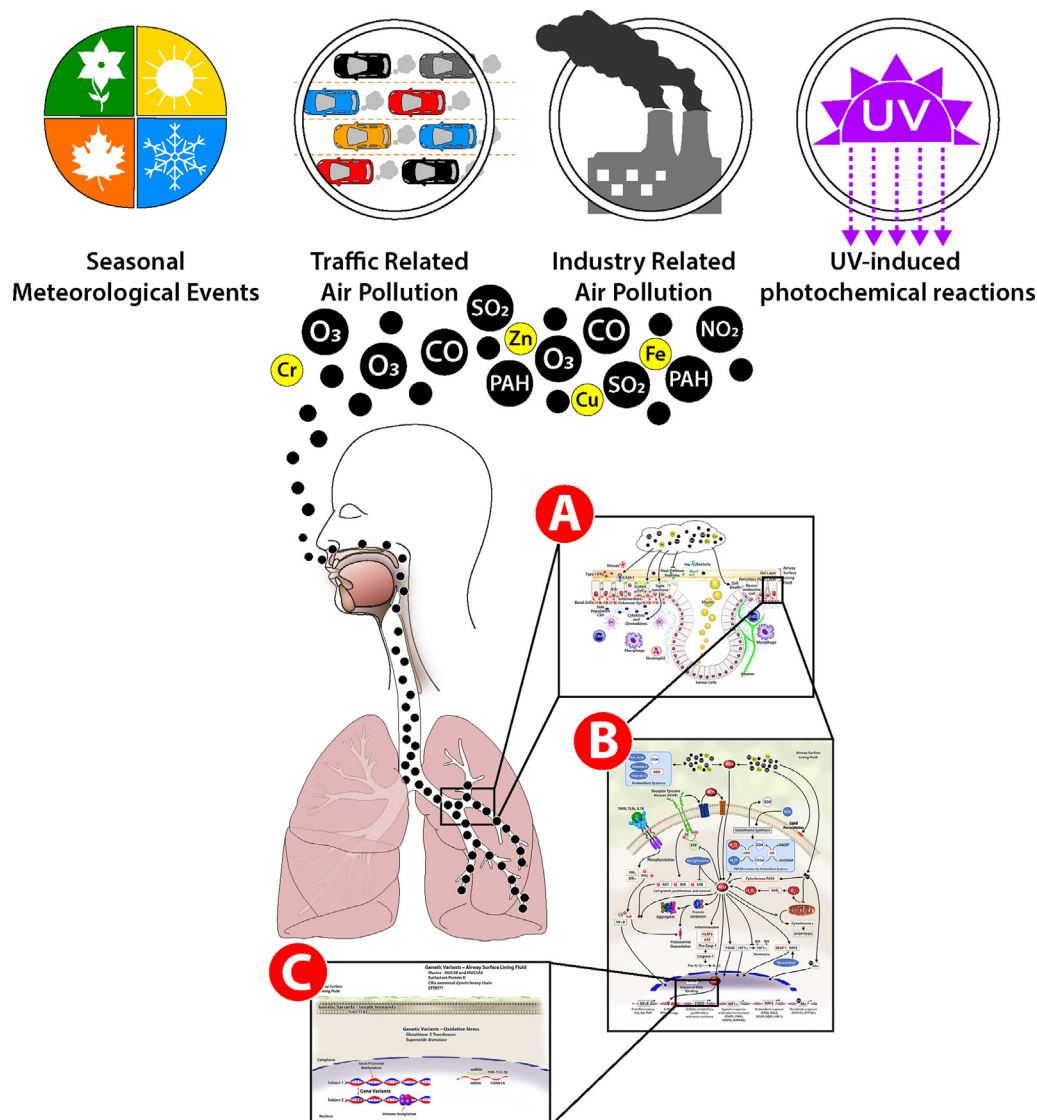


FIG 1. Overview of environmental air pollutants and their effects at the organ, tissue, cell, and genetic levels in the lung. Air pollution composition is a product of manmade and geogenic processes and is influenced by meteorological events, including temperature fluctuations, humidity, and UV light, all of which are affected by geographic location and season. Therefore air pollution composition is varied, but common processes include generation of ozone, sulfur dioxides, nitrogen dioxides, PAHs, carbon monoxide, and particulates of varying dimensions. Airborne particulates are inhaled into the lungs through the upper airway and deposit based on their physical and chemical characteristics (*Box 1*). The first point of contact for air pollution in the respiratory mucosa are the airway surface lining fluid and epithelial cells (*Box 2*). Within the airway surface lining fluid and epithelial cells, air pollution can trigger a variety of redox-sensitive pathways (*Box 3*) that can contribute to gene expression patterns designed to protect the host (*Box 4*). Overarching the host response to air pollution are genetic variants that might contribute to increased susceptibility to exposures.

The first point of contact for air pollution in the respiratory mucosa is the mucus layer of airway surface lining fluids.¹² Secreted mucins are affected by diesel exhaust exposure, with an increase in MUC5B and MUC16 levels observed in atopic patients in bronchoalveolar lavage fluid after 2 hours of exposure to 300 $\mu\text{g}/\text{m}^3$ PM_{2.5} from freshly generated diesel exhaust.¹⁴ MUC5AC is also upregulated by urban particulate matter (SRM-1649B) in a mechanism linked to early growth response gene 1.¹⁵ SRM-1649B exposure-induced activation of early growth response gene 1 leads to downstream activator protein 1 and nuclear factor κB (NF- κB) transcription factor activation,

2 pathways important in promoting proinflammatory immune responses.^{16,17} A reductionist approach using fluoranthene, benzo(a)pyrene, and benzo(b)fluoranthene, individual PAHs contained within SRM-1649B, revealed a consistent induction of MUC5AC. The aryl hydrocarbon receptor (AhR) recognizes diverse PAHs, suggesting that MUC5AC levels might be modulated by the AhR.

Beneath the airway surface lining fluid are differentiated epithelial cells of multiple types that form a tight mechanical impedance through cell-cell junctions.¹⁸ Disruption of tight junctions formed by zonula occludens 1 and E-cadherin

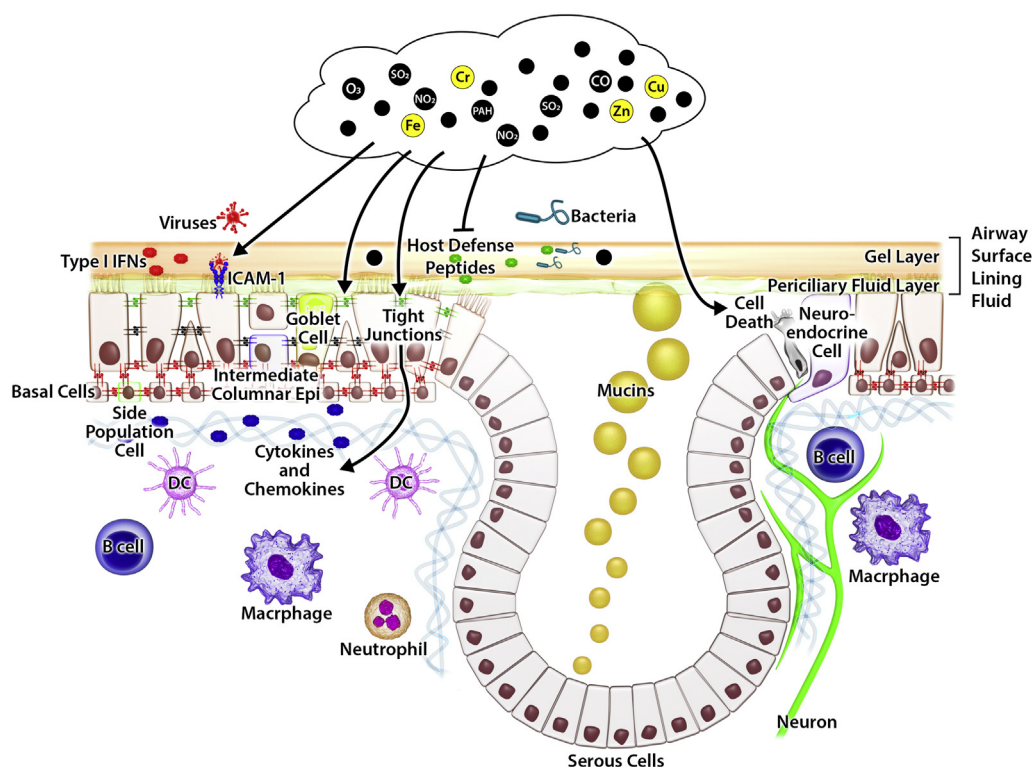


FIG 2. Respiratory mucosa in response to air pollution exposure. Air pollution gaseous and particle components interact with airway surface lining fluid; surfactants; host defense peptides; tight junctions; pathogen recognition receptors, including Toll-like receptors and NOD-like receptors; and redox-sensitive pathways (Fig 3) to modulate the viability and immune responses of airway epithelial cells and recruited immune cells.

complexes between cells can be measured experimentally based on a reduction in *transepithelial electrical resistance* or an increase in diffusion of fluorescently labeled probes (eg, fluorescein isothiocyanate–dextran).⁴ Air pollution exposure can decrease epithelial barrier function associated with cell death, although experimental conditions designed to reproduce the current exposure conditions from a Euro V diesel bus engine or a nonroad diesel engine operating at steady load do not reproduce these findings.^{19,20} Results from the same research group suggest that the effect of air pollution on epithelial barrier function is condition dependent and can only occur at high concentrations.²¹

Consequences for pathogen exposures

Air pollution exposure has been epidemiologically linked to increased respiratory tract infections in patients of all ages and in multiple jurisdictions.^{22–24} The mechanism or mechanisms responsible for these population-level observations of compromised respiratory mucosal immunity are likely multifactorial and could include changes in receptor expression for pathogens, antiviral mechanisms, or host defense peptide biology.

Intercellular adhesion molecule-1 (ICAM-1) is a receptor for human rhinovirus B, which infects epithelial cells of the respiratory mucosa to induce cold symptoms, with more severe consequences for those with underlying chronic respiratory diseases.²⁵ ICAM-1 is upregulated by air pollution in lung epithelial cells mediated by NF- κ B transcriptional events downstream of oxidative stress–induced classical

IL-6/signal transducer and activator of transcription 3 signaling.²⁶ Coexposure of airway epithelial cells to rhinovirus 1B and nitrogen dioxide (NO₂) appears to induce a synergistic upregulation of ICAM-1, which could exaggerate the infectivity of epithelial cells.²⁷ The IL-8 cytokine response induced by rhinovirus 16 and pollution (NO₂ or ozone) can be attenuated by N-acetyl-cysteine (NAC), suggesting that oxidative stress could affect ICAM-1/rhinovirus responses in epithelial cells of the respiratory mucosa. Interactions between air pollution and the low-density lipoprotein receptor, the receptor for rhinovirus-A, or cadherin-related family member 3, the receptor for rhinovirus-C, have not been examined in the context of air pollution to date. Importantly, cadherin-related family member 3 was only discovered on air-liquid interface differentiated primary human airway epithelial cells,²⁸ which emphasizes the value of increasing complexity in cell-culture model systems that more closely recapitulate human physiology.

PAHs are a byproduct of fossil fuel combustion and biomass. PAHs can induce oxidative stress and are ligands for AhR, which subsequently mediates transcriptional responses that affect cellular detoxification and survival pathways.²⁹ In primary human airway epithelial cells, activation of AhR by diesel exhaust particles (NIST SRM1975) is able to attenuate basal and induced levels of CXCL10, a cytokine important in coordinating antiviral responses in immune cells expressing the CXCR3 receptor.³⁰ Activation of AhR by organic combustion is conserved with a variety of biomass materials, including dung from diverse animals with distinct diets, all of which induce concentration-dependent increases in IL-8 and GM-CSF from primary human

airway epithelial cells.³¹ Transcriptional activity related to antiviral responses was further interrogated by using an interferon-sensitive response element luciferase reporter cell line, demonstrating that exposure to dung smoke impaired responses to viral mimic challenge. Furthermore, CXCL10 gene and protein induction by viral mimic challenge was attenuated if prior exposure to dung smoke had occurred. Diverse compositions of the exposures used in these 2 studies converge on a likely mechanism of AhR activation/oxidative stress, attenuated interferon transcriptional activity, and reduced production of mediators of antiviral responses by human airway epithelial cells.

Air pollution also affects the activity of host defense peptides, evolutionary conserved defenses that disrupt pathways important in survival of pathogenic bacteria,³ in airway surface lining fluid.^{8,32,33} Any interference with host defense peptide biology by air pollution could increase host susceptibility to infection. Human airway epithelial cells exposed to residual coal fly ash (NIST SRMs 2689, 2690, and 2691), a byproduct of coal power generation, showed a reduction in host defense peptide expression and activity.⁸ Coal fly ash resulted in decreased killing of *Staphylococcus aureus* in human bronchial explants and reduced LL-37 levels, human β -defensin-3 levels, and lysozyme killing of bacteria in cell-free experiments with recombinant host defense peptides. Interestingly, lysozyme demonstrated complete irreversible neutralization by coal fly ash within minutes, suggesting that acute pollution exposures could result in extended susceptibility of hosts to inhaled pathogens. Urban particulate matter (NIST SRM 1649B) exposures with the human airway epithelial cell line BEAS2B have produced similar results, with an impairment of human β -defensin-2 gene and protein expression associated with impaired *Pseudomonas aeruginosa* killing.³³ Interestingly, reconstitution with human β -defensin-2 or treatment with the antioxidant NAC in conditions of urban particulate matter exposure rescued bacterial killing activity, suggesting that oxidative stress compromises host defense peptide expression and activities.

Diesel exhaust is also able to affect host defense peptide biology in human airway epithelial cells.²⁰ Using primary human airway epithelial cells from pooled healthy and age-matched patients with COPD, diesel exhaust exposure attenuated host defense peptide gene expression levels for human β -defensin-2 and S100A7 in response to nontypeable *Haemophilus influenzae* challenge.²⁰ The results were conserved in independent experiments with diesel engine exhaust from both a cold-start and warmed engine, modeling a diesel city bus driving cycle.¹⁹

The outcome that air pollution exposure has on respiratory mucosal immunity is highly dependent on the composition because exposures to geogenic materials that can contain more microbial components can actually increase host defenses. Primary human airway epithelial cells from healthy subjects exposed to a *farm dust extract*, followed by challenge with human rhinovirus 1B, demonstrated a reduced load relative to control values, which was associated with induction of the interferon-stimulatory gene viperin and interferon regulatory factor 1 independent of type I interferon responses.³⁴ Greater epithelial barrier resistance and capacity for repair after damage also accompanied the increased antiviral responses. The pathways regulating farm dust-induced antiviral immunity are thought to be related to microbial composition, with similar observations observed with the Toll-like receptor 2 agonist

Pam3CSK4. The enhancement of antiviral immunity in response to farm dust exposure further demonstrates the importance that the composition of the pollutant can have on respiratory mucosal immune responses.

Immune mediators and signaling pathways: Beyond IL-6, IL-8, and TNF- α

Air pollution can also affect cytokine production, which can contribute to the development or exacerbation of chronic respiratory diseases beyond the traditionally characterized IL-6, IL-8, and TNF- α .⁵ In the context of asthma, air pollution can induce *thymic stromal lymphopoietin* (TSLP) in airway epithelial cells,³⁵⁻³⁷ which can signal through the TSLP receptor to positively influence the development of T_H2-skewed immune responses.³⁸ *In vitro* diesel exhaust particles induce a concentration-dependent increase in TSLP levels from primary human airway epithelial cells from patients with mild asthma. TSLP induction was dependent on AhR and associated with increased AhR binding to the TSLP promoter. In patients with severe allergic asthma, bronchial biopsy specimens from subjects living in areas with high traffic-related air pollution, as determined by proximity to a major roadway, reveal similar inductions of TSLP protein in airway epithelial cells. Importantly, *in situ* TSLP expression levels were positively associated with AhR nuclear localization, with this patient subset responding to 4 months of anti-IgE therapy, as assessed by using the Asthma Control Test score and FEV₁.³⁷ Throughout this *in vitro* and clinical interrogation, TSLP was accompanied by similar trends in IL-33 and IL-25, additional cytokines potentially important in development and maintenance of the allergic asthma phenotype.³⁹⁻⁴²

In addition to air pollution-associated augmentation of T_H2-skewed immune processes that might be relevant for asthma pathophysiology, evidence also supports that T_H17 responses can be affected in a similar fashion in a distinct patient phenotype. T_H17 immune profiles can be associated with severe steroid-resistant asthma and exposure to diesel exhaust.^{43,44} IL-17A gene and protein expression is upregulated in human airway epithelial cells from patients with mild asthma in response to diesel exhaust particles in an NAC-sensitive manner.⁴⁵ *In situ* gene and protein expression demonstrate that IL-17A expression in airway epithelium is increased in patients with severe asthma who are in close proximity to a major roadway in contrast to patients with severe asthma who are not (defined as 1000 m away from a highway or freeway).⁴⁵ Induction of IL-17A was NF- κ B dependent and attributed to the RelB subunit binding to the IL-17A promoter region.

INTRACELLULAR RESPONSES: OXIDATIVE STRESS

A major theme of recent studies examining air pollution's negative effect on respiratory mucosal immune responses is disruption of oxidation-reduction (redox) homeostasis and the activity of the AhR pathway. Redox signaling and homeostasis consist of a complex network of molecules, signaling proteins, and enzymes that maintain a balance between the production and catabolism of reactive oxygen species (ROS) and reactive nitrogen species (RNS) within and surrounding the cell (Fig 3).^{46,47} ROS is a term that comprises a diverse range of

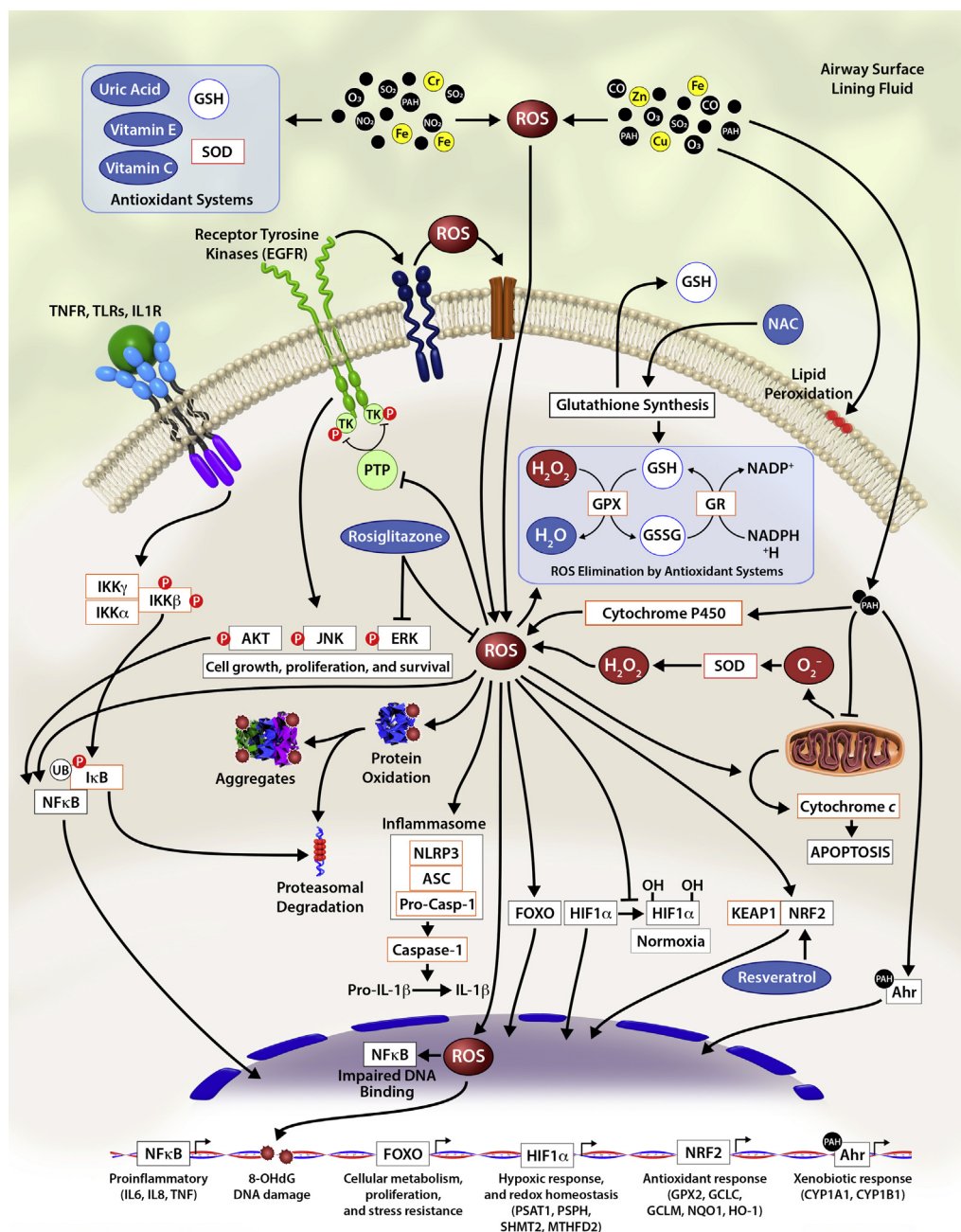


FIG 3. Effect of air pollution on oxidation reduction homeostasis and signaling. Air pollution contains and generates ROS in the respiratory tract lining fluid, as well as in intracellular compartments through NADPH oxidase, SOD, metabolism of PAHs by using cytochrome P450, and redox cycling in the epithelial lining fluid, cytoplasm, and mitochondria. PAHs bind and activate AhR, a transcription factor that induces expression of cytochrome P450 enzymes that amplify intracellular ROS in response to air pollution. ROS can be shuttled between cellular compartments through membrane transporters and pores, such as aquaporins (AQP). Excess ROS is scavenged by antioxidants, such as uric acid, vitamin C, and vitamin E, and catabolized by enzymes, such as glutathione peroxidases (GPX), which oxidizes GSH to glutathione disulfide. Increased ROS values are associated with increased receptor tyrosine kinase and mitogen-activated protein kinase signaling by inhibiting protein tyrosine phosphatases (PTP) through transient oxidation of active site cysteines. ROS-mediated protein oxidation contributes to dissociation of the NF- κ B regulatory subunit I κ B, as well as the NRF2 regulatory subunit Kelch-like ECH-associated protein 1 (KEAP1), leading to transcription of proinflammatory and antioxidant genes. ROS further amplifies inflammation through activation of the NLRP3 inflammasome, leading to increased IL-1 β production. ROS directly activates forkhead box O transcription factors and indirectly increases hypoxia-inducible factor 1 α (HIF-1 α) activation through inhibition of prolyl hydroxylases, resulting in transcription of genes involved in metabolism and redox homeostasis. Further increases in ROS levels oxidize and damage proteins, leading to aggregates; damage cell membranes through lipid peroxidation; damage DNA; and induce apoptosis. ERK, Extracellular signal-regulated kinase; FOXO, forkhead box O; GR, glucocorticoid receptor; GSSG, glutathione disulfide; JNK, c-Jun N-terminal kinase.

molecules, such as superoxide, hydroxyl radicals ($\text{OH}^\bullet$), peroxides (H_2O_2), and ozone, and similarly RNS to molecules, such as nitrate, peroxynitrite, nitrogen monoxide ($\text{NO}^\bullet$), and NO_2 . Accumulation of these species beyond the capacity of the cell's antioxidant systems is generally referred to as oxidative stress. Indeed, mucus, host defense peptides, antiviral responses, and cytokine production all appear to be influenced by oxidative stress and the AhR pathway. We describe below recent advances in our understanding of how oxidative stress and AhR function in human airway epithelial cells in response to air pollution exposure.

ROS are generated from a variety of endogenous sources, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, mitochondrial complexes (I-III), superoxide dismutases (SODs), xanthine oxidases, and cytochromes P450. To prevent oxidation and damage of cellular proteins, lipids, and other macromolecules, the amount of ROS is tightly controlled by antioxidant systems that include enzymes that catabolize the ROS species, such as catalases, thioredoxins, glutathione peroxidases, and peroxiredoxins, scavenging molecules, such as vitamin C and E, as well as transport systems, such as aquaporins and peroxiporins.^{46,47} In the event that cellular antioxidant systems become overwhelmed by ROS, redox-sensitive transcription factors, such as NF- κ B, forkhead box O, hypoxia-inducible factor 1 α , and nuclear factor-like 2 (Nrf2), translocate to the nucleus and orchestrate the expression of proteins involved in antioxidant systems, inflammation, survival, and repair.

Epithelial cells in the respiratory mucosa are also exposed to ROS and RNS from a variety of exogenous sources, including diverse compositions of air pollution. Although the composition and size of air pollution varies depending on the time of year and the proximity to industry and transportation hubs and thoroughfares,^{7,9-11} it is mainly comprised of particles, heavy transition metals, and reactive gases and organic compounds.^{11,21,48-54} Reactive gases and organic compounds, such as ozone, hydroxyl radicals, sulfur and nitrogen species, and PAHs, participate in a number of ROS-generating reactions in the airway surface lining fluid.³² A major redox reaction cycle in the airway surface lining fluid involves the donation of electrons from antioxidant compounds to transition metals, such as Cu^{2+} and Fe^{3+} , as well as a class of PAHs called quinones. The resulting metal ions and semiquinones are regenerated by stripping electrons from O_2 , creating superoxide radicals, which are converted to H_2O_2 by extracellular SODs and Cu^+ and Fe^{2+} in Fenton-like reactions.³² Kinetic models predict the amount of ROS produced in the surface lining fluid from an exposure to $\text{PM}_{2.5}$ of greater than $50 \mu\text{g}/\text{m}^3$ for 2 hours would range from approximately 100 to 250 nmol/L compared with approximately 100 nmol/L found in the bronchial alveolar lavage fluid of healthy human subjects.^{32,55}

Along with the diffusion or import of ROS from airway surface lining fluid, air pollution exposure also stimulates intracellular ROS production. Many recent *in vitro* studies have examined the effect of various models of air pollution, which include ambient outdoor pollution around the world collected on filters,^{11,45,48,49,52-54,56-60} fresh traffic-related air pollution generated by gasoline or diesel engines,^{19-21,50,51,61} SRMs,^{26,62,63} and fresh wood smoke,⁶⁴⁻⁶⁷ and have observed dose- and exposure-dependent increases in intracellular ROS or associated oxidation of proteins or DNA.

Increased production of intracellular ROS on air pollution exposure is thought to be the result of increased activity of xenobiotic metabolism, disruption of the mitochondrial membrane, and increased levels of nitrogen oxides. Xenobiotic metabolism and detoxification of PAHs involves the **cytochrome P450** family of enzymes, such as CYP1A1 and CYP1B1, expression of which is induced by PAH activation of the AhR. Indeed, air pollution and benzo(a)pyrene, a known carcinogenic PAH in air pollution, has induced expression of CYPs in BEAS2B cells,^{50,54,58,61} expression that was blocked by the AhR inhibitor α -NF.⁵⁴ Increased CYP expression has also been observed in the context of wood smoke exposure to A549 cells.⁶⁴

The metabolism of PAHs through cytochrome P450 produces ROS as a byproduct of the catabolic pathway, as well as quinones and semiquinones, which can subsequently produce more ROS through redox cycling.⁶⁸ Further supporting this mechanism, Leclercq et al^{52,62} observed upregulation of many cytochrome genes, benzo(a)pyrene metabolites, OH-benzo(a)pyrene DNA adducts, and ROS-induced modifications of protein and DNA in differentiated primary human bronchial epithelial cells that were grown at the air-liquid interface and exposed to SRM2786. Interestingly, Leclercq et al also found that cells from donors with COPD had significantly more OH-benzo(a)pyrene DNA adducts and ROS-induced macromolecule modifications than those from healthy donors, suggesting that patients with chronic respiratory diseases might be more susceptible to the byproducts and molecular damage caused by increased ROS values.

Another major source of endogenous ROS are mitochondria. Oxidative phosphorylation produces superoxide molecules that are rapidly converted to H_2O_2 by SODs before being further catabolized by antioxidant enzymes. However, alteration of mitochondrial function can lead to increased rates of ROS formation, and disruption of the mitochondrial membrane can release stored ROS.⁴⁷ BEAS2B cells exposed to a specific quinone 1,2-naphthoquinone, which is found on the surfaces of particulate matter, were found to have reduced maximal respiration through a mechanism upstream of pyruvate transport, increased spare respiratory capacity, unaffected ATP production, and increased nonmitochondrial respiration.⁶⁹ The increase in nonmitochondrial respiration coincided with an increase in H_2O_2 levels and was found to be NADPH dependent, suggesting 1,4-naphthoquinone might be driving a redox cycle reaction.

Leclercq et al⁵⁶ also examined the effect of $\text{PM}_{2.5}$ on mitochondrial structure and function in a differentiated primary epithelial model. They found $\text{PM}_{2.5}$ exposure was associated with abnormalities in mitochondrial volumes, matrix brightness, and cristae number. Supporting this observation, they measured concentration-dependent increases in SOD activity and mitochondrial superoxide that corresponded with a decrease in mitochondrial membrane potential, basal oxygen consumption rate, and ATP production. However, these cells did not show signs of activated caspase cascades associated with mitochondrial-induced apoptosis.

Expanding on this work, Song et al⁵⁹ metabolically profiled BEAS2B cells exposed to $\text{PM}_{2.5}$ collected Taiyuan, China. Analysis of the metabolites in $\text{PM}_{2.5}$ -exposed cells revealed increased purine metabolism through the xanthine oxidase pathway (produces ROS as a byproduct) and arginine metabolism. The metabolism of arginine through nitric oxide synthases produces NO, which can be converted to more reactive

RNS that can inhibit or irreversibly damage mitochondrial complexes I to V. Consistent with Leclercq et al.,⁵⁶ metabolic profiling revealed a general shift from oxidative phosphorylation to glycolysis, an observation supported by significantly reduced basal and maximal respiration rates and decreased ATP production. Together, these studies demonstrate the negative effect of air pollution on mitochondrial function and ROS production.

After exposure to air pollution, the first line of defense against ROS and RNS within the respiratory mucosa is a combination of scavenging antioxidant molecules and antioxidant enzymes. Glutathione (GSH) is one of the major antioxidant systems cells use to reduce ROS levels. GSH can be oxidized by glutathione peroxidase to glutathione disulfide, resulting in conversion of H₂O₂ to H₂O. Subsequently, GSH can be regenerated by glutathione reductase by using NADPH.⁴⁶ Oxidation of GSH and subsequent increase in glutathione disulfide levels have been demonstrated in various epithelial cell models exposed to air pollution from a variety of sources.^{11,53,59,60,62} Acute air pollution exposure has also been associated with increased GSH levels in human bronchoalveolar lavage fluid,⁷⁰ where ROS are scavenged by antioxidants, such as vitamin C and E.⁷¹ Exposure to air pollution has been associated with increased uric acid release into the bronchoalveolar lavage fluid in human subjects,⁷⁰ which is likely produced and exported by lung epithelial cells.⁷² Demonstrating the importance of these mechanisms, air pollution exposure has also been correlated with decreases in lung function and circulating α -tocopherol (vitamin E) and threonate (a metabolite of vitamin C) levels.⁷³

As ROS levels continue to accumulate in airway epithelial cells exposed to air pollution, excess ROS triggers mitogen-activated protein kinase signaling cascades, as well as activation of the transcription factors Nrf2 and NF- κ B. Oxidation of Kelch-like ECH-associated protein 1 releases Nrf2, which then translocates to the nucleus and initiates transcription of several antioxidant and detoxifying enzymes. Primary human bronchial epithelial cells exposed to PM_{2.5} were found to have dose-dependent increases in Nrf2 binding activity and transcription of target genes, including *NFE2L2*, the glutathione peroxidases *GPX1* and *GPX2*, and a subunit of a rate-limiting enzyme in glutathione synthesis, *GCLM*.⁵⁶ Interestingly, induction of Nrf2 genes was reduced in cells exposed to the highest dose of PM_{2.5}, possibly through increased oxidation and degradation, suggesting a limit to the antioxidant response lung epithelial cells can mount. Similar results have been observed in a number of studies examining primary human bronchial epithelial cells,^{19-21,66} epithelial BEAS2B cells,^{59,74} and alveolar A549 cells⁵⁷ exposed to air pollution.

The consequences of increased ROS and RNS values that exceed the capacity of antioxidant defenses in airway epithelial cells include DNA oxidation and damage, lipid peroxidation and compromised membrane integrity, protein oxidation and/or nitration, and cell-cycle arrest and apoptosis depending on the severity of the damage. In primary human bronchial epithelial cells air pollution (SRM2786) has been demonstrated to induce production of malondialdehyde (a secondary product of lipid peroxidation) and increased carbonylated protein levels and DNA 8-OHdG adducts.⁶² Consistent with the idea that loss of viability is related to air pollution-driven DNA damage by ROS, Wu et al.⁵³ observed a dose-dependent decrease in S-phase cell counts and an increase in G2/M-phase cell counts in a BEAS2B model, which

was indicative of cell-cycle inhibition at DNA damage checkpoints. Similar results have also been observed in other recent *in vitro* models of air pollution exposure and studies that observed autophagy,^{59,75} cell-cycle arrest,^{76,77} and loss of cell viability at greater exposure concentrations.^{52,53,56,59} Of clinical relevance, ROS and RNS have also been linked to decreased glucocorticoid receptor function,⁷⁸ suggesting air pollution can affect glucocorticoid receptor function. However, a more insidious consequence of air pollution-induced ROS and DNA damage is carcinogenesis,^{79,80} which can be amplified by the known carcinogen benzo(a)pyrene.⁸¹

The possibility of intervening in air pollution-induced ROS signaling and damage has been explored by several groups using compounds that target different aspects of redox signaling or homeostasis. One group found that rosiglitazone, which activates peroxisome proliferator-activated receptors, repressed the activation of extracellular signal-regulated kinase 1/2 and signal transducer and activator of transcription signaling and apoptosis in A549 cells exposed to PM_{2.5}.⁸² Rosiglitazone also reduced ROS production, although it is unclear through which mechanism. Antioxidants, such as chebulic acid⁸³ and gallic acid,⁶⁰ vitamin D,⁸⁴ and the Nrf2 activator resveratrol,⁸⁵ have also been implicated in attenuating air pollution-induced oxidative stress and inflammation. However, the intervention that has been examined the most to date is NAC, which is both an antioxidant and precursor in GSH synthesis. Several studies have demonstrated the protective effect of NAC against air pollution *in vitro*,^{26,74,86,87} and NAC has been shown to be protective *in vivo* against increased airway hyperresponsiveness induced by acute exposure to diesel exhaust.⁸⁸ Although these compounds show some promise, further development of interventions targeting ROS homeostasis in the lung epithelium can have broad clinical applications.

OVERARCHING MECHANISMS: EPIGENETICS AND GENETIC VARIANTS

The reaction of any subject's airway epithelium to air pollution is dictated substantially by a complex interplay between baseline genetic traits and prototypic genomic responses that underlie common pathways in response to the inhaled threat. The role of DNA, its variation across a population, and the relevant downstream machinery leading from it to functional protein in mediating the epithelium's role in subclinical and clinical implications of air pollution is discussed in this section and summarized in Fig 4. Broadly speaking, epithelial genomics wield their influence in 2 ways: by determining, in part, how the cell surface initially handles the incoming pollutant and by mediating intracellular processes downstream of initial contact. The current section focuses on how these pathways are influenced by both inherent genetic variability and epigenetic dynamics.

The first interaction of inhaled pollution and the epithelium will be with mucus, surfactants, cilia, and cell-surface receptors, all of which are influenced by genetic variants that affect function; direct and indirect evidence provide plausibility for how such variants can interact with pollution to influence airway physiology. The genetic influence on mucus is complex, but its relevance in this context is plausible given mounting evidence for the role of mucin gene variants in asthmatic patients⁸⁹ and the role of air pollution-induced mucin production.¹⁴ Surfactants are critical to the epithelial response to the gaseous fraction of

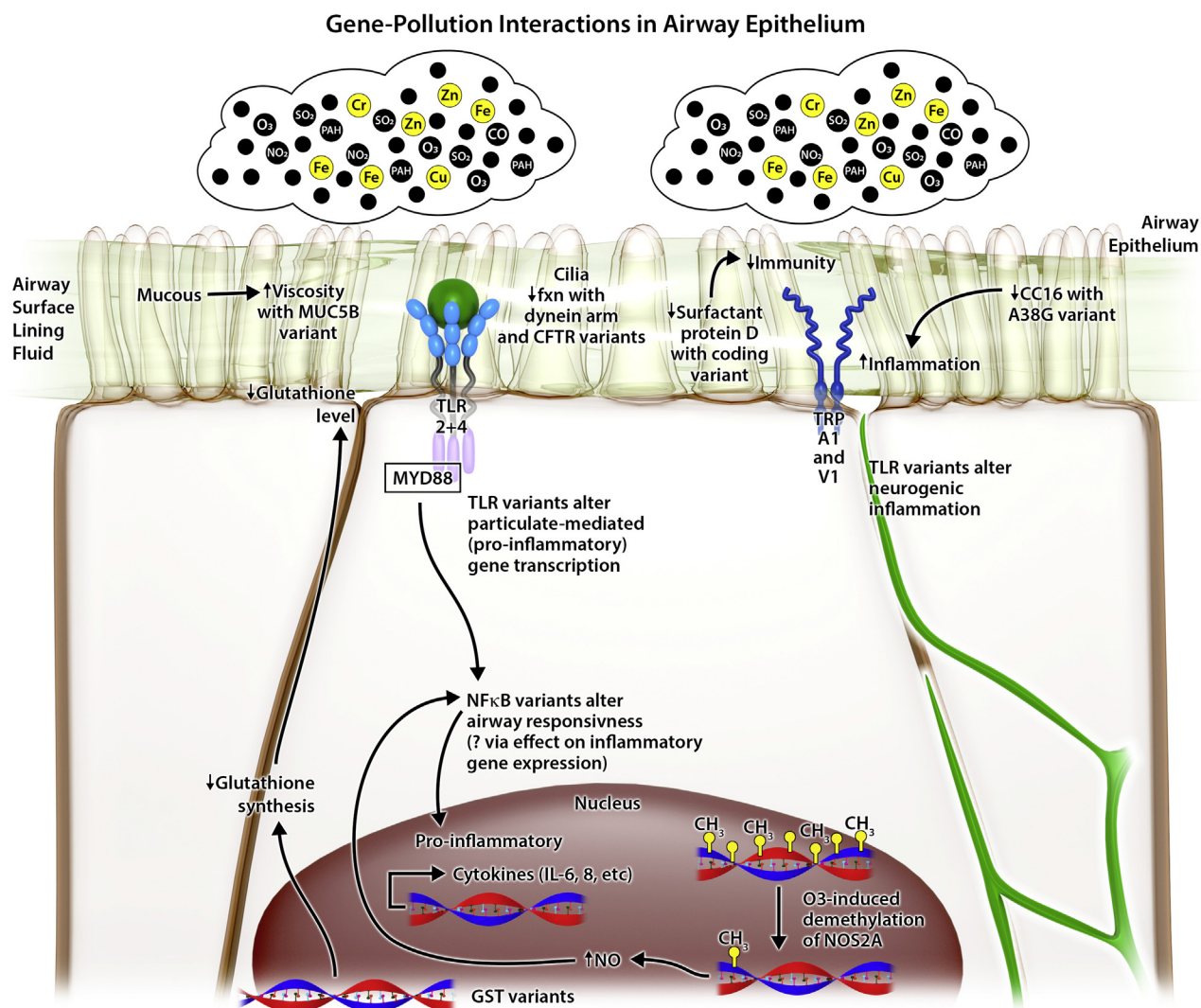


FIG 4. Gene-pollution interactions occur both at the epithelial surface (in terms of mucous, surfactants, antioxidants, cilia, and immune-related receptors) and intracellularly. Depicted are established or plausibly proposed examples of where gaseous and/or particulate pollution exerts variable effects based on its mediation through a gene having a relevant functional variant. *CFTR*, Cystic fibrosis transmembrane conductance regulator; *GST*, glutathione-S-transferase; *MYD88*, myeloid differentiation primary response gene 88; *TLR*, Toll-like receptor.

ambient combustion-derived aerosols, perhaps most notably ozone⁹⁰; surfactant protein D in particular has been shown to modulate the response to ozone and appears to have important genetic variability that influences airways disease.⁹¹ Regarding cilia, variants related to the axonemal dynein heavy chain predict poor lung function,⁹² and adverse effects of such variants can be compounded by exposure to pollution. Regarding surface receptors, transient receptor potential channels and Toll-like receptors 2 and 4 are among those that exist on the airway epithelial surface, appear to be important in mediating responses to traffic-related pollution,^{90,93,94} and have functional variants that alter risk for inflammatory airways disease.^{95,96} Whether PM would interact functionally in variant-induced susceptibility is uncertain,⁹⁵ but this seems most likely for *TRPV1*.⁹⁷ Given recent findings in another disease related to epithelial barrier function (eczema), it might be that a constellation of unfavorable genetic variants need be present to confer detectable risk.⁹⁸

The most substantial influence of genomics within the full cascade of pollution-driven events in the airway epithelium, from initial surface contact to later pathophysiology, is in response to oxidative stress, much of which will be intracellular (Fig 3 and associated text). Gene-ROS interplay can operate at the cell-surface level in terms of antioxidant response to challenge, such as with the *SOD* family.^{99,100} Particularly influential in this regard, given the high concentration of glutathione in airway lining fluid, is the glutathione-S-transferase family, especially *GSTP1*, variability of which has been repeatedly shown to confer risk for decreased airflow in those exposed to air pollution.¹⁰¹ Arguably even more consequential is variability in the genome's ability to mediate exposure-induced intracellular ROS, given lynchpin transcription factors' cascading effects therein. For example, those variant in NF-κB demonstrated markedly increased airway responsiveness on inhaling diesel exhaust relative to those that are normal in NF-κB.⁸⁸ Collectively, insight

into genomic determinants of epithelial defense against ROS might be especially critical in that it can inform personalized preventive and therapeutic strategies.¹⁰²

Finally, in terms of pollution and epithelial epigenetics, a novel human study revealed that diesel exhaust along with allergen alters epithelial cell DNA methylation around pathways determining transcription factor activity, protein metabolism, and cell adhesion.¹⁰³ In terms of gases specifically, others demonstrated how the effects of ozone can be modulated by *NOS2A* hypomethylation in the arginase–nitric oxide synthase pathway, which was associated with an increase in inducible nitric oxide synthase and fraction of exhaled nitric oxide in a human model.¹⁰⁴ Using primary human bronchial epithelial cells removed from their native environment, Bleck et al³⁶ showed miR-375 to regulate TSLP in the context of particulate matter. Subsequently, using an *in vivo* human model and an agnostic approach, others found a broad range of microRNAs within the epithelium to be altered by diesel exhaust.¹⁰⁵ Clinical application of these discoveries remains an opportunity.

In summary, genetic determinants appear to be highly influential in initial and subsequent responses of the airway epithelium faced with air pollution; these effects, which are likely to vary by specific inhalant (eg, by precise mixture of gases and particles), coexposures, and cell-cell interactions, center around response to oxidative stress, but other functions are increasingly plausible. In recent years, this area of research has not received significant attention despite earlier publications demonstrating that host response to ozone exposure is affected by genetics.^{106–108} Overall, the potential for gene-pollution interactions specific to the airway epithelium remains underexplored and yet holds great promise for biological insight, leading to preventive and therapeutic application.¹⁰⁹ A particularly exciting area for ongoing exploration is to delineate how variants in DNA sequences influence epigenetic responses to pollution and how this interaction itself might explain pollution-related changes in downstream functional layers (eg, proteomics).¹⁴

CONCLUSIONS

The respiratory mucosa is a mechanical and immunologic barrier that protects the lung from the 10,000 L of air we breathe each day. Under normal circumstances of high air quality, the airway epithelial mucus and airway surface lining fluid, tight junctions, host defense against bacteria and viruses, and oxidative stress responses all work in tandem to maintain lung health. In periods of poor air quality with high particulate and gaseous air pollution, excessive oxidative stress can occur, overwhelming redox-sensitive pathways, increasing inflammatory cytokine production, attenuating host defense peptide and antiviral responses, and disrupting tight mechanical barriers. Despite the recent advances in understanding how air pollutants affect host responses to induce a proinflammatory environment, it is clear from studies on host defense peptides that pollutants can also attenuate host responses, leading to susceptibility to subsequent exposures. We recommend that future investigations strike a balance between focus on traditional proinflammatory responses (eg, IL-6 and IL-8) and host defenses (eg, β -defensins) after air pollution exposure.

The mechanisms responsible for compromising the epithelium on multiple fronts are not entirely clear, although the use of antioxidants has demonstrated a central role for oxidative stress as

a contributing factor. In select patient populations with underlying genetic variants of molecules in redox pathways that have reduced function, supplementation with antioxidants might be an approach that could benefit susceptible subjects with the aim of regulating the negative effect of air pollution on respiratory mucosal immune responses. The effect of relevant genetic variants on multifaceted responses, including those mediated by epigenetics, appears substantial and might be an opportunity for carefully chosen intervention to attenuate the adverse epithelial responses to air pollution.

KEY CONCEPTS AND THERAPEUTIC IMPLICATIONS

1. Air pollution composition is a product of manmade and geogenic processes and is influenced by meteorological events, including temperature fluctuations, humidity, and UV light, all of which are affected by geographic location and season.
2. The respiratory mucosa has several defense mechanisms, including mucus, tight mechanical barriers, host defense peptides, antiviral pathways, and cytokine responses, that work in tandem to protect the host and are all negatively affected by air pollution exposure.
3. A common mechanism for impairment of respiratory mucosal immunity by air pollution is activation of redox-sensitive pathways and induction of oxidative stress, which can potentially be managed by antioxidant interventions in susceptible populations.
4. Underlying all respiratory mucosal immune responses are individual genetic variations in genes involved in respiratory mucosal defense components, which contribute to diversity in susceptibility to air pollution exposures and represent an opportunity for precision management with antioxidants or other interventions.

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