

# Experimental studies on the effects of diesel exhaust emissions

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Patients with asthma and other airway diseases have been found to be more vulnerable than the normal population to the inhalation of air pollution components like sulfur dioxide (SO<sub>2</sub>), nitrogen dioxide (NO<sub>2</sub>), and ozone (O<sub>3</sub>) (1–4). This review is focused on experimental studies of the health effects of the particulate component of air pollution, especially diesel emissions. However, interactions between particles and photochemical pollutants are likely to be important during natural exposures. The vast majority of published studies deal with the health effects of diesel exhaust particles, and this trend is natural in view of the major contribution of such particles to air pollution in the urban regions of Sweden and the rest of Europe. It would be of interest to compare the effects of diesel exhaust particles with those possibly induced by the inhalation of particles generated during the burning of wood, but the literature on the latter topic is very limited.

Diesel engines produce a mixture of nitrogen oxides (NO<sub>x</sub>), formaldehyde, carbon monoxide, hydrocarbons, ash, transition metals, and carbon particles. Compared with gasoline engines, diesel engines produce far less carbon monoxide but give rise to greater concentrations of NO<sub>x</sub> and aldehydes, which are particularly prone to cause irritation of the upper respiratory tract. Diesel engines also produce submicron soot or black smoke particles (BS) that are believed to mediate several of the observed adverse effects. The recent indications that such ultrafine particles may be particularly harmful are dealt with in the section of this report called "Experimental Studies on the Effects of Ultrafine Particles". In fact, it has been estimated that the particulate emission from diesel engines per mile (1.6 km) of traveled distance is over 100 times higher than that emitted from gasoline engines of equivalent power running on unleaded gasoline (5). The diesel exhaust particles are readily respirable, with a diameter of <0.05 µm, and they consist of a carbonaceous core similar to carbon black, with adsorbed hydrocarbons. In this review, the term diesel exhaust particles is used without further specification, although it is clear that different grades and sources of diesel will result in emissions with different characteristics with regard to both particulate matter (PM) and photochemical components. Variability in the relative composition of diesel exhaust may thus be one cause of variability in results between different investigations. From studies generally carried out on rats there are also reports on carcinogenic effects and, especially, the induction of lung cancer by diesel exhaust particles, but this association has been reviewed previously (6) and will not be discussed in this report.

## Inflammation in lung and airways

As discussed in detail in another section of this report ("Human Experimental Studies on the Effects of Inhaled Particles"), Rudell and co-workers have conducted experiments with human healthy volunteers and described both symptoms and changes in inflammation markers following exposure to diesel exhaust. Therefore, this account is limited to experimental studies with animal models or in vitro systems.

### In vivo studies

#### Rat

Long-term studies of animal exposure to diesel exhaust have shown an accumulation of particles in the lungs and an increased lung burden of soot. Aggregates of macrophages with particles are found in alveoli and peribronchial interstitial tissues and are associated with local inflammation, epithelial proliferation, fibrosis, and emphysematous lesions.

Rats (F344 strain) were exposed to diesel exhaust particles at 350, 3500, or 7000 µg/m<sup>3</sup> for 7 hours a day, 5 days a week for 6, 12, 18, or 24 months (7). A progressive increase in the lung burden of particles was observed in the animals exposed to higher levels of diesel exhaust particles. Tracheal mucociliary clearance [using <sup>67</sup>gallium sesquioxide (<sup>67</sup>Ga<sub>2</sub>O<sub>3</sub>) as the test substance] showed no changes, however. There was significant prolongation of the long-term pulmonary clearance half-times (using <sup>134</sup>cesium-fused aluminosilicate particles) in the two highest exposure groups, with increases from 79 to 264 and 240 days, respectively.

Another study, using the same strain of rats, investigated the effect of low-exposure doses for 52 weeks (diesel exhaust particles 50 µg/m<sup>3</sup>, 20 hours a day, 7 days a week) (8). At 1 year postexposure more than 80% of the particles had been eliminated. Acute high exposure was also tested, with 5700 µg/m<sup>3</sup> for 3 days, and animals exposed to higher concentrations cleared more of the lung burden during the postexposure period. A brief high exposure resulted in a rapid increase and decline in macrophage burden. At the longer low concentration, however, the macrophage burden was intermediate, and it stimulated a steady development of lung maculae. This result may indicate that low continuous exposure may be more detrimental than acute exposure, to even high loads.

Yu & Yoon (9) constructed a mathematical model for the prediction of lung burden and alveolar clearance

from rats. At low lung burdens, the alveolar clearance rate of diesel soot was constant, whereas at a high lung burden the alveolar clearance was determined principally by the capacity for transport to the lymphatic system.

Diesel exhaust particles cause dust overloading and impair pulmonary clearance at high doses in both rats and humans. There appears to be a threshold above which particle retention and inflammation occurs. It has, with most evidence from animal studies, been calculated that the threshold is around a load of 500 µg per gram of lung tissue (10). It is, however, difficult to assess how this value correlates with levels in the inhaled air.

Rats (F344) exposed to diesel emission for 30 months were examined anatomically and pathologically by an electron microscope every 6 months (11). Morphological changes in the lung were seen after 6 months of exposure at concentrations of 1000 µg/m<sup>3</sup>. The main change was anthracosis by inhaled diesel soot. Foci of alveolar macrophages laden with diesel particles were found. Diesel exhaust particles were found in type 1 epithelial cells, and hypertrophy and proliferation were found for type 2 cells. An infiltration of particle-laden macrophages, neutrophils, mast cells, and plasma cells into the interstitium of alveolar septa was seen. However, the most prominent changes found in the airways were focal shortening of cilia and protrusion of nonciliated cells.

Three groups of female Wistar rats were exposed during a lifetime either to clean air or to filtered or unfiltered diesel exhaust particles (4000 µg/m<sup>3</sup>), and another group was exposed to coal oven flue gas mixed with pitch fumes pyrolyzed under nitrogen (12). Most of the animals exposed to diesel exhaust particles had deposits of large amounts of carbonaceous particles phagocytosed by alveolar macrophages, severe chronic inflammation, alveolar septal thickening, bronchiolo-alveolar hyperplasia, and alveolar lipoproteinosis. Rats exposed to coal oven flue gas had much fewer severe inflammatory changes.

*Studies of developing lung in the rat.* Human studies in different areas have raised the concern that children and elderly people are more susceptible to the harmful effects of air pollution. There have been some earlier studies implying that lung development in animals exposed to oxidant gases early in life could be impaired or that young animals would be more susceptible to inhaled toxicants. In one study, however, two groups of rats were exposed 7 hours a day, 5 days a week for 6 months to NO<sub>2</sub> (9.5 ppm), to diesel exhaust diluted to a soot concentration of 3500 µg/m<sup>3</sup>, or to control air (13). The younger group was exposed in utero, by exposing the mother from conception through gestation and then exposing the newborns from birth up to the age of 6 months. The other group was exposed between the ages

of 6 and 12 months and considered to be a model for grown-ups.

Diesel exhaust particles altered the airway fluid constituents and tissue collagen in both groups. There was a sixfold increase in neutrophils, an increase in the number of cells in lung-associated lymph nodes, delayed clearance of particles, and an increase in the lung weight of adult rats. However, none of these changes were found in the rats exposed during development. In adult rats there was a focal aggregation of soot-laden alveolar macrophages but only scattered individual macrophages were found in young rats.

The authors concluded that the effects were equal or less in the developing rats and that there was no evidence for developing rats being more susceptible to the toxic effects of NO<sub>2</sub> or diesel exhaust.

*Studies in animals with experimental lung disease.* Epidemiologic studies have indicated that subjects with pre-existing lung disease may be more susceptible to episodic high levels of airborne pollutants than normal subjects. In an effort to define the mechanisms, this possibility has been studied in detail in an animal model in which lung disease has been experimentally induced (14). In this model, pulmonary emphysema was induced in rats through the intratracheal installation of the proteolytic enzyme elastase and manifested as enlarged alveoli and alveolar ducts and ruptured alveolar septa. These structural changes were not, by themselves, associated with inflammation or alterations of bronchioles. Such emphysematous rats and control rats were then exposed for 24 months to diesel exhaust at 3500 µg/m<sup>3</sup>, 7 hours a day, 5 days a week or to NO<sub>2</sub> at a concentration of 9.5 ppm, or to clean air, as the control. Different parameters were measured, such as the lung burden of diesel soot particles, respiratory function, the composition of bronchoalveolar lavage fluid, the clearance of radiolabeled particles, pulmonary immune response, lung collagen, excised lung weight and volume, and histopathology. The hypothesis was that the effect of pre-existing emphysema and long-term diesel exhaust exposure on lung function and morphology were more than additive and therefore indicated increased susceptibility. The results, however, showed that rats with experimentally induced emphysema were no more susceptible to the inhalation of NO<sub>2</sub> or diesel exhaust than control rats were. In fact fewer soot particles accumulated in the emphysematous lungs (14, 15).

In another study, however, only published in abstract form, rats with pulmonary inflammation induced by monocrotaline (50 mg/kg, administered subcutaneously) or chronic bronchitis induced by SO<sub>2</sub> were reported to have an increase in mortality of 19% and 37%, respectively, after exposure to a particle load when compared with control rats with no lung disease (16).

### Mouse

When the *in vivo* toxicity of diesel exhaust particles was studied in male mice (ICR) (17), the acute intratracheal instillation of diesel exhaust particles (400 to 1000 µg/mouse) caused severe lung injury and a high mortality rate. The cause of death was pulmonary edema mediated by endothelial cell damage. The toxicologic effect and the increased mortality were, to a great extent, prevented by pretreatment with the oxygen radical scavenger superoxide dismutase. This finding supports the hypothesis that the toxicity of diesel exhaust particles is connected to endothelial cell damage through the production of the radical superoxide.

The effect of diesel exhaust particles on allergen-induced bronchial hyperresponsiveness was studied in mice. Mice (C3H/HeN) were made allergic by intraperitoneal sensitization to ovalbumin and thereafter exposed to diesel exhaust (3000 µg/m<sup>3</sup>) by inhalation for 12 hours a day for 3 months (18). Three weeks after the onset of the exposure, there was a challenge with ovalbumin (1% for 6 minutes) every third week. Twenty-four hours after the last challenge the animals were sacrificed and airway responsiveness was measured, inflammatory cells in bronchoalveolar lavage were analyzed, and the lungs were examined histologically. Diesel exhaust exposure combined with antigen challenge induced airway hyperresponsiveness and airway inflammation, including increased eosinophils and mast cells in lung tissue. With only exposure to diesel exhaust particles there was also increased airway hyperresponsiveness but no eosinophil infiltration. In a similar setup, diesel exhaust inhalation (3000 µg/m<sup>3</sup>) in combination with ovalbumin sensitization increased the number of goblet cells in lung tissue, caused increased respiratory resistance, and increased the immune response measured as specific immunoglobulin (Ig)E, IgG<sub>1</sub>, and interleukin-5 (IL-5) in lung tissue (19).

### Guinea pig

A rhinitis model was used to study short-term effects of diesel exhaust (3 hours of exposure at 1000 and 3200 µg/m<sup>3</sup>) on guinea pigs (20). Following diesel exhaust exposure alone, there was no induction of sneezing, nasal secretion, or congestion. However, diesel exhaust particles augmented the sneezing and nasal secretion induced by histamine, but they had no significant effect on histamine-induced nasal congestion. The observations suggest that acute exposure to high levels of diesel exhaust may cause nasal mucosal hyperresponsiveness, but no overt symptoms of rhinitis.

### Cat

Cats have also been exposed to diesel exhaust particles for more than 2 years (21). Cats were exposed for 8

hours a day, 7 days a week. During the initial 61 weeks the concentration of diesel exhaust particles was 6000 µg/m<sup>3</sup>, and during the following 62 weeks it was 12 000 µg/m<sup>3</sup>. During the first period no evident functional changes were seen, but after 2 years a pattern of restrictive lung disease developed.

In another study cats were exposed to diesel exhaust particles for 8 hours a day, 7 days a week for 27 months, and the exposure concentration was nearly doubled after 62 weeks, from 6340 to 11 700 µg/m<sup>3</sup>, and the NO<sub>2</sub> concentration rose from 2.68 to 4.37 ppm (22). The following morphologic findings were reported: lesions in proximal acinar regions of lungs, peribronchial fibrosis, an increase of lymphocytes, fibroblasts and interstitial macrophages containing diesel exhaust particles, and bronchiolar metaplasia. The study implied that chronic exposure to diesel exhaust particles has a persistent fibrogenic effect on the proximal acinar region of the lung.

### Monkey

Male Cynomolgus monkeys were exposed 7 hours a day, 5 days a week for 24 months to ambient air, diesel exhaust particles (2000 µg/m<sup>3</sup>), coal dust (2000 µg/m<sup>3</sup>), or diesel exhaust particles and coal dust in combination (1000 µg/m<sup>3</sup> + 1000 µg/m<sup>3</sup>) and, interestingly enough, compared with rats receiving the same exposures (23). The monkeys retained relatively more particulate material than the rats. The sites of particle retention were the same for the diesel exhaust particles, coal dust, or the combination. Rats retained more material in the lumen of alveolar ducts and alveoli, whereas monkeys retained more in the interstitium. Rats showed significant alveolar epithelial hyperplasia, inflammation, and septal fibrosis. In contrast, such morphological changes were not seen in the monkeys. The results indicate that particle retention patterns and tissue reactions in rats exposed to diesel exhaust particles may not be predictive of the reactions in primates (24), which may be less sensitive to diesel exhaust particles.

### In vitro studies

The effect of diesel exhaust particles on isolated tissues and cultured cells from the respiratory tract of guinea pigs was studied (25). Diesel exhaust particles induced a dose-dependent relaxation of tracheal smooth muscle and time-dependent cytotoxicity of tracheal smooth muscle cells and lung fibroblasts. On the basis of pharmacological interventions, it was suggested that the cytotoxicity of diesel exhaust particles is mediated via the generation of oxygen radicals. Diesel exhaust particles have also been shown to produce aggressive oxygen radicals in a cell-free *in vitro* system in the presence of ap-

propiate electron donors (26), which in part may explain the potential toxicity and mutagenicity of diesel exhaust particles.

### **Allergy, immunology and related mechanisms**

There are reports from Japan suggesting that children living close to roads with heavy traffic are more likely to develop allergies. A higher prevalence of allergy to cedar pollen was thus observed in a population living close to motorways, as compared with a reference group living close to a cedar forest and being exposed to the same number of cedar pollens (27). Therefore, some experimental studies with possible relevance to these epidemiologic observations are reviewed (5, 28–30).

#### **In vivo studies**

##### **Humans**

Studies have indicated that asthmatics are more sensitive to air pollutants and, in particular, acid aerosols (31). Hyperosmolar aerosols also induce bronchoconstriction in asthmatics, and this response is potentiated by acidity. Other studies show that young allergic asthmatics with exercise-induced bronchoconstriction are more sensitive than adult asthmatics to acid aerosols.

There is, however, a remarkable case report of diesel exhaust particles themselves causing asthma (32). Three railroad workers, nonsmokers and without any previous history of asthma, developed persistent asthma after acute exposure to excessive levels of diesel exhaust. This phenomenon occurred in situations where two locomotive units were coupled together and the crew riding in the second locomotive unit was exposed to significant diesel exposure. After 2–5 hours of exposure, there was an acute onset of shortness of breath, chest tightness, burning chest pain, and wheezing. All three subjects developed asthma, which still persisted 1 to 3 years postexposure. Although the actual levels were never assessed, it is likely that such excessive exposures rarely occur even in occupational situations.

Diesel exhaust particles potentiate IgE production in human respiratory mucosal membranes, as shown for example in a study by nasal challenge with varying doses of diesel exhaust particles (33). Four days after a challenge with 0.30 mg of diesel exhaust particles there was a significant increase in nasal IgE but not in the other Ig classes. There was also an increase in the number of IgE-secreting cells in lavage but no increase in IgA-secreting cells.

Diesel exhaust particles appear capable of acting as an adjuvant to allergens. Human nasal provocation studies were performed with challenges with diesel exhaust particles (0.3 mg) or the ragweed allergen *Amb a 1* or

both in a group of ragweed-sensitive subjects (34). When the challenge was with ragweed alone, there was an increase in IgE and IgG<sub>4</sub>, and ragweed-specific IgE was also detectable in the lavage fluid. However, after challenge with the allergen and diesel exhaust particles, there was a 16-fold increase in ragweed-specific IgE. Diesel exhaust particles alone could increase total IgE, but in the presence of allergen there was an increase in antigen-specific IgE and in the expression of T<sub>H0</sub>- and T<sub>H2</sub>-type cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13). It is currently believed that such deviations of the immune system may play a crucial role in the development of an atopic response. These studies suggest that diesel exhaust particles can enhance B-cell differentiation. By initiating and elevating IgE production, diesel exhaust particles may theoretically play a role in the increased prevalence of allergic disease.

The combination of nasal challenge with diesel exhaust particles and allergen has shown that, in comparison with the challenge with only diesel exhaust particles, diesel exhaust particles together with ragweed allergen induces higher ragweed-specific IgE and IgG<sub>4</sub> responses. However the total IgE levels were unchanged (35). Also in this study, there was a change in the cytokine pattern that would favor allergic sensitization. The authors suggested that the synergism between diesel exhaust particles and natural allergens is a key feature in increasing allergen-induced respiratory allergic disease.

Recently there has been evidence of diesel exhaust particles inducing what is called IgE isotype switching (3). Human volunteers were exposed by nasal challenge to diesel exhaust particles together with ragweed allergen. There was a local increase in IgE production, cytokine production, and an even greater increase in mucosa-specific ragweed IgE production. The isotype switching occurred only when diesel exhaust particles and allergen exposure was combined. The findings suggest that increasing environmental diesel exhaust particles with the same environmental load of allergen could be a factor behind the increasing sensitization and prevalence of allergic asthma.

Healthy, nonsmoking human volunteers have been exposed to diesel exhaust particles by intranasal instillation and cytokines in nasal lavage estimated after 18 hours by an indirect approach (mRNA) (36). Before the challenge, most of the subjects had detectable mRNA levels of only a few cytokines [interferon gamma (IFN- $\gamma$ ), IL-2 and IL-13], while after a challenge with 0.3 mg of diesel exhaust particles these three cytokines and a number of additional other cytokines (IL-4, IL-5, IL-6 and IL-10) were found in increasing levels. An increase in such nasal cytokine expression after exposure to diesel exhaust particles could again contribute to enhanced local IgE production.

## Rat and mouse

Bice et al (37) studied the effect of exposure to diesel exhaust particles on the accumulation of particles in lung-associated lymph nodes and the possible effect on the antibody immune response after immunization in rats (F344) and mice (CD-1). Animals were exposed in chambers to diesel exhaust particles, 7000 µg/m<sup>3</sup>, 3500 µg/m<sup>3</sup> or 350 µg/m<sup>3</sup>, 7 hours a day, 5 days a week for 24 months. Control and exposed animals were immunized by the intratracheal instillation of sheep red blood cells after exposure for 6, 12, 18, and 24 months. Maximal pathological changes were found after exposure for 18 to 24 months at 3500 or 7000 µg/m<sup>3</sup>, but there were also some effects in the low-dose group. Microscopic findings were an increase in the particle content of alveolar macrophages, a concentration of macrophages closer to terminal bronchioles, wall fibrosis, bronchial metaplasia, and, in some cases, squamous metaplasia. There were cholesterol clefts and increased interstitial neutrophils and intraalveolar granular eosinophilic material with entrapped free particles. Lung-associated lymph nodes were enlarged and black due to clusters of diesel exhaust particles. The number of lymphoid cells was elevated in rats exposed to the highest dose. However, the levels of specific antibodies were not significantly changed. The findings suggest that the lymph nodes accumulate diesel exhaust particles, and this accumulation leads to an increase in lymphoid cells in the tissue, but this change appears to have no consequence for antigen retention and immune functions.

Diesel exhaust particles show an adjuvant activity on IgE production in mice. Mice injected intraperitoneally with ovalbumin mixed with diesel exhaust particles showed higher IgE levels than mice immunized with ovalbumin alone. A production of Japanese-cedar-pollen-specific IgE was also found in mice immunized with Japanese cedar pollen mixed with diesel exhaust particles but not in animals immunized with Japanese cedar pollen alone (38).

The adjuvant effect on IgE production by different particles induced by carbon black and diesel exhaust particles were also studied in BDF<sub>1</sub> mice (39). Particles (25 µg) were instilled intranasally, and the animals were exposed to an aerosol of Japanese cedar pollen for 30 minutes a day at 1-week intervals for 8 weeks, followed by exposure for 30 minutes every 3 weeks for 9 weeks. There was a significant adjuvant effect of the particles on the production of Japanese-cedar-pollen-specific IgE and IgG. IgE production seemed to occur earlier in mice treated with particles than in mice immunized with allergens alone.

The adjuvant effect of diesel exhaust particles on systemic IgE production has also been studied in mice after intranasal challenge in order to elucidate which

part of the exhaust, the carbon core or the absorbed organic substance, is responsible for the effect (40). Balb/cA mice were immunized four times with ovalbumin alone or in combination with diesel exhaust particles or carbon black particles, and ovalbumin-specific IgE was analyzed. Both diesel exhaust particles and carbon black particles showed an adjuvant activity for specific IgE production after intranasal instillation, the implication being that both the organic matter absorbed on diesel exhaust particles and the nonextractable carbon core were responsible for the effect.

Using another mouse model, the popliteal lymph node assay, Lövik et al (41) showed that the nonextractable particle core contributed substantially to the adjuvant effect of diesel exhaust particles. Mice were immunized with ovalbumin together with diesel exhaust particles in the hind foot pad, and changes in popliteal lymph-node weight, cell numbers, cell proliferation, and specific IgE were measured. Ovalbumin inoculated together with diesel exhaust particles significantly augmented the response.

Combined intratracheal inoculations of diesel exhaust particles and antigen in mice increased airway inflammation induced by antigens and increased antigen-specific IgG<sub>1</sub> production but not antigen-specific IgE after 6 weeks (42). However, after a longer exposure (9 weeks), antigen-specific IgE was also increased. This may be another indication that there are differences between acute and chronic exposure.

Mice from five different strains were injected intratracheally with ovalbumin alone or together with diesel exhaust particles four times at 3-week intervals (43). Significant adjuvant activity of IgE production by diesel exhaust particles (50 µg) was not observed in any strain. However, the degree of eosinophilic inflammation in the airways corresponded with the specific IgG<sub>1</sub> production. There may be differences due to exposure route or dose, as higher doses of diesel exhaust particles (300 µg) showed an increase in specific IgE under similar conditions (44).

In another study, the effect of diesel exhaust particles was tested on two strains of mice, a high IgG responder strain (C3H/He) and a high IgE responder strain (BALB/c) (45). The mice were exposed through the intratracheal instillation of diesel exhaust particles (0.1 ml of 250 µg/ml) once a week for 5 weeks with ovalbumin sensitization intraperitoneally starting after 1 week. Twenty-four hours after diesel exhaust particles were administered, the mice were challenged with an aerosol of 1% ovalbumin. The diesel exhaust particles caused increased IgG<sub>1</sub> production and airway hyperresponsiveness in C3C/He mice and an increase in eosinophils and goblet cells in lung tissue and an increase in cytokines involved in eosinophilic function (IL-2 and IL-5). There was no effect on IgE production in any strain, and only

low effects on other parameters were found in the BALB/c mice. There are thus murine-strain differences, and the effect on mice appears to be related to IgG<sub>1</sub> production.

Takano et al (42) also studied the effect of diesel exhaust particles on ICR mice and found that the intratracheal instillation of diesel exhaust particles increased antigen-induced airway inflammation, as indicated by an increase in eosinophils, lymphocytes, and goblet cells in bronchial epithelium and an increase in IgG and IgE production. There was also an increase in the proallergic cytokines IL-2, IL-4, IL-5, and granulocyte macrophage-colony stimulating factor (GM-CSF) but not in the suppressive cytokine IFN- $\gamma$ . They also found that a combination of diesel exhaust particles and ovalbumin increased the bronchoconstriction response to inhaled acetylcholine in this model (46). Furthermore, daily inhalations of diesel exhaust particles may enhance allergen response, possibly through an increased local expression of proinflammatory cytokines such as IL-5 and GM-CSF (47). In another study, increased IgE production was also found in mice after the intranasal administration of suspended particulate matter (SPM) together with ovalbumin at 3-week intervals until 21 weeks (48).

The role of nitric oxide in asthma-like symptoms induced by diesel exhaust particles has been studied in mice (49). The repeated intratracheal instillation of diesel exhaust particles in mice induced a fourfold increase in macrophages, neutrophils, eosinophils, and lymphocytes in bronchoalveolar lavage fluid. Diesel exhaust particles induced a twofold increase of nitric oxide in the exhaled air and an increase in staining of nitric oxide synthetase in the airway epithelium. The increase in respiratory resistance induced by the installation of diesel exhaust particles was suppressed by N<sup>G</sup>-methyl-L-arginine, an inhibitor of nitric oxide synthetase.

## In vitro studies

### Human cells

Complement activation is a part of the host defense system, and the effect of diesel exhaust particles on the hemolytic activity of human serum complement has been studied (50). Pretreatment of human serum with extracts of diesel exhaust particles (500–2500  $\mu$ g/ml) showed a dose-dependent decrease in hemolytic activity to 20% of the total at the highest tested dose. These studies also indicate that diesel exhaust particles can activate the alternative pathway of the complement system and result in a decrease in the hemolytic activity of the complement.

Studies on isolated human B-lymphocytes have shown that exposure to diesel exhaust particles enhances the synthesis of IgE by these cells. Airway epithelial cells play a prominent role in the pathogenesis of air-

way disease. Various studies have shown that the exposure of nasal or bronchial epithelial cells to diesel exhaust particles results in increased synthesis and a release of proinflammatory mediators, cytokines such as IL-6, IL-8 or GM-CSF, and adhesion molecules (51).

The ability of diesel exhaust particles to induce IgE production has been mimicked by polycyclic aromatic hydrocarbons extracted from diesel exhaust particles and shown to enhance IgE production from purified human B-cells stimulated by IL-4 (52). However, the polycyclic aromatic hydrocarbons from diesel exhaust particles did not induce IgE production in unstimulated B-cells, the implication being that it only enhances ongoing IgE production. Phenanthrene, a major polycyclic aromatic hydrocarbon and an important component of diesel exhaust particles, has shown the same effect on IgE production in a human B-cell line (53).

Other studies were performed using cultured human bronchial epithelial cells exposed to diesel exhaust particles (54). Exposure of these cells to diesel exhaust particles at a concentration of 50  $\mu$ g/ml, a filtered solution of diesel exhaust particles, or diesel exhaust particles at a concentration of 100  $\mu$ g/ml attenuated the ciliary beat frequency dose dependently and increased the release of IL-8, GM-CSF, and soluble intracellular cell adhesion molecule (ICAM-1). The observations support the possibility that exposure to diesel exhaust particles may lead to functional changes and a release of proinflammatory mediators that have the potential to influence the development of airway disease. Specifically, infiltrations of neutrophils and other inflammatory cells would be promoted by the observed changes.

The effect of diesel exhaust particles was also studied in three different in vitro systems of human airway epithelial cells, in epithelial cells from nasal polyps, in human bronchial cells obtained during lung tumor resection or at autopsy, and in a human bronchial epithelial cell line (BEAS-2B) (55). Diesel exhaust particles (10–100  $\mu$ g/ml) induced a dose- and time-dependent stimulation of IL-8 and GM-CSF production by all three kinds of epithelial cells. With the use of double chamber plates, it was shown that the cells could only be stimulated from the apical side. As a control, neither coal nor graphite showed any stimulatory effect, whereas, benzpyrene, a constituent of diesel exhaust particles, did. It thus appears that the exposure of human epithelial cells to diesel exhaust particles may stimulate cytokine production, with possible relevance for allergic inflammation.

In another study the effect of diesel exhaust particles in doses between 40 to 330  $\mu$ g/ml was investigated using a human bronchial epithelial cell line (56). Diesel exhaust particles ranged between 25 to 35 nm and were phagocytized by these cells. There was an increase in IL-6 and IL-8 production (11-fold and 4-fold,

respectively). Exposure of these cells to tumor necrosis factor alpha (TNF- $\alpha$ ) also stimulated a strong increase in IL-6 and IL-8 production. There was an additive effect on the production of IL-6 and IL-8 after TNF- $\alpha$  priming and subsequent exposure to diesel exhaust particles, seen only at low doses of diesel exhaust particles (10–70  $\mu\text{g/ml}$ ) and TNF- $\alpha$  (0.05–0.2  $\text{ng/ml}$ ).

Mechanisms involved in asthma development after exposure to diesel exhaust particles may include a better penetration of allergen across the respiratory mucosa or direct modulation of the immunologic response (57). Particulate air pollution includes particles from diesel and gasoline engine exhaust and biological materials such as plant debris and tire debris (eg, latex) that may be allergens (58). Some allergens, such as the major grass pollen *Lolium perenne*-1 (Lol p1) allergen, are also shown to bind specifically to diesel exhaust particles. This might be a possible mechanism for triggering asthma attacks and one theoretical explanation for the increase in asthma prevalence (59).

Ormstad et al (60) have also studied the allergen-carrying effect of diesel exhaust particles, in an *in vitro* study of allergen adsorption to particles such as indoor SPM and diesel exhaust particles. They found that a cat allergen (Fel d1), dog allergen (Can f1), and birch pollen allergen (Bet v1) were all found on the surface of SPM, whereas house-dust-mite allergen (Der p1) was not. However, all four allergens were found to be absorbed by diesel exhaust particles.

A chemical characterization has been studied of the coating of birch pollen grains collected during the pollen season in the north of Stockholm. The greatest portion (80%) consisted of n-alkanes and n-alkenes, but also methylketones, ethers, alcohols, and amino alcohol were identified (61).

### *Rat*

Yang et al (62) studied the possible role of cytokines for the toxic effect of diesel exhaust particles. Rat alveolar macrophages were incubated with diesel exhaust particles in different concentrations, as well as with methanol, washed diesel exhaust particles, or methanol extracts of diesel exhaust particles. High concentrations of diesel exhaust particles and methanol extracts increased IL-1 secretion by alveolar macrophages, while there was no effect on TNF- $\alpha$ . Diesel exhaust particles inhibited the production of IL-1 and TNF- $\alpha$  stimulated by endotoxin (lipopolysaccharide). This inhibition suggests that the proinflammatory cytokine IL-1 may play a role in the pulmonary response to the inhalation of diesel exhaust particles. Furthermore, pretreatment of alveolar macrophages with diesel exhaust particles suppresses the response to lipopolysaccharide stimulation, which may be a factor contributing to the impairment

of the pulmonary defense system after prolonged exposure to diesel exhaust particles.

### *Rabbit*

The effect of diesel exhaust particles on endogenous production or the action of nitric oxide has been studied by Muto et al (63), who worked with a model using rabbit bronchial or aortic rings. As nitric oxide is present in exhaled air, they studied whether diesel exhaust particles inhibit the synthesis of nitric oxide. It is well known that nitric oxide released from vascular endothelium after stimulation by acetylcholine accounts for the biological activity of endothelium-derived relaxing factor. They found that diesel exhaust particles, as well as N<sup>G</sup>-methyl-L-arginine (an inhibitor of nitric oxide synthetase), alter the effect of acetylcholine, as it abolished the acetylcholine-induced relaxation of aortic rings pre-constricted with phenylephrine. Nitric oxide release by acetylcholine from aortic rings was inhibited by diesel exhaust particles (100  $\mu\text{g/ml}$ , 60 minutes). Nitric oxide released by the bronchial epithelium in rabbit bronchial strips attenuated the bronchoconstriction induced by acetylcholine, and this attenuation was abolished by 60 minutes of preincubation with diesel exhaust particles (100  $\mu\text{g/ml}$ ) or N<sup>G</sup>-methyl-L-arginine ( $5 \times 10^{-4}$  M). Their conclusion was that the inhibition of nitric oxide availability by diesel exhaust particles may be a part of the observed respiratory effects of such particles, but further studies are required to establish the significance of their findings.

### *Cardiovascular effects*

The direct toxic action of diesel exhaust particles has been examined in a model of isolated atria in guinea pigs (64). Diesel exhaust particles in lower doses (between 10 and 500  $\mu\text{g/ml}$ ) induced a transient but dose-dependent increase in contractile force (positive inotropic response). In doses higher than 500  $\mu\text{g/ml}$ , diesel exhaust particles only decreased contractile force (negative inotropic response) and induced cardiac arrest. It was concluded that the cardiac toxicity contributes to the lung edema that is known to be a prominent cause of death of animals exposed to diesel exhaust particles. It appears unlikely, however, that the inhalation of diesel exhaust particles by humans may produce the concentrations employed in these particular experiments.

Epidemiologic studies have shown increased mortality in cardiovascular disease and respiratory disease during heavy air pollution (see the section "Epidemiologic studies on Health Effects of Ambient Particulate Air Pollution"). In a meta-analysis, Seaton et al (65) suggested that ultrafine particles induce inflammation

in the lung in susceptible persons, the release of mediators, and an increase in blood coagulability. There has been a hypothesis of a possible association between occupational exposure to dust and ischemic heart disease, and Sjögren has tried to test this idea in a literature survey (66–68). Coal miners showed manifestations of pneumoconiosis and an increased incidence of ischemic heart disease. Fibrinogen is a risk factor for ischemic heart disease, and there are increased levels of fibrinogen in coal miners with pneumoconiosis. The hypothesis is that long-term inhalation of particles retained in the lung induces inflammation, accompanied by an increase in plasma fibrinogen, which leads to an elevated risk for blood clotting and ischemic heart disease (68). There are, however, no experimental data at the moment which support the possibility that the inhalation of PM by humans has direct effects on fibrinogen levels or activity, and the hypothesis is built on circumstantial evidence.

### Summary and concluding remarks

Diesel exhaust particles can have several effects on the lung and the cardiovascular system and may interfere with immunologic and allergic reactions.

The acute effects include irritation of the nose and eyes by an unpleasant odor, a transient decline in ventilatory capacity, cardiovascular symptoms, headache, fatigue, and nausea. These symptoms are usually reversible within a few days.

Chronic exposure to diesel exhaust induces cough, sputum production, and lung function decrements. It has been speculated that there are exacerbations of asthma and respiratory disease due to the inhalation of PM<sub>10</sub> particles from diesel exhaust particles, but there is no direct experimental support for this view. The relative role of diesel exhaust particles and other particles for the pulmonary effects of exposure to road tunnel air remains to be established (69).

Pathological and histological findings in lung after exposure to diesel exhaust particles have mostly been studied in rats and include increases in lung weight, increases in particles in lung, and an increased burden of BS, associated with alveolar infiltration of macrophages, macrophage aggregation, chronic inflammatory expression, proliferation and hyperplasia of alveolar epithelium and type 2 cells, thickening of alveolar septa, wall fibrosis, and emphysema.

Comparisons of diesel exhaust particles and carbon black in animal inhalation studies show that a reduction in lung function and an accumulation of macrophages appear in parallel, the result suggesting that the toxic effect of diesel exhaust particles is in part coupled to

the carbon core. The toxicity of diesel exhaust particles could in part also be mediated via the production of oxygen radicals.

Diesel exhaust particles introduced by different routes, intraperitoneally, intranasally or intratracheally, may act as an adjuvant to pollen and hence increase the sensitization response. Such a phenomenon has been observed in both human and animal studies, mostly in mice, in which the IgE production in response to allergen has been enhanced by diesel exhaust particles. Diesel exhaust particles can also activate the alternative pathway of complement activation. Diesel exhaust particles affect human B cells and may enhance IgE production through several mechanisms. In addition, diesel exhaust particles show effects on allergic responses that involve inflammatory cells in respiratory mucosa, like T cells, mast cells and epithelial cells, and also on the local production of different proinflammatory cytokines.

Another possible mechanism for diesel exhaust particles in allergic responses may also be to act as a carrier of pollen allergens, allowing enhanced deposition of pollen in the lower airways. Allergen binding to diesel exhaust particles may trigger asthma attacks and may facilitate the penetration of allergen through the airway mucosa.

Published data do not indicate that there is a unifying mechanism of action which can explain different alleged public health effects of particulate air pollution. One major shortcoming of many experimental studies relates to the inability to establish whether the exposures used for the studies are relevant to the background or peak exposures which may occur in real life, acutely or chronically. There is therefore a great need to assess such relations in humans or in animal models that are sufficiently predictive and sensitive.

The particle concentrations that include diesel emissions (ie, particles less than PM<sub>10</sub>) are reported to be 30–100 µg/m<sup>3</sup> in urban situations in Sweden and Europe. The highest exposures occur in occupational situations and have been reported to vary between 4 and 1740 µg/m<sup>3</sup> (for railroad workers, miners, or truck drivers) (70). In experimental situations with animals, the doses have usually varied between 100 and 11 700 µg/m<sup>3</sup> (71). Therefore the effects observed probably have the greatest relevance to situations of relatively high exposure (ie, occupational). Theoretically, acute exposure to high levels of diesel exhaust particles or chronic exposure to low levels may both impair respiratory functions, but the mechanisms may differ. It is, for example, known for a classical trigger of asthma such as allergen that acute exposure to high allergen doses produces effects which differ from those caused by repeated low-dose exposure (72). After the results of chronic-exposure studies on rats have been combined with

mathematical models for lung clearance, it has been proposed that the effects on lung function, especially lung clearance, occur at doses that are 10–50 times lower than those required to induce carcinogenic effects (73). A threshold dose (no observable adverse effect level) has been noted for the appearance of symptoms or effects on lung function, and it is reported to be 2000, 350 and 250  $\mu\text{g}/\text{m}^3$  for monkeys, rats, and guinea pigs, respectively. Such findings, and other data, give the impression that primates are less sensitive than rodents to diesel exhaust particles. Extrapolations from the dose-response relationships in animal studies and transformation to the human situation have been reported. Thus from the NOAEL level in animal studies there have been attempts to calculate a corresponding safe dose for humans, and the Environmental Protection Agency (EPA) in the United States has accordingly established a limit of 5  $\mu\text{g}/\text{m}^3$  for continuous lifetime exposure. It must however be recognized that such calculations have many limitations, as they are based on mathematical modeling and interspecies extrapolations.

Finally, during the compilation of this section, new epidemiologic data appeared in the literature that supported the assumption that atopic children constitute a group with particular risk of developing significant health effects as a result of exposure to airborne particles (74). The existence of such sensitive groups would seem to deserve particular attention in risk assessment.

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