Title
Diesel exhaust and asthma: hypotheses and molecular mechanisms of action.

Journal
Environmental Health Perspectives, 110(suppl 1)

ISSN
1542-4359

Authors
Pandya, Robert J
Solomon, Gina
Kinner, Amy
et al.

Publication Date
2002-02-01

DOI
10.1289/ehp.02110s1103

Peer reviewed

Diesel Exhaust and Asthma: Hypotheses and Molecular Mechanisms of Action

Robert J. Pandya,[1] Gina Solomon,[1,2] Amy Kinner,[2] and John R. Balmes[1]

[1]Division of Occupational and Environmental Medicine, University of California at San Francisco and San Francisco General Hospital, San Francisco, California, USA; [2]Natural Resources Defense Council, San Francisco, California, USA

Several components of air pollution have been linked to asthma. In addition to the well-studied critera air pollutants, such as nitrogen dioxide, sulfur dioxide, and ozone, diesel exhaust and diesel exhaust particles (DEPs) also appear to play a role in respiratory and allergic diseases. Diesel exhaust is composed of vapors, gases, and fine particles emitted by diesel-fueled compression-ignition engines. DEPs can act as nonspecific airway irritants at relatively high levels. At lower levels, DEPs promote release of specific cytokines, chemokines, immunoglobulins, and oxidants in the upper and lower airway. Release of these mediators of the allergic and inflammatory response initiates a cascade that can culminate in airway inflammation, mucus secretion, serum leakage into the airways, and bronchial smooth muscle contraction. DEPs also may promote expression of the T_H2 immunologic response phenotype that has been associated with asthma and allergic disease. DEPs appear to have greater immunologic effects in the presence of environmental allergens than they do alone. This immunologic evidence may help explain the epidemiologic studies indicating that children living along major trucking thoroughfares are at increased risk for asthmatic and allergic symptoms and are more likely to have objective evidence of respiratory dysfunction. *Key words*: **air pollution, allergy, asthma, diesel exhaust, immunology, irritant, particulate matter, respiratory.** *Environ Health Perspect* **110(suppl 1):103–112 (2002).**
http://ehpnet1.niehs.nih.gov/docs/2002/suppl-1/103-112pandya/abstract.html

Medical treatment of asthma and knowledge about asthma's biologic mechanisms have improved in recent years. Yet asthma prevalence, hospitalization rates, and mortality rates continue to rise internationally in both adults and children (*1–5*). According to the Centers for Disease Control and Prevention, the number of individuals with self-reported asthma increased by 75% in the United States from 1980 to 1994 (*6*). The increase was seen in all races, both sexes, and all age groups, but nonwhite children have been particularly affected. The prevalence of pediatric asthma increased by 160% during the same time period in children under 4 years of age and by 74% in children over age 4 (*7*). Not only is the prevalence of asthma rising in industrialized countries, but also the severity among those afflicted has increased. A recent cross-sectional study found that the odds of an adverse outcome (i.e., intubation, cardiopulmonary arrest, or death) among children hospitalized for asthma in California doubled between 1986 and 1993 (*8*).

Asthma is more prevalent in the urbanized areas of industrialized countries (*9*). Numerous studies have demonstrated that specific components of air pollution may be associated with exacerbations of asthma (*10–14*). Although the levels of coarse particulate matter in the atmosphere have decreased over recent decades, the levels of fine particulate matter smaller than 2.5 μm in size ($PM_{2.5}$), such as diesel exhaust particles (DEPs), remain an ongoing problem (*15*). Ambient air pollution has been associated with hospitalizations and deaths due to exacerbations of cardiovascular and respiratory diseases (*15*). Particulate air pollution has also been linked more specifically to asthma (*16,17*).

Some of the evidence linking particulate air pollution and asthma is indirect. For instance, several studies found that children raised in more polluted regions of a country are more likely to develop respiratory diseases and allergies compared with children raised in "cleaner" regions (*18,19*). Within communities, children living on busy streets have a higher likelihood of developing chronic respiratory symptoms than those living on streets with lower traffic volume (*10,20*). When exposed to similar levels of Japanese cedar pollen (a standard allergen), people who live in highly trafficked areas have enhanced allergic reactions compared with people who live in rural areas. This suggests the possibility of a synergistic effect between air pollution and aeroallergens (*21*).

Diesel exhaust and DEPs have previously been associated with asthma (*22–24*). Current evidence supports the hypothesis that components of diesel exhaust worsen respiratory symptoms in individuals with preexisting asthma or allergies, and offers some support for the hypothesis that diesel exhaust and DEPs may play a role in causing asthma (*25–28*). This paper critically analyzes the research relevant to the question of whether diesel exhaust exposure is associated with asthma. We also review molecular mechanisms by which particulate matter in diesel exhaust may facilitate and promote asthmatic symptoms.

Molecular Basis for the Inflammatory Events in Asthma

Asthma is a chronic respiratory disease manifested by bronchial hyperresponsiveness, reversible bronchial constriction, airway inflammation, and respiratory symptoms such as wheezing, dyspnea, coughing, and chest tightness (*29,30*). A complex immunologic cascade, including recruitment of inflammatory cells from the bloodstream to the bronchial mucosa, is characteristic of asthma (*31*).

During asthma attacks, both inflammatory and structural cells of the respiratory tract are activated. Activated cells include T cells, mast cells, eosinophils, macrophages, epithelial cells, fibroblasts, and bronchial smooth muscle cells. By releasing proinflammatory and cytotoxic mediators and cytokines, these cells are all involved in a cascade that leads to the acute and chronic symptoms of asthma (*30*). Figure 1 summarizes the immunologic events involved in asthma.

T lymphocytes appear to play a particularly important role in airway inflammation. T cells have been demonstrated in the airways of patients with fatal asthma (*32*) and appear to be vital for regulating the immune pathways that control allergic immune responses (*31*). In general, T cells can be classified into two major subsets consisting of $CD4^+$ or $CD8^+$ cells. $CD4^+$ T cells

Address correspondence to J.R. Balmes, Division of Occupational and Environmental Medicine, University of California at San Francisco, PO Box 0843, San Francisco, CA 94143-0843 USA. Telephone: (415) 206-8953. Fax: (415) 206-8949. E-mail: JBalmes@itsa.ucsf.edu

We thank E. Arévalo for creation of the graphics and G. de Rita for general assistance. RJP was supported by training grant T42/CCT 910427 from the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute for Occupational Safety and Health. Additional support for this work came from the Rose Foundation for Communities and the Environment, the W. Alton Jones Foundation, the Beldon Fund, and the Richard and Rhoda Goldman Fund.

Received 7 September 2001; accepted 2 November 2001.

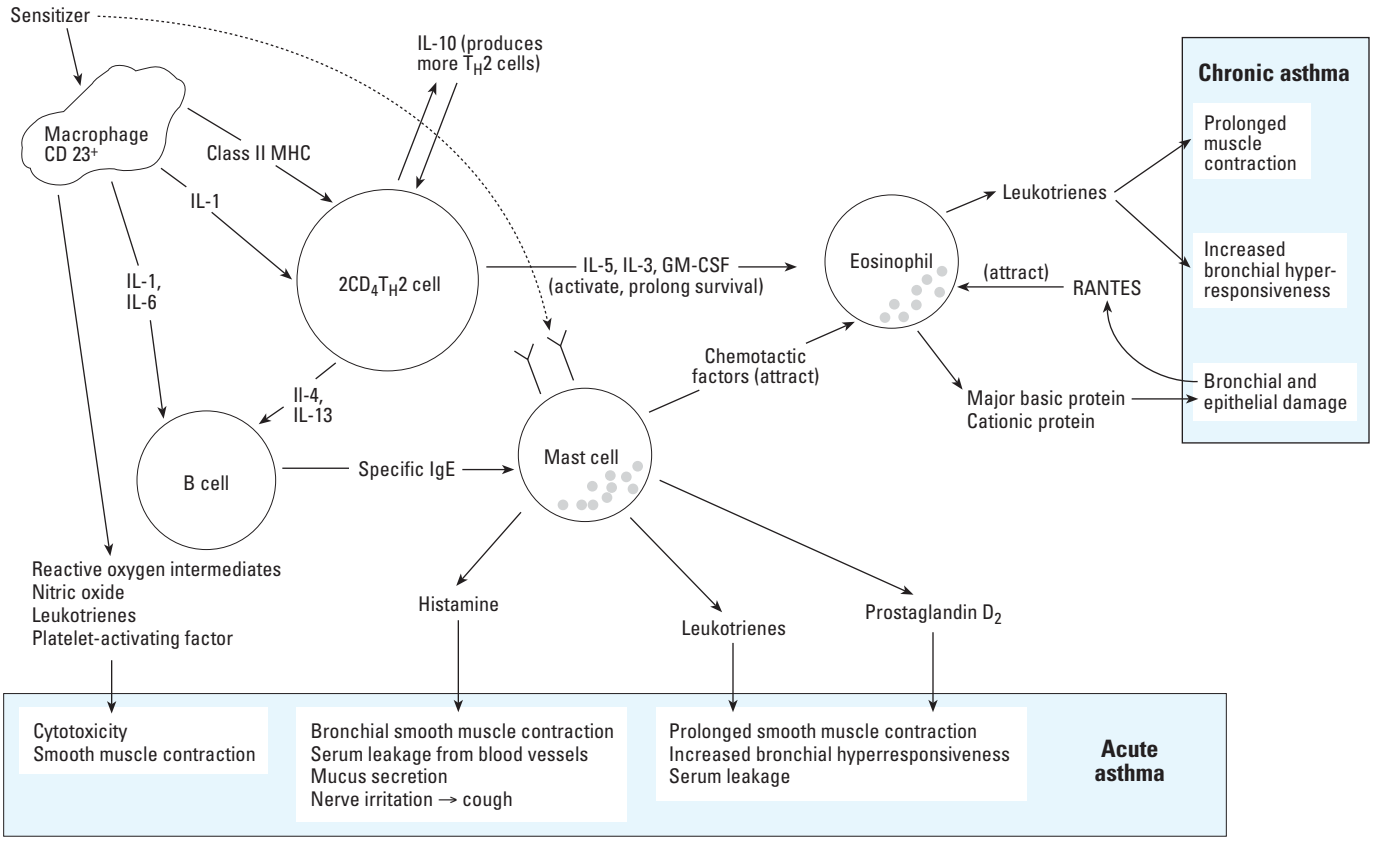

Figure 1. Immunologic pathways in asthma.

differentiate into several phenotypes of T cells, including T helper 1 (T_H1) and T helper 2 (T_H2) (*33*). A shift in the predominant T-cell population from the T_H1 type to the T_H2 type has been associated with asthma (*34*).

T helper cells release specific cytokines that mediate inflammation. T_H1-type cells produce interferon γ, interleukin-2 (IL-2), and tumor necrosis factor β (TNF-β), whereas T_H2-type cells produce IL-4, IL-5, IL-6, IL-10, and IL-13. In addition, both T_H1 and T_H2 produce some common cytokines (i.e., IL-1, IL-3, IL-8, TNF-α, and granulocyte-macrophage colony–stimulating factor (GM–CSF) (*31*). The T_H2 and common cytokines are the signaling molecules that have been most strongly linked to asthmatic responses.

Immunoglobulins, cytokines, and chemokines appear to play important roles in the inflammatory foundation of asthma. For example, IL-5 promotes the development and survival of eosinophils, the cells that help drive the chronic asthmatic response. IL-8 is a potent chemoattractant for neutrophils and primes eosinophil responses. IL-10 builds and prolongs the immune response by stimulating production of more T_H2 cells. IL-4 and IL-13 act on B cells to stimulate production of antigen-specific immunoglobulin E (IgE), and GM–CSF is an important growth and survival factor for neutrophils, eosinophils, and macrophages. The relationship between these molecules and the eventual clinical symptomatology of asthma is illustrated in Figure 1.

Theories on the Etiology of Asthma

Genetic and environmental factors interact to cause asthma (*1*). There is substantial epidemiologic evidence, supported by clinical and toxicologic data, regarding a variety of asthma risk factors. Atopy is a major heritable risk factor for asthma and involves the familial tendency to develop immediate-type hypersensitivity (i.e., IgE-mediated) immune responses to specific allergens (*34*). Although genetic predisposition may be important in the development of asthma, recent increases in the prevalence and severity of asthma seem to have occurred too rapidly to be mediated solely by genetic shifts (*35*).

Environmental factors that have been associated with adult and childhood asthma include allergen exposure, environmental tobacco smoke, socioeconomic status, nutrition, family size, history of infections, and ambient levels of air pollution (*2,7*). Although no consensus exists on the relative importance of each of these factors, the development of asthma is clearly multifactorial. Some scientists have hypothesized that fetuses and infants may take the first steps toward sensitization to environmental allergens during critical windows of susceptibility during early life, perhaps because of an environment that encourages dominance of the T_H2 phenotype beyond fetal life (*9,34*). Because components of diesel exhaust have been shown to affect numerous inflammatory and immunologic pathways in the respiratory tract, including promoting induction of a T_H2 phenotypic response, some researchers hypothesize that exposures to diesel exhaust may play a role in the development or exacerbation of asthma and allergic disorders (*36*).

Composition of Diesel Exhaust

Arising from the combustion of diesel fuel in compression-ignition engines, diesel exhaust consists of a complex mixture of particulate matter, including elemental carbon and polycyclic aromatic hydrocarbons (PAHs; i.e., phenanthrene, fluorenes, naphthalenes, pyrenes, fluoranthrenes), as well as acid aerosols, volatile organic compounds, various hydrocarbons (including highly reactive quinones), and gases, including carbon dioxide (CO_2), carbon monoxide (CO), nitric oxide (NO), nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) (*37*). After combustion of diesel fuel, the exhaust components tend to aggregate into discrete, spherical, respirable particles approximately 0.1–0.5 μm

in diameter (*38*). These particles consist of an inert carbonaceous core with a large surface area, ideal for adsorbing heavy metals and organic compounds such as PAHs. The PAHs are small compounds of three to five benzene rings that can easily diffuse through cell membranes and bind to receptors within the cytoplasm. One such receptor is the aromatic hydrocarbon receptor complex (*36*). In addition, diesel exhaust contains many substances that are listed as toxic air pollutants by the State of California and as hazardous air pollutants by the U.S. Environmental Protection Agency (*37,39*).

Buses, trucks, and other heavy industrial transport vehicles are major sources of ambient diesel exhaust pollution. Utilization of diesel fuel has steadily increased in the United States over the past several decades: the number of miles traveled by commercial trucks in the United States has increased by 235% between 1950 and 1985, and cargo tonnage carried by trucks has increased by 169% (*40*).

DEPs are major sources of ambient $PM_{2.5}$ (*41*). In California, an estimated 26% of all particulate matter from fuel combustion sources arises from the combustion of diesel engines (*41*). In 1996, diesel exhaust also comprised a quarter of the NO smog precursors released nationally in the United States (*39*).

Epidemiologic Studies Linking Diesel Exhaust and Asthma

There is some epidemiologic evidence associating exposure to high levels of diesel exhaust with asthma. Wade and Newman (*42*) describe three railroad workers who traveled in locomotive units directly behind the lead diesel-powered locomotive engine and eventually developed acute or subacute onset of respiratory symptoms. They demonstrated symptoms consistent with asthma, including hyperreactive airways, airflow limitation, and reversibility with bronchodilators. None of these workers had any known preexisting respiratory conditions. Numerous components within diesel exhaust are respiratory irritants (*38*), including some of the acid aerosols, volatile organic compounds, and gases in the mixture. The irritant effect alone could potentially trigger asthmatic symptoms at sufficiently high exposure levels.

Although exposure to acutely high levels of diesel exhaust can produce respiratory symptoms, there is also epidemiologic evidence that chronic exposure to diesel exhaust at lower environmental levels may also be associated with increased levels of respiratory symptoms. For instance, children living near busy diesel trucking routes have decreased lung function in comparison with children living near roads with mostly automobile traffic (*10*). A population-based survey of more than 39,000 children living in Italy found that children living on streets with heavy truck traffic were 60–90% more likely to report acute and chronic symptoms such as wheeze, phlegm, and diagnoses such as bronchitis, bronchiolitis, and pneumonia (*43*). A German study of over 3,700 adolescent students found that those living on streets with "constant" truck traffic were 71% more likely to report symptoms of allergic rhinitis and more than twice as likely to report wheezing (*44*).

Diesel Exhaust Gases and Potential Adverse Respiratory Effects

Diesel exhaust contains many well-known air pollutants that have been associated with asthma exacerbations (*45*), including SO_2, NO_2, and fine particulate matter smaller than 10 μm in size (PM_{10}), which are all criteria air pollutants (*39*).

Several studies have found temporal associations between ambient particulate levels (PM_{10}) and emergency department admissions for exacerbations of asthma (*16,17,46*). Some recent studies have also shown relationships between both daily and long-term levels of SO_2 and child hospital visits for respiratory diseases (*11,47*). SO_2 causes bronchoconstriction in asthmatics during exercise. These effects are above and beyond the effects of exercise alone. Adult asthmatic subjects exposed to ambient concentrations (0.5 ppm SO_2) during just a few minutes of moderate exercise experienced significant drops in forced expiratory volume in 1 sec (FEV_1) (*48,49*). There is also evidence that short-term exposure of asthmatics to NO_2 at ambient atmospheric levels may increase airway responsiveness to SO_2 (*50*). Therefore, it is possible that some of the gases related to diesel exhaust may trigger exacerbations of asthmatic and allergic symptoms in already asthmatic subjects (*51–53*).

Several epidemiologic studies have reported associations between daily and chronic levels of NO_2 and exacerbations of asthma *(12,24,26,54).* Toxicologic evidence indicates that NO_2 is directly harmful to the respiratory system. Normal healthy subjects exposed for 2 hr to 2 ppm NO_2 demonstrated increases in IL-8 and neutrophils (*55*). An *in vitro* study exposed human nasal mucosal tissues to NO_2 and ozone and reported elevated histamine levels (*56*). Another study that exposed mildly asthmatic human subjects to 260 ppb (500 μg/m^3) NO_2 for 30 min found that the response to an inhaled allergen was enhanced after the NO_2 exposure (*57*).

Acute exposures to diesel exhaust, even at low concentrations, have been shown to elicit inflammatory responses. There is some evidence to suggest that the inflammatory response from diesel exhaust may not simply be due to SO_2 and NO_2 exposures. Fifteen nonasthmatic volunteers exposed for 1-hr periods to diesel exhaust (at PM_{10} concentrations of 300 μg/m^3 and NO_2 concentrations of 1.6 ppm) developed elevated levels of neutrophils, macrophages, B cells, mast cells, T lymphocytes, histamine, endothelial adhesion molecules, and lactate dehydrogenase in their airways at 6 hr postexposure (*58*). Such effects do not occur in nonasthmatics exposed to NO_2 alone at comparable concentrations, making the particles the more likely culprit. An *in vitro* study found that exposure of human bronchial epithelial cells to unfiltered diesel exhaust released inflammatory cytokines, whereas diesel exhaust that was filtered (and therefore contained gases but no particulate matter) did not have this effect (*59*). These studies suggest that the particulate components of diesel exhaust may play a more significant role in triggering airway inflammation than the gaseous components.

Molecular Mechanisms of Action of DEPs in the Respiratory Tract

It is not entirely clear which DEP components produce toxicity. Some studies suggest that the majority of the toxicity is attributable to the adsorbed organic compounds (*38,60,61*), whereas others conclude that the most toxic portion of a DEP is the carbonaceous core (*15*). Regardless of which specific components of DEPs are most toxic, it appears that DEPs may be associated with both early and late phases of the inflammatory response in asthma.

Typically, the early asthmatic phase is predominantly IgE mediated, whereas the late phase involves complex networks of inflammatory mediators, including eosinophils, T cells, cytokines, chemokines, and immunoglobulins (*30*).

There are numerous hypothesized interactions of DEPs with the immune and respiratory systems. DEPs may act directly to alter specific immunologic pathways that may precipitate acute exacerbations of asthma. Direct effects of DEPs include stimulation of IgE production, eosinophilic degranulation, augmentation of cytokine and chemokine production and release, free radical formation, and effects on production of NO in the airways (*62*). As an adjuvant with environmental allergens, DEPs appear to enhance the differentiation of $CD4^+$ T lymphocytes into the T_H2 phenotype and enhance allergen-specific IgE and IgG production. The potential pathways by which DEPs may promote asthma are summarized in Table 1.

Table 1. Molecular effects of diesel exhaust particles.

Mechanism of action	Clinical relevance	Category of evidence	Major findings
Increase in IgE production	Stimulates mast cells to release histamine and other mediators of acute hypersensitivity upon exposure to an allergen	Human studies	DEPs alone increase IgE and IgE mRNA in nasal lavage fluid (*63*)
			DEPs and allergen increase IgE and stimulate isotype switching (*45,71,86*)
			Nasal challenge with DEPs results in a de novo IgE response to a neoantigen (*108*)
		Animal studies	No effect on IgE levels post-DEPs alone (*66,68,81*)
			Adjuvant effect of DEPs with allergen on IgE production (*69,72,73,82,87,114–116*)
			No clear adjuvant effect on IgE in mice exposed to DEPs and allergen (*68,70,77*)
		In vitro studies	Phenanthrene and other PAHs from diesel exhaust increase IgE production by B cells (*45,64,67*)
Enhanced IgG production	Association with delayed and chronic asthmatic responses	Human studies	DEPs with allergen increases allergen-specific IgG_4 and IgE levels (*45,86*)
			No effect on IgG_4 from DEPs alone (*63*)
		Animal studies	DEPs with allergen results in greater antiallergen IgG_1 antibody levels (*72,81,82,85,87,115–117*)
			No adjuvant effect on immunoglobulins observed (*68,77*)
Enhanced activity of eosinophils	Mediator of chronic bronchial inflammation, including prolonged muscle contraction, increased bronchial hyperresponsiveness, and mucosal damage	Human studies	No increase in eosinophils after DEP exposure alone (*75*)
		Animal studies	DEPs alone increase eosinophil infiltration in mouse airways—blocked by treatment with superoxide dismutase (*99,104*)
			Exposure to DEPs and allergen enhances eosinophil recruitment (*68,77,81,82,87,115*)
		In vitro studies	DEPs enhance eosinophil adhesion to nasal epithelial cells and induce eosinophil degranulation (*76*)
Effect on T-lymphocyte differentiation and promotion of T_H2-type cytokine production	T_H2-type phenotype is associated with a propensity to asthmatic and allergic responses	Human studies	Induction of T_H2-type cytokine expression (i.e., IL-4, IL-5, IL-6, IL-10, IL-13) by DEPs with or without allergen (*45,65,79,86*)
	Cytokines mediate immunologic pathways involved in acute and chronic asthmatic and allergic symptoms	Animal studies	Increased T_H2 cytokines in the airway and the gut (*77,80–82,85,87,117*)
Increased levels of specific cytokines (interleukins)			
IL-4	Mediates immunoglobulin class switching of B cells from IgM to IgE	Human studies	DEPs alone increase IL-4 in nasal lavage fluid (*65,79*)
	Stimulates differentiation of T cells into a T_H2 phenotype		Combination of DEPs and allergen increases IL-4 levels (*45,71,86*)
	Activates eosinophils	Animal studies	Intratracheal exposure to DEPs and allergen enhances IL-4 production (*80,87*)
		In vitro studies	Pyrene induces expression of IL-4 protein in human T cells (*78*)
			DEPs and antigen stimulate IL-4 production in mouse spleen cells (*69,72*)
IL-5	Growth factor for eosinophils	Human studies	DEPs increase IL-5 levels (*65*)
			DEPs plus allergen increase IL-5 and other T_H2 cytokines in the nasal lavage fluid of healthy humans (*45,86*)
		Animal studies	DEPs plus allergen increase IL-5 in lung tissue of mice (*77,81,82,85*)
IL-8	An important mediator in neutrophil recruitment to the respiratory tract in acute, severe asthma	Human studies	DEPs increase IL-8 in the bronchial wash and epithelium (*84*)
			No change in IL-8 in bronchioalveolar lavage fluid of normal subjects with DEPs alone (*58*)
		In vitro studies	DEPs enhance IL-8 release from human nasal and bronchial epithelial cells (*38,59,60,91–94,96*)
Effects on other inflammatory mediators			
GM–CSF	Prolongs the survival of eosinophils, neutrophils, and macrophages	Human studies	No effect on GM–CSF levels after exposure to diesel exhaust (*84*)
	Augmented GM–CSF production observed in cells involved in asthmatic activity	Animal studies	Increase in GM–CSF mRNA expression in the lungs of mice intranasally exposed to DEPs (*66*)
			Increase in GM–CSF in mice exposed to DEPs and allergen (*77,87*)
		In vitro studies	Endocytosis of organic compounds in DEPs by human respiratory epithelial cells causes secretion of GM–CSF (*38,60,93,94,96,97*)
RANTES	Chemokine central to the delivery of eosinophils to the airway	*In vitro* studies	DEPs increase expression of the RANTES gene in human bronchial epithelial cells (*92,96*)
TNF-α	Proinflammatory cytokine that influences eosinophil recruitment	Human studies	No change in levels of TNF-α after exposure to DEPs alone (*84*)
		Animal studies	Increased synthesis and secretion of TNF-α from macrophages (*61*)

(continued)

Table 1. *Continued.*

Mechanism of action	Clinical relevance	Category of evidence	Major findings
Enhanced superoxide production and inhibition of antioxidant effects	Reactive oxygen molecules that directly injure airway epithelium and promote apoptosis in macrophages, thereby generating more free radicals	Animal studies	Increased superoxide production via P450 reductase after intratracheal treatment with DEPs (*101*)
	Superoxide levels increase in response to the adsorbed organic molecules on DEP	*In vitro* studies	Inhibition of superoxide dismutase activity and catalase activity by DEPs (*102,103*)
			Organic components in DEPs induce apoptosis in macrophages (*100*)
Effects on NO	Important mediator of airway inflammation	Animal studies	Increased inducible and constitutive NOS in murine airways after DEP exposure (*101*)
			Aggravation of airway inflammation in mice by DEP-mediated increase in the inducible form of NOS (*105*)
		In vitro studies	Inhibition of NO release from bronchial and aortic ring cells by DEPs (*106*)
DEPs and allergen binding	Promotes synergistic response due to co-delivery to the mucosa	*In vitro* studies	Pollen and other allergens bind to DEPs (*70,110,111*)

Direct Immunologic Effects of DEPs

Enhanced IgE Production by Effects on B Lymphocytes

DEPs consistently enhance the production of IgE in the airways (*63–65*). IgE is produced by activated B cells in response to a specific allergen. Once produced, IgE attaches to mast cells and, when cross-linked by allergen, induces mast cells to release histamine and leukotrienes. The chemicals released from mast cells cause constriction of bronchial smooth muscle, mucus secretion, and serum leakage into the airways and result in acute asthma symptoms (*30*). The mast cell is often considered the central cell type in the acute asthmatic response, and IgE is the critical immunoglobulin driving the mast cell response.

In a study of eleven nonsmoking, nonallergic volunteers, Diaz-Sanchez et al. (*65*) showed that exposure to DEPs significantly increases IgE levels in nasal fluids by greatly increasing the numbers of IgE-secreting cells and by altering the expression of IgE mRNA isoforms. In comparison, there was no effect on IgG, IgA, or IgM antibody production. This suggests that DEP exposure *in vivo* induces both a quantitative increase in IgE production and a shift in the type of IgE that is produced. Although most studies support the finding that DEPs increase IgE synthesis (*63–65*), one study in mice failed to find an increase in IgE synthesis from DEPs alone (*66*).

In vitro evidence suggests that IgE-secreting B cells may be directly stimulated by DEPs. For instance, PAHs from DEPs were able to induce production of IgE in purified human B cells treated with IL-4 and CD-40 (*67*). Another study demonstrated that phenanthrene, a major PAH in DEPs, increased IgE in human B cells transformed by Epstein-Barr virus (*64*). The IgE stimulation by phenanthrene was accompanied by an increased expression of total IgE mRNA. In addition, several studies have found that the DEP-mediated increase in IgE synthesis may be amplified when DEPs act as an adjuvant to environmental allergens (*68–73*).

Stimulation of Eosinophils

Diesel exhaust may also stimulate the proliferation of eosinophils. Eosinophil production is regulated by IL-3, IL-5, and GM–CSF. The granules of mature eosinophils contain chemokines, leukotrienes, and toxic proteins. Degranulation of eosinophils in mucosal tissues results in bronchial inflammation and contributes to asthmatic symptoms (*74*). Just as mast cells are regarded as the central cell for the acute asthmatic response, eosinophils are often regarded as the critical cell type in chronic asthma.

DEPs may enhance eosinophilic infiltration into the respiratory tract and subsequent degranulation. Healthy human volunteers exposed to diesel exhaust had increased eosinophils and other inflammatory molecules on bronchial biopsies 6 hr after exposure (*58*). However, a similar study did not detect increased eosinophils in induced sputa 4 hr after exposure to DEPs (*75*). Induced sputa are less sensitive than bronchial biopsies at detecting subtle inflammatory changes in the lower airway. Eosinophils incubated with DEPs had enhanced adherence to human nasal epithelial cells and enhanced levels of degranulation (*76*). In animal assays, the DEP-induced eosinophilia is enhanced in the presence of allergens such as ovalbumin (OVA) and is accompanied by enhanced airway hyperresponsiveness to acetylcholine challenge (*68,77*).

Influence on Cytokine Expression

Exposure to DEPs may augment levels of many different cytokines (soluble protein immune mediators such as interleukins) and chemokines (attractant proteins that induce migration of different cell types). These molecules are key chemical messengers in the inflammatory processes of asthma. Various interleukins stimulate T-cell switching between T_H1 and T_H2 subtypes, stimulate B cells, attract and prolong the survival of eosinophils, and play other roles orchestrating the immunologic cascade that results in an allergic or asthmatic response.

Augmentation of interleukin levels. DEPs and associated polyaromatic hydrocarbons may increase levels of some interleukins. For example, healthy humans exposed nasally to 0.15 mg of DEPs suspended in 200 µL of saline expressed T_H2-type cytokines (i.e., IL-4, IL-5, IL-6, IL-10) in their nasal mucosal cells 18–24 hr after exposure (*65*).

IL-4 production may be enhanced by pyrene, a PAH found in DEPs (*78*). The molecular mechanism of this effect may be upregulation of IL-4 mRNA transcription. IL-4 is a T_H2-type cytokine that induces isotype switching in B cells to alter antibody production from the IgM to IgE isotype and is also central to the production of IgE (*79*). DEPs may enhance IL-4 production more effectively with allergen than it does alone. Mice injected intratracheally with DEPs plus Japanese cedar pollen manifested an IL-4 production about twice as high as that seen in mice injected with Japanese cedar pollen alone (*80*). This enhancement in IL-4 production increased to an 8-fold level in mice injected with OVA and DEPs compared with mice receiving only OVA. A later study examining cytokine production in DEP-exposed and control mice sensitized with OVA found that IL-4 and IL-10 production in spleen cells was significantly increased in the group of DEP-exposed mice (*69*). In addition, humans challenged with DEPs plus ragweed antigen had enhanced local IgE, IL-4, and IL-13 production accompanied by isotype switching from IgM or IgD to IgE antibody in nasal lavage cells (*71*). In comparison, isotype switching did not occur

in those challenged with ragweed antigen or DEPs alone.

Levels of IL-5 are also increased after DEP exposure. IL-5 is an important factor for the proliferation and activation of eosinophils after exposure to certain allergens such as OVA and pollen (*81,82*). A recent study found that mRNA expression for IL-5 was significantly lower in patients who had no nasal symptoms when compared with those who required medicines to control allergic symptoms during pollen season (*83*). Two human studies found that exposure to DEPs resulted in increased levels of IL-5 (*65,84*). However, other human, animal, and *in vitro* studies found that diesel exhaust alone did not result in any IL-5 response (*38,66,81,82,85*).

Despite the conflicting results about the effect of DEPs alone on IL-5, DEPs consistently increase IL-5 levels in the presence of environmental allergens. For instance, healthy human subjects exposed to DEPs with ragweed antigen had significantly increased levels of IL-5 and other T_H2 cytokines in nasal lavage fluid (*86*). Mice exposed to diesel exhaust combined with OVA sensitization had increased expression of IL-5 in lung tissue and developed airway inflammation and hyperresponsiveness (*77,81,82,87*). Instillation of OVA and DEPs together produced a 3- to 4-fold increase in IL-5 in mouse lung tissue compared with the levels in mice exposed to OVA or DEPs alone (*77*). DEPs may enhance the symptoms of allergic rhinitis by a synergistic effect with pollen to increase IL-5 secretion (*86*).

DEPs also increase the presence of IL-8, a member of the CXC chemokine family. Produced primarily by macrophages, IL-8 is one of the most important mediators in the recruitment of neutrophils to the respiratory tract (*88*). Neutrophils appear to be important inflammatory leukocytes in airway secretions of patients with acute severe asthma (*89*). IL-8 appears to play an important role in augmenting the numbers of activated eosinophils in asthmatic patients (*90*).

Increased IL-8 levels are found in bronchial washings and bronchial tissues of healthy humans exposed to diesel exhaust levels similar to those in the ambient air of many cities (*84*). *In vitro* exposure to DEPs has also been found to enhance the release of IL-8 from various types of airway cells, including human bronchial epithelial cells (*38,60,91–93*), human mucosal microvascular endothelial cells (*94*), and human nasal epithelial cells (*38,94*).

Effect on other inflammatory mediators. DEPs may enable the release of several additional molecules involved in airway inflammation. In animal and *in vitro* models, DEPs increase GM–CSF. In both animals and humans, GM–CSF is thought to sustain the asthmatic response by prolonging the survival of eosinophils and neutrophils (*95*). Mice intranasally exposed to DEPs developed bronchial constriction associated with increased levels of GM–CSF in bronchial epithelial cells; blocking the GM–CSF response abolished the DEP-evoked airway hyperresponsiveness (*66*). DEP-induced increases in GM–CSF were also shown *in vitro* in exposed human bronchial epithelial cells (*60,93,96*), human mucous membrane epithelial cells, and human nasal epithelial cells (*38,94*). However, no effect on GM–CSF levels in bronchial cells was found in one study of human volunteers exposed to diesel exhaust (*84*).

Proposed mechanisms by which DEPs may increase GM–CSF include increased expression of the histamine H_1 receptor (*94*) and free radical production, which may independently elevate GM–CSF levels (*97*). A recent study demonstrated that free radical scavengers inhibit the DEP-mediated GM–CSF release in airway epithelial cells (*97*), providing some support for the latter hypothesis. Free radical production is part of the inflammatory pathway discussed in more detail below.

Expression of Chemokines

DEPs have been shown to increase the expression of RANTES (regulated upon activation, normal T-cell expressed and secreted), a chemokine that is central to the delivery of eosinophils to the airway (*30*). RANTES also plays a role in attracting leukocytes during the inflammatory response (*98*). Upon exposure to DEPs, expression of the gene for RANTES was increased in the bronchial epithelial cells of asthmatic (*96*) and nonasthmatic individuals (*92*). Although DEPs enhance both IL-8 and RANTES, an inhibitor of p38 mitogen-activated protein (MAP) kinase apparently prevents these effects. p38 MAP kinase is thought to be important in the signal transduction pathway leading to upregulation of nuclear factors (e.g., activator protein 1 [AP-1] and nuclear factor kappa B [NFκB]) that activate the transcription of genes for IL-8 and RANTES. Thus, DEPs may enhance IL-8 and RANTES through activation of the p38 MAP kinase pathway in human bronchial epithelial cells, which leads to upregulation of nuclear transcription factors AP-1 and NFκB (*92*).

Inflammatory Effects of DEPs

Although DEPs may have numerous effects on the immunologic cascade involved in allergy and asthma, there is also some evidence that these particles may have a more direct irritant or cytotoxic effect in the respiratory tract. Although there is overlap between the two pathways, this inflammatory mode of action is somewhat distinct from the more immunologic effects described above. The inflammatory pathway in asthma is shown in Figure 2.

Enhanced Superoxide Production

DEPs may induce the production of oxidants such as superoxide (O_2^-) and hydroxyl radical (OH^-), reactive compounds that can cause direct damage to the pulmonary epithelium (*99*). Superoxides appear to be part of a cellular response against the adsorbed organic molecules on DEPs and may promote apoptosis in macrophages (*100*), thereby causing release of more inflammatory and cytotoxic molecules. Intratracheal DEP exposure in mice enhances the activity of P450 reductase, an enzyme that increases production of superoxide. This provides a possible mechanism by which DEPs may stimulate superoxide production (*101*). While increasing superoxide production, DEPs may also reduce the superoxide scavenging activities of superoxide dismutase (SOD) and glutathione *in vitro*. For example, when the antioxidant catalase was exposed to the oxidant stress of hydrogen peroxide (H_2O_2) in the presence of DEPs and chlorine, the activity of the catalase was inhibited dose dependently (*102*).

This type of inhibitory activity by DEPs can reduce the capacity of the body to counteract oxidants (e.g., H_2O_2), thereby providing another mechanism for cellular injury. Lim et al. (*101*) provide evidence for this by demonstrating that the activity of CuZn–superoxide dismutase (SOD) and Mn–SOD was decreased after intratracheal exposure to DEPs in mice. DEPs may also inhibit the activity of antioxidants through a deactivating reaction between SOD and quinones, which are present on the surface of DEPs (*103*). Therefore, DEPs appear to increase the superoxide load yet decrease the body's innate superoxide scavenging activity, which leads to potentially higher levels of cytotoxicity.

Increases in superoxides may be a key factor in asthmatic and allergic responses. For instance, pretreatment with polyethyleneglycol-conjugated SOD suppressed DEP-related airway alterations in mice, including infiltration of inflammatory cells, mucus hypersecretion, and airway constriction (99,*104*). This illustrates that direct cellular toxicity by superoxides may play a role in asthma. Superoxides may also activate intracellular signaling pathways, including those involving NFκB and AP-1, that upregulate chemokine and cytokine expression. This may help mediate and sustain inflammatory responses in asthma.

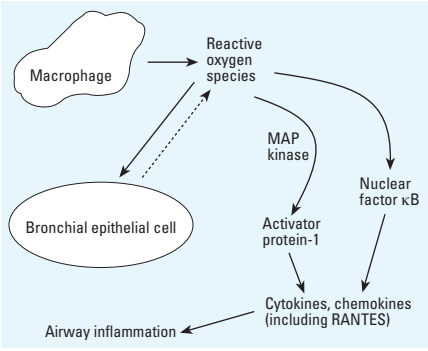

Figure 2. Diesel exhaust particles and inflammatory pathways in asthma.

Effect on the Nitric Oxide Pathway

DEPs may be capable of influencing NO production. NO is elevated in asthmatic patients and has been proposed as a biologic marker for airway inflammation (*105,106*). NO is synthesized from the amino acid arginine by the enzyme nitric oxide synthase (NOS). NO is normally released constitutively by one isoform of NOS, but NO may also be produced from augmented expression of inducible forms of NOS by various stimuli. However, the precise role of NO in asthma is not clear. Interestingly, it appears that NO produced by constitutive NOS may have anti-inflammatory effects, whereas NO produced from inducible forms of NOS may have proinflammatory effects (*105*).

DEPs may affect both the constitutive and inducible NOS pathways. Intratracheal exposure of mice to DEPs increased production of both the constitutive and inducible NOS isoforms (*101*). However, another study found that DEP-induced airway inflammation was aggravated by NO generated from the inducible form of NOS (*105*). This study suggested that DEPs may aggravate airway inflammation by inhibition of NO production by the constitutive form of NOS.

Although DEPs may alter the NO pathway, the implications for asthma are not clear. One theory is that NO may react with superoxide to form a compound called peroxynitrite that may play a key role in the development of airway inflammation and hyperresponsiveness (*101*).

Adjuvant Immunologic Effects of DEPs

Although DEP exposure alone can elicit adverse biologic effects in the airway, the effect of DEPs has been repeatedly shown to be even greater in conjunction with allergens (*82,87*). For example, mice exposed intranasally to DEPs and OVA have far greater levels of anti-OVA IgE than mice exposed solely to DEPs or OVA alone (*73*). Guinea pigs exposed for 4 weeks to diesel exhaust and challenged with histamine experienced nasal mucosal hyperresponsiveness, sneezing, and nasal secretion, while those exposed to either diesel exhaust or histamine alone had far weaker responses (*107*).

An innovative study of 10 nonsmoking atopic human subjects tested the potential for DEPs to create a brand new immune response to an allergen. The investigators exposed the atopic subjects on three occasions to the neoantigen keyhole limpet hemocyanin (KLH), a compound to which humans are not normally sensitized. Twenty-four hours prior to each exposure to the new antigen, the subjects were exposed nasally to a concentration of DEPs roughly equivalent to 1–3 days of breathing Los Angeles air. Subjects exposed to KLH alone did not develop IgE antibodies to this compound, whereas subjects exposed to DEPs followed by KLH developed KLH-specific IgE and mounted a T_H2-type cytokine response with increased levels of IL-4. This important study indicates that DEPs may promote new allergic sensitization to antigens in addition to aggravating existing allergic diseases (*108*).

Theories as to how DEPs may have adjuvant effects include stimulation of a T_H2-type immune response, by acting as delivery agents for coallergens, and by increasing allergen-specific IgE and IgG production.

DEPs and Induction of a T_H2 Phenotypic Response

Exposure to diesel exhaust may induce T cells to differentiate into a T_H2 phenotype (*34*). Rather than a direct effect of DEPs alone, this shift toward a T_H2 phenotype seems to occur as an adjuvant effect of DEPs with allergens. In the presence of allergen, DEPs stimulate the release of T_H2-specific cytokines (i.e., IL-4, IL-5, IL-6, IL-10, and IL-13). These cytokines appear to play a major role in the molecular pathophysiology underlying the clinical manifestations of asthma and allergies. Increased levels of T_H2-type cytokines have stimulatory effects on B cells, enhancing IgE production, as discussed above. In a study of 13 nonsmoking volunteers, Diaz-Sanchez et al. (*86*) found that exposure to DEPs plus ragweed results in increased expression of all of the T_H2-type cytokines in nasal lavage fluid and decreased expression of T_H1-type cytokines. A study of 27 nonsmoking volunteers with known allergies found that intranasal coadministration of DEPs and an allergen to which the subjects are sensitized stimulates a dramatic increase over 18 hr of T_H2-type cytokines such as IL-4 and IL-6. The initial production of these cytokines appears to derive from mast cells in the mucosa (*79*).

The precise mechanism of how DEPs stimulate the T_H2 pathway has not been determined. However, the time during development when an organism is exposed to DEPs may be vital in priming the immune system for development and maintenance of the T_H2 pattern. Exposure to DEPs and environmental allergens during early life may predispose individuals to asthma and allergic disorders later in life by promoting the expression of T_H2 phenotypic responses (*34,109*).

Physical Interactions between DEPs and Allergens

DEPs may enhance the immune response to allergens by physically binding with them. By this mechanism, DEPs may be transported with allergens such as pollen grain fragments into human airways, where both agents may be deposited on the mucosa at the same location. This proximity may facilitate synergistic immunologic responses and respiratory symptoms. DEPs bind strongly with certain allergens. For instance, a study that incubated DEPs with purified natural grass pollen allergen, Lol p 1, for 30 min found that this compound was bound to DEPs with sufficient strength that it could not be removed by washing methods (*110*). Another study used immunogold labeling to demonstrate the presence of the allergens Can f 1 (dog) and Bet v 1 (birch pollen) on the surface of suspended particulate matter, similar to DEP, which was collected from the indoor environment. In addition, the allergens Fel d 1 (cat) and Der p 1 (house dust mite) both attached to DEP when incubated with DEP *in vitro* (*111*).

However, actual binding of DEP to allergen does not appear to be necessary to the immune response. For instance, pollen grains from timothy grass do not adhere significantly to DEP *in vitro,* but the combination does induce synergistic inflammatory changes (i.e., influx of macrophages, eosinophilic granulocytes, and granuloma formation) in the lungs of rats (*112*). Another study demonstrated that the capacity of a particle to adsorb antigens was not related to its ability to enhance allergic responses (*113*). Thus, the binding or adsorption of DEP to antigen may be less important than the physical proximity of the two agents on the mucosal surface.

Enhancement of IgE and IgG Production

Exposure to DEP and many environmental allergens has been shown to augment both IgE and IgG production. Both IgE and IgG_1 antibodies are the result of T_H2 cytokine environments. Research in mice has demonstrated that DEP produces allergen-specific

IgG_1 prior to enhancing IgE production (*85*). Production of IgG_1 antibodies is dependent on T_H2 lymphocytes in mice, and has been linked in humans to delayed asthmatic reactions. Human nasal instillation studies involving exposures to 0.30 mg DEP (equivalent to total exposure on 1–3 average days in Los Angeles) along with a ragweed antigen challenge showed that ragweed-specific IgE levels peaked far higher in the presence of DEP, with a maximum level 4 days postexposure. The levels of ragweed-specific IgG_4 (an isoform of IgG that is linked to IgE expression) also increased in these studies, although other forms of IgG were not affected (*45,86*).

Adjuvant IgE antibody responses were observed in mice exposed by intraperitoneal injection to OVA and DEPs (*114*). However, another study measured IgE and IgG responses to intratracheal instillation of diesel exhaust and OVA sensitization in strains of mice that were either high IgG responders or high IgE responders. In contrast to the previous study, IgE production did not change in either strain, but the combined exposure dramatically increased IgG_1 production and IL-2 and IL-5 levels in the high IgG responders (*85*). Similar studies (*81,82,87*) found that inhaled exposure to diesel exhaust with OVA sensitization for 5–6 weeks increased both IgG_1 and IgE levels. Studies in mice using other allergens such as house dust mite antigen and Japanese cedar pollen were consistent with the literature using OVA. Mice immunized with either of these antigens mounted a much greater IgG_1 response with exposure to DEPs than mice exposed to the same level of allergen without DEPs. A similar response was found for IgE synthesis, indicating that both antibodies play a role in the adjuvant effects of DEPs on the immune response (*72,113*).

Guinea pigs exposed to DEPs for 5 weeks with OVA sensitization once per week developed 7-fold greater anti-OVA IgG antibody than guinea pigs exposed only to filtered air, indicating that the response is not specific to mice. The exposed guinea pigs also experienced slight concentration-dependent increases in IgE antibody (*115*). Similar results have been seen in rats, where intranasal or intratrachial co-exposure to DEPs and pollen grains resulted in a much greater serum level of specific IgE and IgG_1 antibodies than exposure to either alone. Electron microscopy revealed pollen grains in the alveoli surrounded by DEP-loaded macrophages (*116*). One interesting study examined the effects of oral ingestion of DEPs in mice because it is known that airborne particulate reaches not only the lung but also the mucosa of the gastrointestinal tract. DEPs in the gut mucosa also appear to act as an adjuvant, enhancing both T_H1- and T_H2-type responses to allergen and enhancing production of allergen-specific IgG_1 (*117*).

Conclusions and Considerations for Further Research

Rising rates of asthma and allergies create a public health imperative to identify any modifiable environmental factors that may cause or contribute to these diseases. Abundant evidence suggests that components of diesel exhaust can cause biologic responses that are related to asthma. Although evidence from research cited in this article indicates that exposures to diesel exhaust and DEPs are associated with the inflammatory and immune responses involved in asthma, some questions remain regarding the underlying molecular mechanisms.

DEPs alone may augment levels of IgE, trigger eosinophil degranulation, and stimulate release of numerous cytokines and chemokines. DEPs also may play a role in unleashing the cytotoxic effects of free radicals in the airways. All of these cellular mechanisms would be expected to produce airway inflammation, bronchial smooth muscle contraction, serum leakage, and mucus production, thereby resulting in the clinical symptoms of asthma. Interestingly, DEPs appear to have a far greater impact as an adjuvant with allergens than it has alone.

The immune events leading to the asthmatic response are intertwined, and DEPs likely act at numerous points on the pathway. Stimulation of the T_H2-type pathway and increase in IgE production are two of the most important and likely mechanisms by which DEPs may generate and sustain an asthmatic response. The timing of exposure to air pollutants such as DEPs during early life may also be critical in fostering the persistence of the T_H2 phenotype.

DEPs also have other biologic effects, such as increasing superoxide and NO levels. However, the evidence for these effects is currently found only in a few animal or *in vitro* studies, and key questions remain. Although exposure to diesel exhaust appears capable of inducing inflammatory changes in the respiratory tract, this area is poorly understood. Most important, the epidemiologic evidence linking diesel exhaust and asthma is distressingly sparse because of a paucity of studies that have collected relevant exposure data.

More research is needed to investigate the mechanism and the clinical relevance of the observed adjuvant effect of co-exposure to DEPs and allergens. One study demonstrated that this adjuvant effect results in increased respiratory resistance in mouse airways after acetylcholine challenge (*118*). This line of research will help to link the observed immunologic alterations with clinical relevance. The question of windows of vulnerability in early life and the induction of an allergic phenotype also requires further investigation. Research is needed to demonstrate more clearly the effect of DEPs on reactive oxygen species, superoxide, and NO production. Epidemiologic research on allergic and/or asthmatic human populations would be particularly valuable. Observational studies of children, including quantitative assessment of DEP exposure and airway function, would remove some of the uncertainties associated with the epidemiologic research to date.

Despite the need for further research, it is biologically plausible that diesel exhaust and associated particles are associated with asthma and other allergies in humans. In light of these findings, public health efforts to reduce exposures to diesel exhaust are warranted. In particular, reducing the exposure of infants and children should be a priority as part of a coordinated effort to improve the prevention and management of childhood asthma.

References and Notes

1. Eggleston PA, Buckley TJ, Breysse PN, Wills-Karp M, Kleeberger SR, Jaakkola JJK. The environment and asthma in U.S. cities. Environ Health Perspect 107(3):1–21 (1999).
2. Davies RJ, Rusznak C, Devalia JL. Why is allergy increasing? Environmental factors. Clin Exp Allergy 28(6):8–14 (1998).
3. Weiss KB, Sullivan SD, Lyttle CS. Trends in the cost of illness for asthma in the United States, 1985–1994. J Allergy Clin Immunol 106:493–499 (2000).
4. Passalacqua G, Ciprandi G, Canonica GW. United airways disease: therapeutic aspects. Thorax 55(suppl 2):S26–S27 (2000).
5. Millar WJ, Hill GB. Childhood asthma. Health Rep 10(3):9–21 (1998).
6. Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E, Kang DS. Surveillance for asthma—United States, 1960–1995. Morbid Mortal Wkly Rep CDC Surv Summ 47:1–27 (1998).
7. Clark NM, Brown RW, Parker E, Robins TG, Remick DG, Philbert MA, Keeler GJ, Israel BA. Childhood asthma. Environ Health Perspect 107(3):421–429 (1999).
8. Calmes D, Leake BD, Carlisle DM. Adverse asthma outcomes among children hospitalized with asthma in California. Pediatrics 101(5):845–850 (1998).
9. Bjorksten B. The environmental influence on childhood asthma. Allergy 54:17–23 (1999).
10. Brunekreef B, Janssen NA, de Hartog J, Haressema H, Knape M, van Vliet P. Air pollution from truck traffiic an lung function in children living near motorways. Epidemiology 8:298–303 (1997).
11. Buchdahl R. Association between air pollution and acute childhood wheezy episodes: prospective observational study. Br Med J 312:661–665 (1996).
12. Studnicka M, Hackl E, Pischinger J, Fangmeyer C, Haschke N, Kuhr J, Urbanek R, Neumann M, Frischer T. Traffic-related NO_2 and the prevalence of asthma and respiratory symptoms in seven year-olds. Eur Respir J 10(10):2275–2278 (1997).
13. Krankenhaus G. Airways response of asthmatics after a 30 min exposure, at resting ventilation, to 0.25 ppm NO_2 or 0.5 ppm SO_2. Eur Respir J 3(2):132–137 (1990).
14. Jenkins HS, Devalia JL, Mister RL, Bevan AM, Rusznak C, Davies RJ. The effect of exposure to ozone and nitrogen dioxide on the airway response of atopic asthmatics

to inhaled allergen. Am J Respir Crit Care Med 160:33–39 (1999).
15. Samet JM. Fine particulate air pollution and mortality in 20 U.S. cities. New Engl J Med 343(24):1742–1749 (2000).
16. Norris G, YoungPong SN, Koenig JQ, Larson TV, Sheppard L, Stout JW. An association between fine particles and asthma emergency department visits for children in Seattle. Environ Health Perspect 107(6):489–493 (1999).
17. Lipsett M, Hurley S, Ostro B. Air pollution and emergency room visits for asthma in Santa Clara County, California. Environ Health Perspect 105(2):216–222 (1997).
18. Van Niekerk C, Weinberg E, Shore S, Heese HV, van Schalkwyk D. Prevalence of asthma: a comparative study of urban and rural Xhosa children. Clin Allergy 9:319–324 (1979).
19. Heinrich J, Hoelscher B, Wjst M, Ritz B, Cyrys J, Wichmann HE. Respiratory diseases and allergies in two polluted areas in East Germany. Environ Health Perspect 107(1):1–17 (1999).
20. Oosterlee A, Drijver M, Lebret E, Brunekreef B. Chronic respiratory symptoms in children and adults living along streets with high traffic density. Occup Environ Med 53:241–247 (1996).
21. Ishizaki T, Koizomi K, Ikemori R, Ishiyama Y, Kushibiki E. Studies of prevalence of Japanese cedar pollinosis among residents in a densely cultivated area. Ann Allergy 58:265–270 (1987).
22. Nel AE, Diaz-Sanchez D, Ng G, Hiura T, Saxon A. Enhancement of allergic inflammation by the interactio between diesel exhaust particles and the immune system. J Allergy Clin Immunol 102(4 pt 1):539–554 (1998).
23. Peden DB. Mechanisms of pollution-induced airway disease: *in vivo* studies. Allergy 52(suppl 38):37–44 (1997).
24. McConnell R, Berhane K, Gilliland F, London SJ, Vora H, Avol E, Gauderman WJ, Margolis HG, Lurmann F, Thomas DC, et al. Air pollution and bronchitic symptoms in southern California children with asthma. Environ Health Perspect 107(9):1–9 (1999).
25. Rusznak C, Devalia JL, Davies, RJ. The impact of air pollution on allergic disease. Allergy 49:21–27 (1994).
26. Hajat S, Haines A, Goubet SA, Atkinson RW, Anderson HR. Association of air pollution with daily GP consultations for asthma and other lower respiratory conditions in London. Thorax 54:597–605 (1999).
27. Nicolai T. Environmental air pollution and lung disease in children. Monaldi Arch Chest Dis 54(6):475–478 (1999).
28. D'Amato G. Outdoor air pollution in urban areas and allergic respiratory diseases. Monaldi Arch Chest Dis 54(6):470–474 (1999).
29. American Thoracic Society. Guidelines for the evaluation of impairment/disability in patients with asthma. Am Rev Respir Dis 147:1056–1061 (1993).
30. Busse WW, Rosen FS. Asthma. New Eng J Med 344(5):350–362 (2001).
31. Kemeny DM. The effects of pollutants on the allergic immune response. Toxicology 152:3–12 (2000).
32. Azzawi M, Johnston PW, Majumdar S, Kay AB, Jeffery PK. T lymphocytes and activated eosinophils in airway mucosa in fatal asthma and cystic fibrosis. Am Rev Respir Dis 145:1477–1482 (1992).
33. Mosmann T, Cherwinski H, Bond M, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I: Defiinitio according to profiles of lymphokine activities and secreted proteins. J Immunol 136(7):2348–2357 (1986).
34. Peden DB. Development of atopy and asthma: candidate environmental inflluences and important periods of exposure. Environ Health Perspect 108(suppl 3):475–482 (2000).
35. Von Mutius E. The environmental predictors of allergic disease. Curr Rev Allergy Clin Immunol 105(1):9–19 (2000).
36. Peterson B, Saxon A. Global increases in allergic respiratory disease: the possible role of diesel exhaust particles. Ann Allergy Asthma Immunol 77:263–268 (1996).
37. Air Resources Board. Proposed identification of diesel exhaust as a toxic air contaminant. As approved by the Scientific Review Panel on April 22, 1998. Sacramento, CA:California Environmental Protection Agency.
38. Boland S, Baeza-Squiban A, Fournier T, Houcine O, Gendron MC, Chevrier M, Jouvenot G, Coste A, Aubier M, Marano F. Diesel exhaust particles are taken up by human airway epithelial cells *in vitro* and alter cytokine production. Am J Physiol Lung Cell Mol Physiol 276(4):L604–L613 (1999).
39. U.S. EPA. National Air Pollutant Emission Trends. Offiic of Air Quality Planning and Research, 1900–1996, Appendix A. Washington, DC:U.S. Environmental Protection Agency, 1997.
40. Gross M, Feldman RN. National Transportation Statistics 1997. Washington, DC:Bureau of Transportation Statistics, U.S. Department of Transportation, 1996.
41. Air Resources Board. Draft diesel exposure assessment. In: Motor Vehicles Facts and Figures 1997. Detroit, MI:American Automobile Manufacturers Association, 1998;78.
42. Wade JF, Newman LS. Diesel asthma: reactive airways disease following overexposure to locomotive exhaust. J Occup Med 35(2):149–154 (1993).
43. Ciccone G, Fostastiere F, Agabati N, Biggeri A, Bisanti L, Chellini E, et al. Road traffic and adverse respiratory effects in children. SIDRIA Collaborative Group. Occup Environ Med 55(11):771–778 (1998).
44. Duhme H, Weiland SK, Keil U, Kraemer B, Schmid M, Stender M, Chambless L. The association between self-reported symptoms of asthma and allergic rhinitis and self-reported traffic density on street of residence in adolescents. Epidemiology 7(6):578–582 (1996).
45. Diaz-Sanchez D. The role of diesel exhaust particles and their associated polyaromatic hydrocarbons in the induction of allergic airway disease. Allergy 52(suppl 38):52–56 (1997).
46. Schwartz J, Slater D, Larson TV, Pierson WE, Koenig JQ. Particulate air pollution and hospital emergency room visits for asthma in Seattle. Am Rev Respir Dis 147:826–831 (1993).
47. Romieu I, Meneses F, Sienra-Monge JJ, Huerta J, Valesco SR, White MC, Etzel RA, Hernandez-Avila M. Effects of urban air pollutants on emergency visits for childhood asthma in Mexico City. Am J Epidemiol 141:546–553 (1995).
48. Balmes JR, Fine JM, Sheppard D. Symptomatic bronchoconstriction after short-term inhalation of sulfur dioxide. Am Rev Respir Dis 136(5):1117–1121 (1987).
49. Trenga CA, Koenig JQ, Williams PV. Sulfur dioxide sensitivity and plasma antioxidants in adult subjects with asthma. Occup Environ Med 56:544–547 (1999).
50. Jorres R, Magnussen H. Airways response of asthmatics after a 30-min exposure, at resting ventilation, to 0.25 ppm NO_2 or 0.5 ppm SO_2. Eur Respir J 3(2):132–137 (1990).
51. Devalia JL, Rusznak C, Herdman MJ, Trigg CJ, Davies RJ. Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. Lancet 344(8938):1668–1671 (1994).
52. Huang JL, Wang SY, Hsieh KH. Effect of short-term exposure to low levels of SO_2 and NO_x on pulmonary function and metacholine and allergen bronchial sensitivities in asthmatic children. Arch Environ Health 46(5):296–299 (1991).
53. Jorres R, Magnussen H. Airways response of asthmatics after a 30 min exposure, at resting ventilation, to 0.25 ppm NO_2 or 0.5 ppm SO_2. Eur Respir J 3(2):132–137 (1990).
54. Hirsch T, Wieland SK, von Mutius E, Safeca AF, Grafe H, Csaplovics E, Dhume H, Keil U, Luepold W. Inner city air pollution and respiratory health and atopy in children. Eur Respir J 14:669–677 (1999).
55. Blomberg A, MT Krishna, FJ Kelly, AJ Frew, ST Holgate, T Sandstrom. Effects of 2ppm NO_2 on cytokines, airway lavage fluid and the bronchial mucosa of healthy nonsmokers. Eur Respir J 9(suppl 23):447s (1996).
56. Schierhorn K, Zhang M, Matthias C, Kunkel G. Inflluenc of ozone and nitrogen dioxide on histamine and interleukin formation in a human nasal mucosa culture system. Am J Respir Cell Mol Biol 20:1013–1019 (1999).
57. Strand V, Svartengren M, Rak S, Barck C, Bylin G. Repeated exposure to an ambient level of NO_2 enhances asthmatic response to a nonsymptomatic allergen dose. Eur Respir J 12(1):6–12 (1998).
58. Salvi S, Blomberg A, Rudell B, Kelly F, Sandstrom T, Holgate ST, Frew A. Acute inflammatory responses in th airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am J Respir Crit Care Med 159(3):702–709 (1999).
59. Abe S, Takizawa H, Sugawara I, Kudoh S. Diesel exhaust (DE)-induced cytokine expression in human bronchial epithelial cells: a study with a new cell exposure system to freshly generated DE *in vitro*. Am J Respir Cell Mol Biol 22(3):296–303 (2000).
60. Ohtoshi T, Takizawa H, Okazaki H, Kawasaki S, Takeuchi N, Ohta K, Ito K. Diesel exhaust particles stimulate human airway epithelial cells to produce cytokines relevant to airway inflammation *in vitro*. J Allergy Clin Immunol 101(6 pt 1):778–785 (1998).
61. Yang HM, Barger MW, Castranova V, Ma JKH, Yang JJ, Ma JYC. Effects of diesel exhaust particles (DEP), carbon black, and silica on macrophage responses to lipopolysaccharide: evidence of DEP suppression of macrophage activity. J Toxicol Environ Health 58(pt A):261–278 (1999).
62. Casillas AM, Hiura T, Li, Nel AE. Enhancement of allergic infllammation by diesel exhaust particles: permissive rol of reactive oxygen species. Ann Allergy Asthma Immunol 83:624–629 (1999).
63. Diaz-Sanchez D, Dotson AR, Takenaka H, Saxon A. Diesel exhaust particles induce local IgE production *in vivo* and alter the pattern of IgE messenger RNA isoforms. J Clin Invest 94:1417–1425 (1994).
64. Tsien A, Diaz-Sanchez D, Ma J, Saxon A. The organic component of DEP and phenanthrene, a major polyaromatic hydrocarbon constituent enhances IgE production by IgE-secreting EBV-transformed human B cells *in vitro*. Toxicol Appl Pharmacol 142(2):256–263 (1997).
65. Diaz-Sanchez D, Tsien A, Casillas A, Dotson AR, Saxon A. Enhanced nasal cytokine production in human beings after *in vivo* challenge with diesel exhaust particles. J Allergy Clin Immunol 98:114–123 (1996).
66. Ohta K, Yamashita N, Tajima M, Miyasaka T, Nakano J, Nakajima M, Ishii A, Horiuchi T, Mano K, Miyamoto T. Diesel exhaust particulate induces airway hyperresponsiveness in a murine model: essential role of GM-CSF. J Allergy Clin Immunol 104(5):1024–1030 (1999).
67. Takenaka A, Zhang K, Diaz-Sanchez D, Tsien A, Saxon A. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: direct effects on B-cell IgE production. J Allergy Clin Immunol 95(1 pt 1):103–115 (1995).
68. Ichinose T, Takano H, Miyabara Y, Sagai M. Long-term exposure to diesel exhaust enhances antigen-induced eosinophilic inflammation and epithelial damage in the murine airway. Toxicol Sci 44:70–79 (1998).
69. Fujimaki H, Saneyoshi K, Shiraishi F, Imai T, Endo T. Inhalation of diesel exhaust enhances antigen-specific IgE antibody production in mice. Toxicology 116(1–3):227–233 (1997).
70. Steinsvik TE, Ormstad H, Gaarder PI, Aaberge IS, Bjonness U, Lovik M. Human IgE production in hu-PBL-SCID mice injected with birch pollen and diesel exhaust particles. Toxicology 128(3):219–230 (1998).
71. Fujieda S, Diaz-Sanchez D, Saxon A. Combined nasal challenge with diesel exhaust particles and allergen induces *in vivo* IgE isotype switching. Am J Respir Cell Mol Biol 19(3):507–512 (1998).
72. Suzuki T, Kanoh T, Ishimori M, Ikeda S, Ohkuni H. Adjuvant activity of diesel exhaust particulates (DEP) in production of anti-IgE and anti-IgG1 antibodies to mite allergen in mice. J Clin Lab Immunol 48:187–199 (1996).
73. Takafuji S, Suzuki S, Koizumi K, Tadokoro K, Miyamoto T, Ikemori R, Muranaka M. Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. J Allergy Clin Immunol 79(4):639–645 (1987).
74. Erjefält JS, Persson CGA. New Aspects of degranulation and fates of airway mucosal eosinophils. Am J Respir Crit Care Med 161(6):2074–2088 (2000).
75. Nightingale JA, Maggs R, Cullinan P, Donnelly LE, Rogers DF, Kinnersley R, Chung FC, Barnes PI, Ashmore M, Newman-Taylor A. Airway inflammation after controlled exposure to diesel exhaust particles. Am J Respir Crit Care Med 162:161–168 (2000).
76. Terada N, Maesako K, Hiruma K, Hamano N, Houki G, Konno A, Ikeda T, Sai M. Diesel exhaust particles enhance eosinophil adhesion to nasal epithelial cells and cause degranulation. Int Arch Allergy Immunol 114(2):167–174 (1997).
77. Takano H, Ichinose T, Miyabara Y, Shibuya T, Lim HB, Yoshikawa T, Sagai M. Inhalation of diesel exhaust enhances allergen-related eosinophil recruitment and airway hyperresponsiveness in mice. Toxicol Appl Pharmacol 150:328–337 (1998).
78. Bömmel H, Li-Weber M, Serfling E, Duschl A. The environmental pollutant pyrene induces the production of IL-4. J Allergy Clin Immunol 105:796–802 (2000).
79. Wang M, Saxon A, Diaz-Sanchez D. Early IL-4 production

driving T_{H2} differentiation in a human *in vivo* allergic model is mast cell derived. Clin Immunol 90(1):47–54 (1999).
80. Fujimaki H, Nohora O, Ichinose T, Watanabe N, Saito S. IL-4 production in mediastinal lymph node cells in mice intratracheally instilled with diesel exhaust particles and antigen. Toxicology 92:261–268 (1994).
81. Miyabara Y, Ichinose T, Takano H, Lim HB, Sagai M. Effects of diesel exhaust on allergic airway infllammatio in mice. J Allergy Clin Immunol 102(5):805–812 (1998).
82. Miyabara Y, Takano H, Ichinose T, Lim H, Sagai M. Diesel exhaust enhances allergic airway inflammation and hyperresponsiveness in mice. Am J Respir Crit Care Med 157:1–7 (1998).
83. Kakinoki Y, Ohashi Y, Nakai Y, Washino Y, Nasako A, Tanaka A, Nakai Y. Allergen-induced mRNA expression of interleukin-5, but not interleukin-4 and interferon-gamma, in peripheral blood mononuclear cells obtained before the pollen season predicts the clinical effiicacy o immunotherapy for seasonal allergic rhinitis. Scand J Immunol 51:202–208 (2000).
84. Salvi S, Nordenhall C, Blomberg A, Rudell B, Purazar J, Kelly FJ, Susan W, Sandstrom T, Holgate ST, Frew AJ. Acute exposure to diesel exhaust increases IL-8 and GRO-α production in healthy human airways. Am J Respir Crit Care Med 161(2):550–557 (2000).
85. Miyabara Y, Yanagisawa R, Shimojo N, Takano H, Lim HB, Ichinose T, Sagai M. Murine strain differences in airway inflammation caused by diesel exhaust particles. Eur Respir J 11(2):291–298 (1998).
86. Diaz-Sanchez D, Tsien A, Flemming J, Saxon A. Combined diesel exhaust particulate and ragweed allergen markedly enhances *in vivo* nasal ragweed-specific IgE and shows cytokine production to a T_H2-type pattern. J Immunol 158(5):2406–2413 (1997).
87. Takano H, Yoshikawa T, Ichinose T, Miyabara Y, Imakaoka K, Sagai M. Diesel exhaust particles enhance antigen-induced airway infflammation and local cytokin expression in mice. Am J Respir Crit Care Med 156(10):36–42 (1997).
88. Lezcano-Meza D, Teran LM. Occupational asthma and interleukin-8. Clin Exp Allergy 29:1301–1303 (1999).
89. Ordoñez CL, Shaughnessy TE, Matthay MA, Fahy JV. Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma: clinical and biological significance. Am J Respir Crit Care Med 161(4):1185–1190 (2000).
90. Norzila MZ, Fakes K, Henry RL, Simpson J, Gibson PG. Interleukin-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma. Am J Respir Crit Care Med 161(3):769–774 (2000).
91. Takizawa H, Ohtoshi T, Kawasaki S, Kohyama T, Desaki M, Kasama T, Kobayashi K, Nakahara K, Yamamoto K, Matsushima K, et al. Diesel exhaust particles induce NF-kappa B activation in human bronchial cells *in vitro*: importance in cytokine transcription. J Immunol 162(8):4705–4711 (1999).
92. Hashimoto S, Gon Y, Takeshita I, Matsumoto K, Jibiki I, Takizawa H, Kudoh S, Horie T. Diesel exhaust particles activate p38 MAP kinase to produce interleukin 8 and RANTES by human bronchial epithelial cells and *N*-acetylcysteine attenuates p38 MAP kinase activation. Am J Respir Crit Care Med 161:280–285 (2000).
93. Bayram H, Devalia JL, Sapsford RJ, Ohtoshi T, Miyabara Y, Imaoka K, Sagai M, Davies RJ. The effect of diesel exhaust particles on cell function and release of infflamatory mediators from human bronchial epithelial cells *in vitro*. Am J Respir Cell Mol Biol 18(3):441–448 (1998).
94. Terada N, Hamano N, Maesako KI, Hiruma K, Hohki G, Suzuki K, Ishikawa K, Konno A. Diesel exhaust particulates upregulate histamine receptor mRNA and increase histamine-induced IL-8 and GM-CSF production in nasal epithelial cells and endothelial cells. Clin Exp Allergy 29(1):52–59 (1999).
95. Sampson AP. The role of eosinophils and neutrophils in infflammation. Clin Exp Allergy 30(suppl 1):22–27 (2000).
96. Devalia JL, Bayram H, Abdelaziz MM, Spasford RJ, Davies RJ. Differences between cytokine release from bronchial epithelial cells of asthmatic patients and non-asthmatic subjects: effect of exposure to diesel exhaust particles. Int Arch Allergy Immunol 118:437–439 (1999).
97. Boland S, Bonvallot V, Fournier T, Baeza-Squiban A, Aubier M, Marano F. Mechanisms of GM-CSF increase by diesel exhaust particles in human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 278:L25–L32 (2000).
98. Lee AH, Hong JH, Seo YS. Tumour necrosis factor-alpha and interferon-gamma synergistically activate the RANTES promoter through nuclear factor kappa B and interferon regulatory factor 1 (IRF-1) transcription factors. Biochem J 350(pt 1):131–138 (2000).
99. Sagai M, Saito H, Ichinose T, Kodama M, Mori Y. Biological effects of diesel exhaust particles. I: In vitro production of superoxide and *in vivo* toxicity in mouse. Free Radic Biol Med 14:37–47 (1993).
100. Hiura TS, Kaszubowski MP, Li N, Nel AE. Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. J Immunol 163(10):5582–5591 (1999).
101. Lim HB, Ichinose T, Miyabara Y, Takano H, Kumagai Y, Shimojyo N, Devalia JL, Sagai M. Involvement of superoxide and nitric oxide on airway infflammation and hyperresponsiveness induced by diesel exhaust particles in mice. Free Radic Biol Med 25(6):635–644 (1998).
102. Mori Y, Murakami S, Sagai T, Hayashi H, Sakata M, Sagai M, Kumagai Y, Sagae T. Inhibition of catalase activity *in vitro* by diesel exhaust particles. J Toxicol Environ Health 47:125–134 (1996).
103. Kumagai Y, Taira J, Sagai M. Apparent inhibition of superoxide dismutase activity *in vitro* by diesel exhaust particles. Free Radic Biol Med 18:365–371 (1995).
104. Sagai M, Furuyama A, Ichinose T. Biological effects of diesel exhaust particles (DEP). III: Pathogenesis of asthma like symptoms in mice. Free Radic Biol Med 21(2):199–209 (1996).
105. Takano H, Lim HB, Miyabara Y, Ichinose T, Yoshikawa T, Sagai M. Manipulation of the L-arginine-nitric oxide pathway in airway inflammation induced by diesel exhaust particles in mice. Toxicology 139:19–26 (1999).
106. Muto E, Hayashi T, Yamada K, Esaki T, Sagai M, Iguchi A. Endothelial-constitutive nitric oxide synthase exists in airways and diesel exhaust particulates inhibit the effect of nitric oxide. Life Sci 59(18):1563–1570 (1996).
107. Kobayashi T, Ikeue T, Ikeda A. Four-week exposure to diesel exhaust induces nasal mucosal hyperresponsiveness to histamine in guinea pigs. Fundam Appl Toxicol 38:166–172 (1998).
108. Diaz-Sanchez D, Garcia MP, Wang M, Jyrala M, Saxon A. Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. J Allergy Clin Immunol 104:1183–1188 (1999).
109. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. Science 275:77–79 (1997).
110. Knox RB, Suphioglu C, Taylor P, Desai R, Watson HC, Peng JL, Bursill LA. Major grass pollen allergen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. Clin Exp Allergy 27:246–251 (1997).
111. Ormstad H. Suspended particulate matter in indoor air: adjuvants and allergen carriers. Toxicology 152:53–68 (2000).
112. Streenberg PA, Dormans JAMA, van Doorn CCM, Middendorp S, Vos JG, van Loveren H. A pollen model in the rat for testing adjuvant activity of air pollution components. Inhal Toxicol 11:1109–1122 (1999).
113. Maejima K, Tamura K, Taniguchi Y, Nagase S, Tanaka H. Comparison of the effects of various fiine particles on Ig antibody production in mice inhaling Japanese cedar pollen allergens. J Toxicol Environ Health 52:231–247 (1997).
114. Muranaka M, Suzuki S, Koisumi K, Takafuji S, Miyamoto T, Ikemori R, Tokiwa H. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. J Allergy Clin Immunol 77:616–623 (1986).
115. Kobayashi T. Exposure to diesel exhaust aggravates nasal allergic reaction in guinea pigs. Am J Respir Crit Care Med 162:352–356 (2000).
116. Steerenberg PA, Dormans JAMA, van Doorn CCM, Middendorp S, Vos JG, van Loveren H. A pollen model in the rat for testing adjuvant activity of air pollution components. Inhal Toxicol 11:1109–1122 (1999).
117. Yoshino S, Sagai M. Induction of systemic Th1 and Th2 immune responses by oral administration of soluble antigen and diesel exhaust particles. Cell Immunol 192:72–78 (1999).
118. Takano H, Ichinose T, Miyabara Y, Yoshikawa T, Sagai M. Diesel exhaust particles enhance airway responsiveness following allergen exposure in mice. Immunopharmacol Immunotoxicol 20:329–336 (1998).