

The Impact of Air Pollutants as an Adjuvant for Allergic Sensitization and Asthma

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The current global epidemic of atopy and asthma has been related to the changes in environmental exposures brought about by the development and expansion of industrialized societies. This article reviews the evidence supporting the fundamental role of air pollutants in fostering allergic inflammation of the airways, with emphasis on the molecular and genetic pathways that link ambient particulate matter (PM) exposure to the induction of proinflammatory changes and proallergic effects in the respiratory tract. We propose that the link between PM exposure and proallergic effects involves organic PM components that generate oxygen radicals capable of perturbing the redox equilibrium mucosal immune cells.

Introduction

A wealth of epidemiological studies has consistently demonstrated a link between air pollution and increased morbidity and mortality from respiratory disorders. Acute increases in air pollutants have been implicated as a cause of worsening symptoms in asthmatic children and in adults with chronic obstructive pulmonary disease. Chronic exposure to high levels of ambient pollution is associated with impaired lung development in children, leading to an overall decrease in pulmonary function that persists well into adolescence. In recent years, much effort has been devoted to understand the mechanisms by which air pollution causes respiratory disease. In the context of

asthma and allergic respiratory disease, the observations that ambient particulate matter (PM) is capable of inducing inflammatory responses in humans and animal models are of special interest. PM exposure produces systemic and pulmonary inflammation, enhances bronchial hyper-responsiveness in patients with asthma, and increases sensitization to airborne allergens. PM can target various cellular components of the respiratory wall, including bronchial epithelial cells, macrophages, dendritic cells (DCs), and lymphocytes. Although the molecular pathways whereby PM promotes allergic inflammation are still a matter of debate, it is increasingly recognized that the generation of reactive oxygen species (ROS) and oxidative stress plays an important role in this process.

Air Pollution and Allergen Sensitization

Numerous observations and epidemiologic studies have demonstrated a strong association between exposure to air pollution and increased rates of allergen sensitization [1–5,6••,7–20,21•,22–32,33•,34–40,41••,42–65,66•]. In 1987, Ishizaki et al. [3] studied the prevalence of Japanese cedar pollinosis in densely planted areas. They noted that despite identical pollen counts, there was a higher incidence (13.2%) of cedar pollinosis in individuals living adjacent to intercity main roads with heavy daytime vehicular traffic than in individuals living in less dense traffic zones (5.1%). Similarly, children living in urban areas exhibit higher rates of atopy compared with those from rural areas [4]. In 2000, Wyler et al. [5] examined the association between the presence of an allergic sensitization and the exposure to road traffic in Basel, Switzerland. They observed a positive association with sensitization to pollen that was more pronounced among individuals who had resided in the highly trafficked areas for at least 10 years.

Of the different types of particles found in polluted air, the particulates generated from vehicular emissions are particularly relevant to the increased prevalence of atopy in polluted urban environments. These particulates are generally small particles in the ultrafine range (particulates with an aerodynamic diameter < 100 nm) and

include primary particle emissions from the combustion of diesel fuel and secondary particles that result from the condensation of vapors containing semivolatile organics or sulfuric acid. Diesel exhaust particles (DEPs) account for more than 90% of the ultrafine emissions in most urban areas. These ultrafines are highest on or close to freeways and tend to agglomerate with themselves to form larger transition-phase particulates. Ultimately, ultrafines can end up in the fine particle fraction, also known as $PM_{2.5}$. The latter has an average aerodynamic diameter of 2.5 μm and is currently the particle type that is being used as the PM standard for the regulation of air pollution by the US Environmental Protection Agency. $PM_{2.5}$ is mostly comprised of crustal elements from soil and sea water, and only a small fraction of these particles constitute ultrafine particles (UFPs). In our opinion, UFPs pose greater danger to human health in urban areas due to their small size, large surface area, ability to be retained in the lung, and high content of redox-cycling organic chemicals [6••].

Fossil fuel combustion is responsible for a considerable amount of the adverse health effects due to ambient PM exposure. Janssen et al. [7] investigated the rates of allergen sensitization in children (ages 7–12) attending 24 schools located within 400 m from busy motorways in the Netherlands. They performed air pollution measurements over a 1-year time period at the schools and calculated the annual concentrations of particles smaller than 2.5 μm , soot, and NO_2 . This study showed an increased rate of outdoor allergen sensitization in relation to truck traffic and $PM_{2.5}$ levels, with odds ratios of 2.83 and 1.9, respectively. Children attending school in proximity to high traffic motorways had significantly increased respiratory symptoms characterized as current wheeze. Nordling et al. [8] explored this further in a prospective study of 4089 infants (ages 1–4). They estimated pollution levels by looking at emission databases describing traffic-related nitric oxides, PM_{10} (particles with an average aerodynamic diameter of 10 μm) and SO_2 as a proxy for home heating. These authors reported a significant association between persistent wheezing, decreased peak expiratory flow at age 4, and highest rate of sensitization to pollen in relation to high traffic and PM_{10} exposures. Similar results were demonstrated in another study in which children ages 4 years (2680 subjects) and 6 years (3061 subjects) were assessed under conditions of chronic $PM_{2.5}$ and NO_2 exposure [9]. $PM_{2.5}$ was significantly associated with asthmatic bronchitis, hay fever, and allergic sensitization to pollen. Similar associations were not observed in response to NO_2 exposure.

All considered, these studies provide strong evidence for increased risk of atopic disease and allergic sensitization in children exposed to ambient PM. Infants and children could have heightened sensitivity to PM, adverse health effects due to the immaturity of their respiratory tracts and higher ventilation rates. Whether these early onset effects could lead to long-lasting respiratory morbidity is indeed a matter of great public health concern.

Particulate matter and allergic inflammation

Although the pathogenetic mechanisms that foster allergic sensitization and asthma by air pollutants have yet to be fully elucidated, there is mounting experimental evidence that DEPs act as an adjuvant that promotes allergen sensitization and allergic inflammation in upper and lower airways. Intranasal coadministration of allergens and DEPs in asymptomatic allergic subjects has been shown to increase ragweed-specific IgE and mRNA transcripts of the T-helper 2 (Th2) cytokines interleukin (IL)-4, IL-5, IL-6, IL-10, and IL-13 [10,11]. Not only was this recall on secondary immune response significantly augmented by the DEP co-challenge, but the nasal IgE production persisted for days longer than the response to ragweed alone. This adjuvant effect could also extend to the primary immune response as demonstrated by animal and human exposure studies [12,13]. In one human study, 25 allergic subjects were intranasally exposed to a neoantigen, keyhole limpet hemocyanin (KLH), in the absence or presence of DEPs [13]. This study demonstrated that DEP administration at the same time as KLH exposure led to primary sensitization and production of anti-KHL-specific IgE in nine of 15 atopic individuals.

Animal studies have provided further evidence of the positive association between DEP exposure and allergic sensitization. In Norway rats, both intranasal and intratracheal sensitization with DEPs resulted in pollen-specific IgE production, which was associated with increased inflammation in the lung [14]. In mice, DEP exposure concurrent with an allergen resulted in increased antigen-specific IgE levels and Th2 cytokine expression (IL-5, IL-4, granulocyte-macrophage colony-stimulating factor [GM-CSF]), concomitantly with a decrease in the Th1 cytokine, $INF-\gamma$ [15–20,21•,26]. DEP exposure promotes infiltration of the airway by inflammatory cells, enhances airway hyperreactivity, and increases goblet cells and mucin production. Alessandrini et al. [21•] specifically addressed the effects of UFP exposure in ovalbumin-sensitized mice and nonsensitized controls. Particle exposure was performed 24, 96, or 168 hours before or 24 or 72 hours after ovalbumin aerosol challenge. The authors found that exposure of sensitized mice to UFPs for 24 hours before allergen challenge led to a significant increase in inflammatory cells in the bronchoalveolar lavage (BAL), characterized by an early boost of neutrophils (24 hours) and a later expansion in the number of eosinophils (7 days). UFP-exposed animals had increased airway mucous production, enhanced airway hyperresponsiveness, and increased expression of IL-4, IL-5, and IL-13 proteins. These adjuvant effects were still present if particle exposures were conducted up to 4 days prior to allergen challenge. In contrast, particle exposure in sensitized mice after allergen challenge resulted only in mild downmodulation of BAL fluid cytokines, lower inflammatory scores, and no significant alteration in mean score for airway hyperreactivity.

Relevant cellular responses involved in the adjuvant effects of particulate matter

Following inhalation, PM can target various cellular components of the respiratory wall, including bronchial epithelial cells, macrophages, DCs, and lymphocytes. Among these, two cellular types (epithelial cells and DCs) are largely responsible for the adjuvant effects.

Airway epithelium

Accumulating evidence suggests that airway epithelial cells, which constitute the interface between the internal and external environment, play a crucial role as both mediators and regulators of the innate and adaptive immune response in asthma and other respiratory diseases [22]. DEPs exert an immunomodulatory effect on the epithelial cells by augmenting innate proinflammatory responses and promoting initiation of adaptive responses. DEPs induce upregulation of endothelial adhesion molecules (intracellular adhesion molecule-1 and vascular cell adhesion molecule 1) in healthy human airways, thereby providing a mechanism for the rapid influx of neutrophils and other inflammatory cells into the airway [23]. In addition, DEP exposure results in the release of chemokines and cytokines that are important for the recruitment of an inflammatory cell milieu [24–26]. These include IL-8 and GRO- α -CXC, strong chemotactic factors for neutrophils, eosinophils, and T lymphocytes, and amphiregulin (an epidermal growth factor ligand that induces the release of GM-CSF), as well as macrophage inflammatory protein (MIP) 3 α /CCL20 and GM-CSF [26,27,35–37]. These cytokines/chemokines promote recruitment and local survival of immature DCs. DEP induction of cytokine/chemokine release proceeds, in part, through the activation of the MAPK pathways, specifically the ERK 1 and 2 pathways and the p38 pathway, which have been demonstrated to be involved in DEP-induced secretion of MIP 3 α , GM-CSF, IL-8, and amphiregulin from epithelial cells [28–31].

Exposure to DEPs induces the secretion of epithelial-derived cytokines that can promote the maturation of naïve T cells toward the Th2 and possibly the Th17 phenotype. Bleck et al. [32] demonstrated that immature monocyte-derived DCs (iMDDCs) isolated from the peripheral blood of healthy human volunteers failed to undergo phenotypic maturation (defined by the presence of CD80, CD83, CD86) in response to DEP exposure. In contrast, DEP exposure of primary human epithelial bronchial cultures was able to induce iMDDC maturation. They also were able to demonstrate T-cell activation only in cultures of human bronchial cells treated with DEPs. In subsequent studies, the authors showed that DEP-treated human bronchial epithelium secrete increased amounts of thymic stromal lymphopoietin (TSLP) compared with lipopolysaccharide and carbon black-treated cultures [33•]. TSLP is an IL-7-like cytokine that has been shown to activate DCs to polarize T cells to produce Th2 cytokines IL-4, IL-13, and tumor necrosis factor (TNF)- α . Accordingly, DCs

exposed to DEP-treated human bronchial epithelial cells induced Th2 polarization in a TSLP-dependent manner. Finally, multiple studies have shown that DEP exposure results in airway epithelial cell secretion of IL-6 [34,35]. This increased IL-6 production seen in DEP-exposed airway epithelial cells could conceivably promote Th17 differentiation that would synergize with Th2 responses to further drive allergic airway inflammation [36].

Dendritic cells

PM exposure has been shown to alter the function of several types of immune cells resident in the respiratory tree, including alveolar macrophages, DCs, and lymphocytes. DCs, antigen-presenting cells distributed throughout the lung, play a pivotal role in controlling the immune response to inhaled antigen. Located in close proximity to the mucosal surfaces, DCs rapidly respond to environmental cues such as infection or tissue damage, linking innate and adaptive immunity through their ability to induce the maturation and activation of naïve T cells. Chan et al. [37] first reported that DEPs induce oxidative stress that interferes with the Th1, promoting response pathway in murine bone marrow-derived DCs and in a homogeneous myeloid DC line. DC exposure to DEPs impaired DC maturation and IL-12 production in response to various Toll-like receptor agonists and decreased IFN- γ secretion following antigen stimulation. Notably, the DEP-induced alteration in DC function was shown to be secondary to oxidative stress and could be neutralized by the administration of the antioxidant, N-acetylcysteine. Similar observations were reported by Inoue et al. [38], who demonstrated that PM regulates DC activation/maturation and proliferation of antigen-specific T cells. In vitro treatment of human DCs with DEPs results in increased expression of costimulatory molecules CD40 and major histocompatibility complex class II (MHC II) [39]. Significantly, costimulation of DEP-exposed DCs and allo-reactive CD4 T cells increased production of the Th2 cytokines IL-13 and IL-18 while suppressing that of IFN- γ .

These studies illustrate the impact of PM on DC maturation and function, a property that is central to the promotion of atopy and asthma by environmental pollutants. The precise mechanisms behind the adjuvant effects of PM remain to be established; however, we discuss below that pro-oxidative components may alter the antigen-presenting function of the DCs by changing their redox equilibrium. PM characteristically has a high content of organic chemicals that include redox cycling compounds capable of generating ROS in target cells. DCs are likely to be one such target.

Particulate Matter–induced Oxidative Stress

Although the molecular mechanisms whereby PM promotes allergic inflammation are still a matter of debate, it is increasingly recognized that the generation of ROS

and oxidative stress play an important role in this process [40]. In addition to the contribution of the particles themselves, redox-cycling organic chemical compounds, such as polycyclic aromatic hydrocarbons and quinones, generate oxygen radical production in target cells such as the epithelium, macrophages, and DCs. Cellular production of oxygen radical species such as superoxide and hydroxyl radicals can induce oxidative damage to proteins, DNA, and membrane lipids. Radical production also leads to glutathione depletion (GSH) and can change the redox equilibrium of the cellular targets such as DCs. The changes in the cellular GSH/GSSG (glutathione disulfide) ratio lead to oxidative stress and constitutes a homeostatic mechanism that initiates a cellular protective response. When this protective response fails to restore the redox equilibrium, injurious cellular effects ensue.

In a series of groundbreaking studies, Li and Nel ([41••] and references therein) have provided evidence that PM-induced oxidative stress is a hierarchical event, in which protective cellular responses at lower levels of stress yield to proinflammatory effects at higher levels of oxidative stress. The dynamic equilibrium between protective and proinflammatory effects may determine, for instance, whether epithelial cells produce proinflammatory cytokines (eg, IL-8) or DCs that are capable of promoting Th1 or Th2 immune response. One mechanism by which this is achieved is the activation of the transcription factor nuclear factor erythroid 2–related factor (Nrf2). Nrf2 has been shown to induce the expression of protective phase II enzymes, such as heme oxygenase 1 (HO-1) and catalase, which exert antioxidant, detoxification, and anti-inflammatory effects. These protective cellular effects are capable of interfering in IL-2 production and Th1 immunity. Higher levels of oxidative stress activate the nuclear factor (NF) κ B and mitogen-activated protein kinase–signaling cascades that are responsible for transcriptional activation of proinflammatory cytokines, chemokines, and adhesion molecule genes. These pathways and products contribute to promoting allergic inflammation at many levels, including favoring Th2 immunity and turning off regulatory T-cell circuits.

Genetics and Epigenetics of Asthma and Oxidative Stress

Although asthma has heritable traits, it is a non-Mendelian disorder, which has been the focus of a multitude of genome-wide linkage studies, candidate-gene association studies, and more recently, genome-wide association studies. Following our improved understanding of the mechanisms by which air pollutants promote allergy and asthma, some recent efforts have centered on identifying susceptibility loci that confer altered lung responses to oxidative stress [42]. These studies highlight the intricate role of gene–gene and gene–environment interactions contributing to the asthma phenotype. Interestingly, the genetic footprint that has emerged in endothelial cells exposed to DEP extracts,

together with oxidized low-density lipoprotein, demonstrated clustering of antioxidant, proinflammatory, and apoptotic gene modules [43]. This footprint mirrors the hierarchical oxidative stress profile that was also confirmed by proteome analysis [44]. The differences in phenotype depend not only on the specific polymorphisms involved, but also on the specific ethnic population being investigated and the local air quality.

Current gene candidates important for understanding the role of PM-induced oxidative stress in asthma include glutathione-S-transferases (GSTs), NAD(P)H:quinone oxidoreductase 1 (NQO1), TNF- α , and Nrf2. GSTs are phase II oxidative stress enzymes important in combating the effects of xenobiotic compounds and ROS. In northern Europeans, the GSTP1 Val/Val105 polymorphism has been associated with protection against asthma compared with GSTP1 Ile/Ile [45]. A study of 158 asthmatic children in Mexico City showed that those with the GSTM1 null homozygous deletion polymorphism, present in 39% of the cohort, had significant decreased lung function with increasing ozone exposure [46]. Antioxidant supplementation with vitamins C and E in children with a GSTM1 null phenotype protected these subjects from an ozone-related decrement. Increased respiratory symptoms with increased ozone exposure also were found in children with GSTP1 Val/Val and GSTM1 null genotypes. Children with both genotypes had even greater breathing difficulties. Ozone delivers an oxidative stress stimulus to the lung similar to DEPs, and individuals with both NQO1 Pro/Pro 187 wildtype and GSTM1 null genotypes had decreased lung function and increased production of Clara cell protein, CC16, after exercise at high ozone levels of more than 80 ppb [47]. Similar effects were seen in studies by David et al. [48] and Chen et al. [49]. Importantly, the NQO1 wildtype isoform is a more active enzyme than the serine polymorphism and allows for increased production of ROS in the lung epithelium with ozone exposure.

TNF- α polymorphisms also have been shown to affect pulmonary responses to oxidative stress in children. TNF- α is a classical cytokine that is induced by oxidative stress in macrophages, including in response to DEP, UFP, and organic PM extracts. The -308 G/G TNF genotype was protective against current and lifetime asthma, especially in regions with lower ozone exposure [50]. This association did not differ between non-Hispanic whites and Hispanic whites. The protective effects were less pronounced or even absent (for lifetime asthma) in high ozone communities. Children with GSTP1 Ile/Ile or GSTM1 null genotypes also had a reduction in the protective effects of -308 G/G TNF. Of note, the -308 G/G TNF genotype did not confer protection with other pollutants, such as PM₁₀, PM_{2.5}, NO₂, and second-hand smoke.

Finally, Nrf2 plays an important role in the transcriptional activation of more than 200 antioxidant, anti-inflammatory, and cytoprotective genes that express an antioxidant response element (ARE) in their promoter [51]. Marzec et al. [52] identified an Nrf2 single nucleotide

polymorphism (SNP), -617 C/A, that resulted in a loss of function in vitro compared with the -617 C/C wildtype. Trauma patients with the -617 C/A SNP had a significantly higher risk for acute lung injury than those with the wildtype genotype. Further work on Nrf2 polymorphisms are needed to confirm their possible role in promoting oxidative stress effects in humans.

Aside from elucidating mechanisms of disease, the identification of at-risk polymorphisms for oxidative stress should allow for tailored treatments in specific populations, including the possibility to use antioxidant therapy for those that live in high pollution communities. The ability of nutraceutical chemicals, such as sulforaphane (present in broccoli), to promote Nrf2 release to the nucleus provides a natural means of boosting protective phase II responses. There is now compelling evidence of the efficacy of sulforaphane and broccoli extracts in boosting Th1 immunity in old mice [53] and suppressing inflammation in a variety of tissues [54]. We are currently conducting studies to assess the usefulness of broccoli extracts and sulforaphane in suppressing the proinflammatory and proallergic effects of prooxidant chemicals in ambient air pollution particles.

Epigenetic regulation describes both meiotically and mitotically heritable changes in gene expression that are not encoded in the DNA sequence [55]. Findings from multiple epidemiologic studies indicate that asthma risk may be modified by epigenetic regulation after early and later environmental exposures [56–60]. Additionally, experimental animal models demonstrate that intrauterine exposure to particulate air pollutants increases the risk for respiratory disease in the offspring. Prenatal exposure to an ambient PM surrogate, residual oil fly ash (particulate contaminants of an oil-fired power plant), led to a greater susceptibility in young mice to airway hyperresponsiveness and inflammation (ie, eosinophil infiltration and goblet cell hyperplasia) [61]. Most recently, prenatal exposure to DEPs or “inert” titanium dioxide was shown to promote increased airway inflammation and hyperresponsiveness in mice [62].

Mechanisms of epigenetic regulation include DNA methylation, changes to chromatin packaging of DNA by post-translational histone modification, and RNA interference. Growing evidence suggests that the Th2 polarization seen in asthma is driven, in part, by changes in DNA methylation and histone modification. DNA methylation involves the covalent addition of a methyl group to the cytosine residue in CpG sites mediated by DNA methyl transferases and accessory proteins, resulting in gene silencing. CpG sites are found in 5' CpG clusters termed 5' CpG islands (5' CGI), which range in size from 200 bp to 5 kb and have a high percentage of CpG (> 60%) [63]. Conversely, DNA demethylation results in transcriptional activation of genes. Various in vitro studies have shown that demethylation of the proximal promoter region of the IL-4 locus and hypermethylated IFN- γ gene locus polarizes lymphocytes to Th2 phenotype and promotes IL-4

production [64,65]. Importantly, a recent study showed that DEP-exposed mice sensitized to the powerful adjuvant *Aspergillus fumigatus* had hypomethylation of CpG sites in the IL-4 promoter region and hypermethylation of the IFN- γ promoter region. These changes in methylation patterns correlated with higher antigen-specific IgE levels in the DEP-exposed group [66•]. These studies suggest that epigenetic regulation may be one of the mechanisms by which DEP exposure facilitates the development of allergic inflammation in the airway and thus contributes to increased risk of atopy and asthma associated with environmental pollutants.

Conclusions

A growing body of evidence indicates that air pollutants are capable of inducing proallergic and proinflammatory responses in the airway, and that they do so, in part, by altering the redox equilibrium in target cells of the respiratory tree. Although the negative impact of air pollution on lung function is seen in the population as a whole, pollutant-induced inflammatory effects are subject to substantial interindividual variability. These differences likely respond to environmental and genetic factors. Oxidative stress and inflammation, processes involved in the response to air pollutants, are known to be under genetic regulation. A complete understanding of the molecular mechanisms involved in the proinflammatory effects of air pollutants will help identify those individuals who would particularly benefit from preventive or therapeutic modalities, including the use of antioxidant compounds.

Disclosure

No potential conflicts of interest relevant to this article were reported.

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