Air pollution and allergy: you are what you breathe

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How does air pollution affect asthma and allergic rhinitis? Particulate and gaseous pollution drive proallergic inflammation through the generation of oxidative stress, which is regulated by individual genetic susceptibility.

Airborne particulates and gases related to human activities, or ambient air pollution, are important environmental issues that affect human health. The use of fossil fuels (**Fig. 1**) has increased to the point that the resulting airborne particulates reflect enough energy to affect models of global warming and alter worldwide rainfall patterns. Despite considerable recent advances in controlling fossil fuel emissions, the unabated increase in the number of cars and trucks and the resulting increase in road miles have largely overshadowed these gains. Concurrently, refinement in epidemiological tools and air exposure assessments has provided more conclusive associative studies, leaving little doubt that air quality affects respiratory disorders and, in particular, allergic airway diseases. Thus, traffic-related pollutants such as ozone and nitrogen dioxide (NO_2) and particulate matter less than 10 μm and less than 2.5 μm in size have been linked to allergic responses, asthma exacerbation, wheezing and lung development in children[1]. Recent studies focusing on the involvement of pollutants in the development of asthma and the identification of susceptible populations have given a fresh impetus to laboratory-based studies aimed at dissecting the pathophysiology underlying the relationship between pollution and these diseases.

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Figure 1 Air pollution from increased use of fossil fuels is an important factor in the long-term increase in allergic airway disease. Photo: Ken Kiser (morguefile.com).

Nature of air pollution

Although we will focus on outdoor ambient pollutants, other factors such as occasional industrial-related release of local allergic or irritant material can also have profound effect on local ambient conditions and can lead to outbreaks of asthma and respiratory distress. Moreover, 'indoor' pollutants such as second-hand smoke and cooking combustion products can have similar or even greater effects on human respiratory health. Unlike ambient air, for which exposure is mostly unavoidable, the best strategy in dealing with indoor pollutants is probably to limit personal exposure.

Humans are not 'allergic' to pollutants; that is, people do not generate adaptive immune responses to pollutants *per se*. Thus, the issues facing immunologists relate to how airborne 'pollution' interacts with mucosal surfaces and underlying immune tissues to modulate the adaptive immune responses leading to adverse health outcomes. Such effects are separate although not necessarily distinct from the irritant effects of acute large amounts of pollutants, an effect that may be more pronounced in people with underlying airway disease, whether allergic or not. Although opinions have 'seesawed' over the greatest threat to respiratory health from air pollution, gaseous versus particulate pollutants, and coarse versus fine versus ultrafine particles, there is a general mechanistic commonality among pollutants,

so that regardless of the source, similar outcomes arise. We suggest that most airborne pollutants function as mucosal adjuvants and, in interacting with both innate and adaptive immune cells, skew the immune response to inhaled antigens toward a T helper type 2–like phenotype. When this occurs in the 40% or so of people who are 'genetically programmed' to make allergic responses (that is, atopic people), it can lead to increased expression of both the allergic response and allergic disease. In this context and given the ubiquitous nature of air pollution in the world today, it is critical to remember that the immune system always 'sees' inhaled cognate allergen in the context of a mucosal adjuvant, pollution.

The best-studied particulate pollutant is diesel exhaust particles (DEPs). The 3.3 million diesel trucks and buses in the US contribute 58% of respirable particulates produced from highway sources, although they make up less than 2% of the total vehicles[2]. In other parts of the world, the use of diesel vehicles is far more common. Of the gaseous pollutants, the main focus has been on ozone. The principal source of lower-atmosphere ozone is as an indirect product from automobiles as a consequence of photochemical reactions of nitrogen oxides and volatile organic compounds.

Pollution and airway inflammation

Several lines of evidence have shown that both particulate and gaseous pollutants can act both on the upper and lower airways to initiate and exacerbate cellular inflammation. Increased neutrophil, B cell and alveolar macrophage recruitment is seen in bronchoalveolar lavage fluid of both healthy and asthmatic people exposed to diesel exhaust in chamber studies or in nasal washes after nasal provocation challenges. Similar increases in inflammatory cells are found in bronchoalveolar lavage fluid after exposure to ozone, sulfur dioxide (SO_2) or NO_2. Presumably as a consequence of this inflammation, altered lung function including increased nonspecific airway hyper-responsiveness and increased airway resistance have been reported in humans, most notably after ozone exposure[3].

A common finding in these exposure studies is increased amounts of proinflammatory cytokines, chemokines and adhesion molecules. *In vitro* studies have demonstrated a direct effect of pollutants on the expression of these factors[4]. Ozone, SO_2 and DEPs can all cause release of soluble intercellular adhesion molecule 1 and proinflammatory cytokines such as GM-CSF and interleukin 8 (IL-8) from human normal bronchial epithelial cells *in vitro*. DEPs and their chemical constituents can also have similar effects on transformed bronchial and nasal polyps–derived upper airway epithelial cells. The production of GM-CSF and IL-8 by airway epithelial cells can be regulated by the cytokines IL-1 and tumor necrosis factor (TNF), whereas both DEPs and DEP extracts can induce *in vitro* production of these cytokines from pulmonary alveolar macrophages. Furthermore, supernatants from peripheral blood mononuclear cells stimulated with DEP extracts demonstrate enhanced chemotactic activity for neutrophils and eosinophils. Thus, there is abundant evidence that pollution can stimulate an inflammatory response through interaction with the innate immune system. In contrast to the findings with lipopolysaccharide or bacterial DNA, there is no clear evidence as yet of specific direct triggering of innate immune system receptors.

Interaction with allergen

As noted, in the real world, antigen is 'seen' in the context of pollutants. The study of this interaction of pollutant and allergen using human nasal provocation models has produced some of the most illuminating results[5]. DEPs can both induce and exacerbate *in vivo* allergic responses in the human upper respiratory tract. In atopic patients, in contrast to the two- to threefold increase in allergen-specific immunoglobulin E (IgE) produced with allergen alone, combined challenge with DEP plus allergen enhances local IgE production 20- to 50-fold. This potentiation of the IgE is accompanied by increased expression of IL-4, IL-6 and IL-13 but decreased expression of the T_H1 cytokine IFN-γ. Similar deviations into a T helper type 2 milieu have been seen in mice repeatedly challenged intratracheally

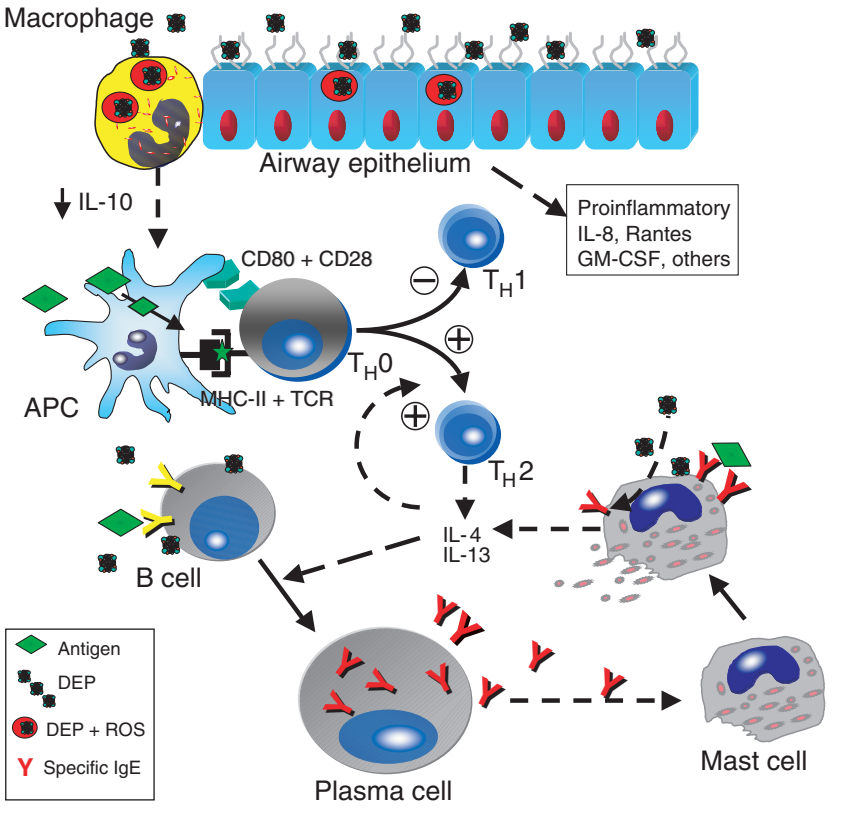

Figure 2 How DEPs modify the immune response to allergen. DEPs impact and are internalized by the airway epithelium and macrophages. The ensuing generation of oxidative stress causes the release of proinflammatory cytokines from both cell types. One outcome is increased antigen presentation because of decreased IL-10 plus upregulation of costimulatory molecules such as CD80 on antigen-presenting cells (APCs). In this setting, the APCs seem to favor the development of a T helper type 2 (T_H2) cytokine milieu, which has a positive feedback loop on IL-4 production. DEPs have indirect effects on B cell development and isotype switching through the secretion of IL-4 and IL-13 from T_H2 cells. DEPs also have a direct effect on B cells that can drive enhanced IgE isotype switch and production. The IgE produced by the resulting antigen-specific plasma cells leads to the priming of mast cells that after antigen exposure degranulate and release cytokines such as IL-4 that produce positive feedback on both the B and T cells. Finally, DEPs themselves can enhance mast cell and basophil degranulation and cytokine release. MHC-II, major histocompatibility complex class II; TCR, T cell receptor.

or via inhalation with DEPs and ovalbumin. In these mice, eosinophil infiltration of the submucosa of medium and small bronchioles is also enhanced, as are goblet cell numbers in the bronchi and bronchioles and airway hyperresponsiveness. Intranasal coexposure to DEPs and allergen also results in increased symptoms and a corresponding threefold higher histamine concentration than obtained with allergen alone. Thus, DEPs are able to turn a minimal mucosal response to allergen challenge into a robust one (**Fig. 2**).

In addition to augmenting the effect of the allergen on the immune system, pollution can also modify the immune system's handling of the allergen. Pollutants modify antigen presentation by upregulation of costimulatory molecules such as CD80 and CD86 on macrophages. In some models, increases in allergen-induced macrophage-derived chemokine production by DEP extracts can be completely blocked by inhibition of the B7-CD28 pathway[6]. Furthermore, because DEPs may inhibit IL-10 and transforming growth factor-β while increasing IL-1 and soluble CD23, exposure to DEPs has been suggested to be important in increasing allergenicity during the critical early phase of allergen presentation. This proposition is supported by *in vivo* human data showing that primary human mucosal allergic sensitization (IgE) can be driven by antigen administered in the context of DEPs in conditions in which the antigen alone leads only to a protective IgG response[7].

Although not as well studied, it seems that gaseous pollutants also modify responses to allergen. In animal models, ozone, SO_2 and NO_2 can all augment allergic antibody production and pulmonary inflammation after allergen challenge. Results of controlled human exposure studies have been more equivocal. Whereas some studies have reported enhanced airway responses to inhaled allergen after exposure to ozone and the combination of NO_2 and SO_2, others have found enhanced late inflammatory or early bronchoconstrictor responses to inhaled antigen in only a subgroup of allergic asthmatic patients[8].

Mechanisms

Despite our knowledge that air pollutants interact with both the innate and adaptive immune system to alter immunophysiologic outcomes, the mechanisms that underlie these outcomes are only now being elucidated. The most plausible model to explain these effects involves hierarchical oxidative stress[9]. This model was originally proposed to explain the dose-dependent protein expression profile noted in macrophages after *in vitro* exposure to oxidant chemicals present in diesel particles. It postulates that with low exposure amounts, the formation of reactive oxygen species (ROS) leads to the activation of antioxidant response elements, followed by transcription of enzymes important in detoxification, cytoprotective and antioxidant responses, such as phase II enzymes. At higher exposures, the transcription factors NF-κB and AP-1 response elements are activated, leading to NF-κB and mitogen-activated protein kinase signaling and resulting in increased expression of proinflammatory and other asthma- and allergy-related genes. Enhanced inflammation leads to additional generation of ROS. Although this cycle of ROS generation and inflammation is normally curtailed by antioxidant defenses, when they are overwhelmed, a robust inflammatory response becomes evident. With even greater exposure, cytotoxic effects may occur because of mitochondrial perturbation.

This model builds on the multitude of studies that have suggested that the effects of DEPs and other particles are driven by increased cellular oxidative stress. The underlying evidence comes mainly from *in vitro* studies in which ROS are generated by macrophages, neutrophils, eosinophils and epithelial cells after stimulation with DEPs or their chemical constituents. Increases in markers of oxidative stress have also been reported in both humans and animals after exposure to diesel exhaust or particles. In murine models, treatment with antioxidants can block DEP-enhanced IgE production and mitigate its proinflammatory and adjuvant effects. Ultrafine particles, because of their potentially richer oxidative chemical composition, may well have greater proinflammatory and more deleterious effects than larger particles. Further studies of ultrafine particles exposures will test this hypothesis; the outcome of these studies may have important public health consequences, as ultrafine particles are neither monitored nor regulated under current air quality standards[3].

This hierarchical oxidative stress model will probably extend to gaseous pollutants and thus underlies the adjuvant effects of air pollution in general. Ozone is a potent oxidant that produces free radicals and ROS. The epithelial surface of the respiratory tract is particularly rich in antioxidants such as glutathione and ascorbate. In controlled exposure studies, ozone causes considerable depletion of this store of protective antioxidants. Similarly, SO_2 inhalation affects the intracellular glutathione redox state in airway epithelial cells[10].

Genetics

These results suggest that the key to protection from the harmful effects of pollutants is to mount an effective cytoprotective response. It follows that people with diminished ability to detoxify xenobiotics and metabolize ROS are at increased risk for adverse outcomes from pollutant exposure. This idea of a 'pollution-susceptible population' is not new. Controlled human exposure studies to ozone, SO_2, DEPs and secondhand smoke have all shown large inter-individual variation in responses to pollutants; variable responses tend to be reproducible in and intrinsic to each person. Thus, the idea of pollutant 'susceptibility genes' has gained prominence. The identification of candidate genes is now an area of intense research interest.

Members of the glutathione *S*-transferase superfamily of phase II xenobiotic metabolizing enzymes, *GSTP1* and *GSTM1*, are proving to be ideal candidate genes. Their gene products conjugate reactive intermediates with glutathione; they are present in the respiratory tract and they have common functional variant alleles. Epidemiological studies have shown that *GSTP1* and *GSTM1* variants that result in absence of or decrease in enzyme function are associated with airway hyper-responsiveness and asthma and increased lung cancer risk among smokers[11]. In one study, people with the nonfunctional *GSTM1* gene variant (*GSTM1* null) had a 3.5-fold higher risk of developing asthma, suggesting a heightened allergic airway response as a consequence of their decreased capacity to mount an effective cytoprotective response to pollutants. What gives these findings added significance is the very high frequency of polymorphisms of *GSTM1* and *GSTP1* genes in most populations in which the frequency of either the homozygous *GSTM1*-null genotype or the *GSTP1*-Ile/Ile (decreased function) genotype is approximately 40% (ref. 12).

Direct support for this idea comes from human nasal provocation studies examining variation in responsiveness to DEPs[13]. Nasal allergic responses were measured in allergen-sensitive people after challenge with allergen alone and, at a separate visit, after allergen plus DEPs. Individuals with low responsive genotypes (*GSTM1*-null or *GSTP1*-Ile/Ile) showed enhanced susceptibility to the adjuvant effects of DEPs but not to the allergen alone. Furthermore, the greatest susceptibility was seen in people who had both *GSTM1*-null and *GSTP1*-Ile/Ile genotypes. Additional proof for involvement of these genes comes from a randomized clinical trial of children who live in high-ozone areas in Mexico[14]. Asthmatic children with the *GSTM1*-null genotype (but not the functional variant) who received placebo had greater ozone related decreases in forced expiratory flow than children who received antioxidant supplementation with

vitamins C and E. Other epidemiological studies have shown that *in utero* exposure to secondhand smoke is associated with increased prevalence of early onset asthma in *GSTM1*-null children.

It is almost certain that *GSTM1* and *GSTP1* are not the only relevant genes involved in conferring susceptibility to pollutants. In mice, a quantitative trait locus analysis located susceptibility genes involved in lung hyperpermeability in response to ozone on chromosome 4 (ref. 15). What makes this finding potentially exciting is that there are very few practicable candidate genes on chromosome 4 except one of the members of the Toll-like receptor (TLR) gene family: TLR4. This raises the fascinating possibility that a gene thought to be key in innate immunity by mediating responses to endotoxin or lipopolysaccharide can also regulate responses to an environmental pollutant. Support for the involvement of TLR4 in ozone responses comes from studies of TLR4-deficient mice that fail to respond to inhaled lipopolysaccharide. In these mice, airway hyper-reactivity after subchronic ozone exposure was notably ablated compared with that of wild-type control mice[16]. These studies are still in their infancy and many unanswered issues remain, such as why analogous differences in airway permeability are not apparent in TLR4-deficient mice and how TLRs interact with particulate pollution. There is a definite need for human controlled exposure studies to determine whether the human TLR4 polymorphism thought to regulate innate immune responses and linked to the development of asthma is actually involved in airway responses to ambient air pollutants.

Cellular inflammatory responses to ozone have been mapped to chromosome 17, bringing renewed attention to TNF, a proinflammatory cytokine that is increased after ozone exposure in bronchoalveolar lavage fluid in human, mouse and rat models and after direct exposure of human nasal epithelia or blood to ozone. Pretreatment of rats with antibody to TNF can reduce ozone-induced inflammation and lung damage, and acute ozone-induced airway hyper-reactivity is reduced in TNF receptor–deficient mice[17]. A *TNF*-308 polymorphism was found to be associated with lung function changes after ozone exposure (although actual amounts of TNF were not reported in bronchoalveolar lavage fluid)[18]. Epidemiological studies associate this same polymorphism with an increased risk of both asthma and wheezing and have suggested that ozone can modify this effect[19]. A caveat to those findings is that unlike the situation with *GSTM1* or *GSTP1*, physiologically different changes in protein amounts resulting from polymorphisms in TNF remain to be proven conclusively.

As research proceed to define pollutant susceptibility, it seems that there will be a hierarchy of genes determining susceptibility, rather than one gene driving this process. For example, in the study mentioned above[18], TNF genotype was thought to account for only 8% of the variance seen in change of lung function. Given their frequency and penetrance, antioxidant genes such as *GSTM1* and *GSTP1* may well be at the apex of this hierarchy, with inflammatory genes such as that encoding TNF and possibly genes involved in the adaptive response (such as IL-4R) below. An example of this interaction can be seen in a study examining asthma risk in children with high lifetime exposure to ozone that showed no correlation between asthma risk and *NQO1* (another phase II enzyme gene with functional polymorphisms)[20]. Yet among people with the null genotype for GSTM1, the risk of asthma was significantly associated with the *NQO1* genotype.

Future directions

So what can be done to minimize the risk for airway disease associated with ambient airway pollution? Although improving air quality is an obvious answer, this is likely to be a difficult long-term goal that will require a genuine commitment at the local, national and global level. Should we screen everyone, no one or only those people with allergies, asthma and/or chronic obstructive pulmonary disease for 'susceptibility genes' as part of their health assessment, to provide them with more specific advise about avoidance? These are difficult questions but they will need to be faced in the future. For the susceptible patient, it is tempting to recommend an antioxidant-rich diet; however, we still lack evidence for effective over-the-counter or even prescription medications with good systemic antioxidant effects. The development and testing of potent and safe antioxidants is a goal that should not be too far in the future. One thing is for certain: the answer is not to simply 'hold your breath'.

1. Heinrich, J. & Wichmann, H.E. *Curr. Opin. Allergy Clin. Immunol.* **4**, 341–348 (2004).
2. *National Air Pollutant Emission Trends* (US Environmental Protection Agency, 1997).
3. Bernstein, J.A. *et al. J. Allergy Clin. Immunol.* **114**, 1116–1123 (2004).
4. Takizawa, H. *Curr. Opin. Allergy Clin. Immunol.* **4**, 355–359 (2004).
5. Diaz-Sanchez, D., Tsien, A., Fleming, J. & Saxon, A. *J. Immunol.* **158**, 2406–2413 (1997).
6. Fahy, O. *et al. J. Immunol.* **168**, 5912–5919 (2002).
7. Diaz-Sanchez, D., Garcia, M.P., Wang, M., Jyrala, M. & Saxon, A.J. *Allergy Clin. Immunol.* **104**, 1183–1188 (1999).
8. Chen, L.L. *et al. Chest* **125**, 2328–2335 (2004).
9. Xiao, C.G., Wang, M., Li, N., Loo, J.A. & Nel, A.E. *J. Biol. Chem.* **278**, 50781–50790 (2003).
10. Todokoro, M. *et al. Inflammation* **28**, 105–114 (2004).
11. Gilliland, F.D. *et al. Am. J. Respir. Crit. Care Med.* **166**, 457–463 (2002).
12. London, S.J. *et al. J. Natl. Cancer Inst.* **85**, 1246–1252 (1995).
13. Gilliland, F.D., Li, Y-F., Saxon, A. & Diaz-Sanchez, D. *Lancet* **363**, 119–125 (2004).
14. Romieu, I. *et al. Thorax* **59**, 8–10 (2004).
15. Kleeberger, S.R., Reddy, S., Zhang, L.Y. & Jedlicka, A.E. *Am. J. Respir. Cell Mol. Biol.* **22**, 620–627 (2000).
16. Hollingsworth, J.W. *et al. Am. J. Respir. Crit. Care Med.* **170**, 126–132 (2004).
17. Shore, S.A., Schwartzman, I.N., Le Blanc, B., Murthy, G.G. & Doerschuk, C.M. *Am. J. Respir. Crit. Care Med.* **164**, 602–607 (2001).
18. Yang, I.A. *et al. Am. J. Respir. Crit. Care Med.***171**, 171–176 (2004).
19. Bayley, J.P., Ottenhoff, T.H. & Verweij, C.L. *Genes Immun.* **5**, 315–329 (2004).
20. David, G.L. *et al. Am. J. Respir. Crit. Care Med.* **168**, 1199–1204 (2003).