Effect of pollutants upon patients with respiratory allergies

I Dávila,[1] J Mullol,[2] J Bartra,[3] A del Cuvillo,[4] M Ferrer,[5] I Jáuregui,[6] J Montoro,[7] J Sastre,[8] A Valero[3]

[1] Service of Immunoallergy, Hospital Clínico, Salamanca, Spain
[2] Rhinology Unit, ENT Service (ICEMEQ), Hospital Clínic, Barcelona, Spain
[3] Allergy Unit, Service of Pneumology and Respiratory Allergy, Hospital Clínic (ICT), Barcelona, Spain
[4] Clínica Dr. Lobatón, Cádiz, Spain
[5] Department of Allergology, Clínica Universitaria de Navarra, Pamplona, Spain
[6] Service of Allergy, Hospital de Basurto, Bilbao, Spain
[7] Allergy Unit, Hospital La Plana, Villarreal (Castellón), Spain
[8] Service of Allergy, Fundación Jiménez Díaz, Madrid, Spain

Summary

Epidemiological studies have revealed an association between pollution and allergic respiratory diseases. The main pollutants in this sense are nitric oxide, ozone, and particulate matter. The present review on one hand addresses the chemical characteristics of each of these three groups of pollutants and their main sources, and on the other examines their effects upon allergic respiratory diseases - placing special emphasis on the effects of diesel exhaust particles. For each of the pollutants, the underlying mechanisms capable of influencing allergic respiratory diseases are commented. Lastly, an evaluation is made of some of the genetic aspects related to the response to pollutants.

Key words: Allergy. Ozone. Nitric oxide. Particulate matter. Asthma.

Resumen

Los estudios epidemiológicos muestran una asociación entre la contaminación y las enfermedades alérgicas respiratorias. Los principales agentes contaminantes son el óxido nítrico, el ozono y las partículas en suspensión. En esta revisión se comentan, por un lado, las características químicas de cada uno de esos tres grupos de contaminantes y sus fuentes principales y, por otro, los efectos que ejercen sobre las enfermedades alérgicas respiratorias , prestando especial atención a los efectos de las partículas derivadas de la combustión de los motores diesel. Para cada uno de los agentes contaminantes, se comentan los posibles mecanismos subyacentes capaces de influir sobre las enfermedades alérgicas respiratorias. Por último, se evalúan algunos aspectos genéticos relacionados con la respuesta a los agentes contaminantes.

Palabras clave: Alergia. Ozono. Óxido nítrico. Partículas en suspensión. Asma.

Introduction

Technical and industrial development has led to a significant increase in the world population and its quality of life, though with considerable differences depending on the geographical location. However, industrialization and the technological revolution have not taken place without collateral effects for the environment in which such activities occur. Globally, such effects can be considered to result from what we call environmental pollution. The dictionary defines (as first option) the term "pollution" as the "alteration or damage of some substance, or its effects upon the purity or state of something". In its third defining option, pollution is taken to be "intense and harmful damage to water or air, caused by the waste of industrial or biological processes". In the medical setting, pollution refers to the effects upon human health of such alterations in water or air. In allergology, pollution can have repercussions in two ways: upon people, altering their health; or upon allergens, altering their composition, allergenicity or production. The present review will center on the first of these aspects.

In the last quarter of a century, there have been important advances in our knowledge of the effects of pollutants upon health considered globally, and upon certain allergic disease in particular. This knowledge has in part reached the general public, which is showing a growing interest in the subject. Thus, an informed patient may for example ponder whether pollution can increase the risk of developing asthma or allergy, or of exacerbating such a disorder; or how to prevent the effects of environmental pollution (questions, for example, regarding whether air pollution can influence childhood asthma, or how to avoid or prevent its effects). It is therefore important for the physician to be able to answer these and other patient concerns.

Air pollutants

Environmental pollutants can be classified in different ways: 1) according to whether they require transformation or not (primary and secondary); 2) according to their composition (gases or particulate matter); or 3) according to their location (outdoor and indoor) [1]. Depending on whether they require prior transformation or not, pollutants are classified as primary agents - these being the contaminants released directly into the atmosphere, such as SO_2 - or secondary agents (i.e., those formed in the atmosphere as a result of chemical reaction with other contaminants or gases such as ozone) (Table 1). Particulate matter (PM) is usually also classified according to size (see below).

The main sources of pollution tend to be human, i.e., anthropogenic. Of these sources, the most important is the combustion of fossil fuels. In urban areas and in much of the suburban setting, the main source of pollutants is motor vehicle combustion. These emissions (vehicle exhaust) constitute a complex mixture of chemical compounds, including for example organic compounds derived from diesel exhaust, PM and irritating gases such as NO_2, SO_2 and O_3 [2]. Other sources of pollution are industries and energy producing facilities - particularly those that use coal. The most important natural source of pollution is represented by fires.

The fundamental pathway for exposure to these pollutants is inhalation [3] - hence their importance in relation to allergic respiratory diseases such as asthma. Other less important sources are water and soil pollution secondary to accumulation and rainfall (precipitation) - taking into account that some pollutants can accumulate and persist in these settings, since their degradation takes place at a slower rate.

A description is provided below of the main pollutants, NO_2, ozone and PM, with an account of their main characteristics and an analysis of their effects upon allergic respiratory diseases - placing special emphasis on asthma, since this is the most widely studied and best known representative of such disorders. In general, studies conducted in animals will be avoided, centering attention on the review of the available data obtained from human studies. Nevertheless, it should be mentioned that under conditions of normal clinical practice, particles and gases form a complex mixture, and close interactions may exist among the different pollutants. Tables 2 and 3 report the limiting values for these pollutants according to current Spanish legislation.

Table 1. Classification of air pollutants

Need (or not) for prior transformation
Primary
SO_2
Some reactive NO_X compounds
CO
Particulate matter (PM)
Secondary
Ozone (O_3)
Some reactive NO_X compounds
Certain particles
Gaseous or particles
Gaseous
SO_2, NO_X, CO, Ozone, CO, SVOC *
Particles
Large or PM_{10} (2.5-10 mm)
Fine or $PM_{2.5}$ (0.1-2.5 mm)
Ultrafine (<0.1 mm)

(*) SVOC: specific volatile organic compounds

1. Nitrogen compounds

Nitrogen compounds are present in the atmosphere in both oxidized and reduced form. The reduced forms include ammonia (NH_3) and ammonium (NH_4). The oxidized forms in turn include nitric oxide (N_2O), nitrogen oxide (NO), nitrogen dioxide (NO_2), nitrous acid (HNO_2), nitric acid (HNO_3), peroxyacetylnitrate (PAN) and particulate nitrates (NO_3)[4]. NO_X is defined as NO + NO_2 [4]. Most nitrogen oxides are released into the atmosphere in the form of NO, which is transformed relatively quickly into NO_2 through reaction with ozone, or with radicals such as HO_2 or RO_2. Of the different nitrogen compounds, NO_2 is the most important in terms of impact upon health.

NO_2 can be generated from natural sources (bacterial activity in the soil, volcanoes, lightning) - though in the European setting anthropogenic sources are the most important. Motor vehicles - both diesel and gasoline - together with coal- and petroleum-burning energy plants are the main

Table 2. Threshold values for certain environmental pollutants in Spain, according to Royal Decree 1073/2002

Pollutant	Type of limit	Averaging period	Limiting value	Date for reaching limiting value
NO_2	Hourly limiting value for protection of human health	Hour	200 μg/m3, value not to be exceeded more than 18 times per calendar year	1 January, 2010
	Annual limiting value for protection of human health	Calendar year	40 μg/m^3	1 January, 2010
	Limiting value for protection of vegetation (NO_X)	Calendar year	30 μg/m^3	19 July, 2001
SO_2	Hourly limiting value for protection of human health	Hour	350 μg/m^3, value not to be exceeded more than 24 times per calendar year	January 1, 2005
	Day limiting value for protection of human health	24 hours	125 μg/m^3, value not to be exceeded more than 3 times per calendar year	January 1, 2005
	Limiting value for the protection of ecosystems	Calendar year and winter (1 October - 31 March)	20 μg/m^3	July 19, 2001
CO	Limiting value for the protection of human health	Mean 8 hours maximum in one day (stepwise)	10 mg/m^3	January 1, 2005
PM_{10}	Day limiting value for the protection of human health	24 hours	50 μg/m^3, value not to be exceeded more than 7 times per year	1 January, 2010
	Annual limiting value for the protection of human health	One calendar year	20 μg/m^3	1 January, 2010
BENZENE	Limiting value for the protection of human health	Calendar year	5 μg/m^3	1 January, 2010

Table 3. Thresholds for tropospheric ozone in Spain, according to Royal Decree 1796/2003

Threshold	Value	Reference period
Threshold for population information	180 μg/m^3	Hourly average
Population alert threshold	240 μg/m^3	Hourly average. For immediate action plans, evaluation is made during 3 consecutive hours
Protection of health	120 μg/m^3	Mean mobile 8-hourly without maximum recovery of each day, cannot be exceeded more than 25 days per calendar year on average in a period of 3 years
Protection of vegetation	AOT40 = 6.000 μg/m^3 h	Hourly values from May to July
Protection of forests	AOT40 = 20.000 μg/m^3 h	Hourly values from April to September
Material damage	40 μg/m^3	Calendar year

AOT40 will be the sum of the difference between hourly concentrations in excess of 80 μg /m^3 (= 40 parts per 1000 million) and 80 μg /m^3 in the course of a given period, using only the hourly values measured between 8.00 and 20.00 hours.

source of nitrogen oxides. The higher the temperature and combustion pressure, the greater the production of nitrogen oxides. Nitrogen dioxide (NO_2) is a brownish-red and toxic gas responsible for the photochemical haze known as smog. In association with SO_2, it is also responsible for acid rain. The gas concentrates mainly in the urban setting, though the levels are highly variable, depending on the zone. The levels of NO_2 are a reasonable marker of exposure to motor vehicle traffic. In the rural setting the concentrations are much lower and depend on the distance to the source. The cycle of nitrogen compounds in the urban setting is complex, and is shown in Figure 1. There is no linear correlation between the levels of NOx and NO_2. In general, indoor concentrations are usually 30-100% of the outdoor concentrations.

1.1. Effects of NO_2. NO_2 exerts its effects upon health over both the short and long term. Short-term exposure to very high levels of NO_2 can have important pulmonary repercussions in healthy subjects. In people with chronic lung diseases such as asthma or chronic obstructive pulmonary disease (COPD), the threshold is lower, and responses can be seen at short term, such as increased bronchial hyper-responsiveness [4]. Chronic exposure to NO_2 can be associated with increased respiratory symptoms. Nevertheless, it may be difficult to distinguish whether the observed effects are the result of short- or long-term exposure to the pollutant.

Some epidemiological studies have associated NO levels to visits to the emergency service due to asthma attacks [5,6], or NO_2 indoor concentrations to asthma exacerbation [7,8]. However, there is a disparity of results in the literature. Thus, a recent study found no association between personal exposure to NO_2 and peak respiratory flow values in asthmatic adults [9], while in other cases the results were inconclusive [10,11]. In addition, it must be taken into account that the effect of a given pollutant is difficult to distinguish from the effects of other pollutants to which the former is often associated. In Valencia (Spain), Tenias et al. [12] found an association between

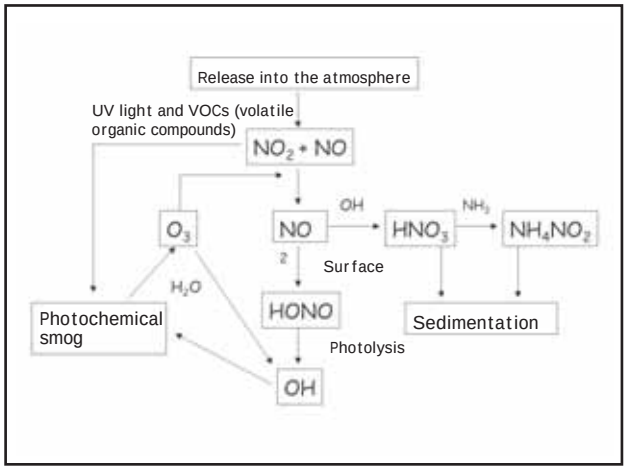

Figure 1. Simplified representation of the nitrogen compounds cycle in an urban environment (taken from [4]).

NO_2 and ozone levels and visits to the emergency service due to asthma attacks. In Madrid (Spain), Galán et al. [13] reported an identical association with the same two pollutants and with PM_{10}; in addition, these authors simultaneously monitored pollen levels - concluding that the observed effect is independent of the atmospheric concentrations of pollen.

The experimental studies conducted on NO_2 have yielded a range of results. Thus, for example, it has been reported that brief exposure to low levels of NO_2 can induce changes in bronchial hyper-responsiveness in some asthmatic patients, but not in all of them [14] - though these findings have not been reproduced in other studies using similar methodology [15] or higher concentrations of NO_2 [16]. This could be due at least in part to the concentration used, since high concentrations (up to 910 mm/m^3) yield effects in terms of bronchial hyper-responsiveness and lung function in both asthmatics and normal subjects [17]. In a later study, the same authors concluded that exposure to up to 500 mm/m^3 of NO_2 has little effect upon bronchial hyper-responsiveness in patients with mild asthma [18]. Another study, conducted in patients with moderate and severe asthma and involving NO_2 concentrations of up to 0.6 ppm, in another different zone, reported no effect [19]. On the other hand, it has been reported that prior exposure to NO_2 is able to increase late bronchial responses induced by allergen [20].

1.2. Mechanisms of action. Although there are studies indicating that exposure to NO_2 - a compound with important oxidative capacity - can induce airways inflammation, there is no clearly demonstrated underlying mechanism of action capable of accounting for such effects. Some studies in healthy volunteers have shown that repeated exposure to NO_2 induces neutrophilic inflammation of the airways that remains for as long as the changes in pulmonary function persist [21,22]. In an *in vitro* study involving human bronchial cells from healthy volunteers, it was seen that NO_2 exposure induced an increase in the levels of NO and IL-8, and the appearance of other inflammatory cytokines [23]. The role of NO in the induction of cytokines and proinflammatory molecules has recently been confirmed [24]. In an interesting study in healthy non-smoking volunteers exposed to NO_2 and subjected to fibrobronchoscopy with the obtainment of a biopsy, increased expression of IL-5, IL-10, IL-13 and ICAM-1 was recorded [25]. The authors suggested that the observed increase in Th2 cytokines could exert a "proallergic" effect upon the respiratory epithelium, while the increase in ICAM-1 could facilitate the development of viral processes - since this adhesion molecule is the principal receptor for rhinoviruses and respiratory syncytial virus (RSV).

2. Ozone

Tropospheric ozone is principally formed from a series of reactions involving the participation of ultraviolet light, nitrogen oxides and organic compounds. These reactions can last from hours to days, depending on the organic compounds involved. Once formed, ozone can persist for several days; as a result, the levels found in a given area may depend upon the emissions of NO_X and organic compounds that may have taken place hundreds or even thousands of kilometers away. At local level, ozone can react with NO to produce NO_2, resulting in a reduction in ozone levels. This is why ozone concentration tends to be lower in the center of cities with intense traffic, and higher in nearby suburban and rural zones. Normally, the highest ozone concentrations are found downwind from the point where ozone is produced. In some regions, atmospheric recirculation phenomena may cause the air to remain in one given zone for several days. As a result of its photochemical origin, ozone concentrations are higher in the summer months and in the afternoon hours. Figure 2 depicts the ozone formation process.

In most of Europe, the mean annual baseline levels range from 60-80 mg/m^3, with scant variation, and are independent of the overlying photochemical peaks [26]. To these levels photochemical episodes characterized by high ozone concentrations may be added. This is partly dependent upon the ozone transported from the stratosphere, and in part upon the ozone produced in the troposphere from both anthropogenic and natural sources (in approximately equal proportions).

2.1. Effects of ozone. Ozone is a potent antioxidant that can react with an important range of cellular components. Specifically, in the respiratory setting, its capacity to induce alterations depends on the concentration, duration of exposure, pattern of exposure, and respiratory minute volume [27]. Generally, the effects are more notorious with intermittent exposures than as a consequence of continuous exposure.

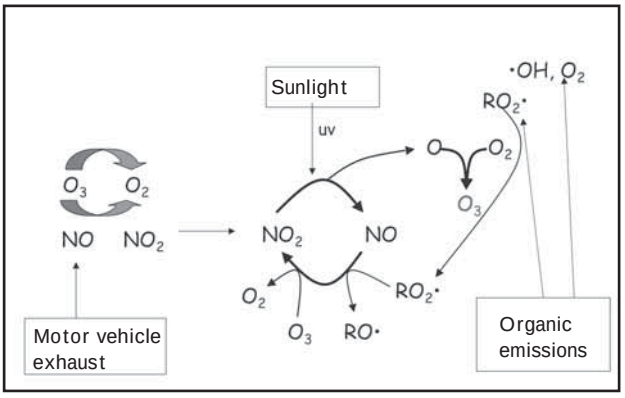

Figure 2. Ozone formation. Ozone is produced by a complex atmospheric process involving the participation of nitrogen oxides, organic compounds, and sunlight. Abundant nitrogen monoxide (NO) is produced in cities - the great majority by motor vehicles. NO reacts with ozone (O_3) present in the air, giving rise to nitrogen dioxide (NO_2). This effectively gives rise to a decrease in ozone levels. Winds in turn can transport this NOX - rich air towards rural areas. In this setting, organic compounds of both natural and anthropogenic origin form peroxy radical (RO_2·), with an uncoupled electron, which makes them enormously reactive. In the rural setting, sunlight breaks down NO_2, giving rise to NO and O. The latter in turn reacts with molecular oxygen to yield O_3. On the other hand, NO reacts with the peroxy radicals to give rise to NO_2. Part of the O_3 is removed through reaction with NO. The amount thus lost depends on the concentration of RO_2 radicals.

The studies that evaluate the effects of ozone generally indicate that exposure to this form of oxygen impairs lung function. Thus, in studies among healthy volunteers, and in addition to irritative cough and retrosternal pain during inspiration, a reduction is seen in vital capacity (VC), forced expiratory volume in one minute (FEV1) and lung resistances, together with an increase in respiratory frequency [26] - though these responses show important interindividual variability [28]. The effects of ozone exposure increase with physical exercise [29]. This happens, for example, on sunny days in summer, in the presence of high ozone pollution levels.

Patients with respiratory diseases are more susceptible to the effects of ozone. Epidemiological studies show that exposure to ozone - in particular under conditions of oxidizing air pollution, as in summer - is associated with asthma exacerbation, an increased number of hospital admissions and emergency care visits because of asthma, and with increased medicine use among asthmatics [12,30-34]. The available data suggest that chronic ozone exposure can reduce the pulmonary function values in children [35].

Prior ozone exposure produces an increase in bronchial or nasal response to allergens [36]. It also has been reported that ozone exposure prior to a bronchial provocation or challenge test with allergen increases the number of eosinophils in the induced sputum [37]. Nevertheless, it also has been reported that exposure to ozone induces no changes in bronchial hyper-responsiveness to methacholine [38].

2.2. Mechanisms of action. Regarding the possible mechanisms, it has been seen that the inhalation of ozone is able to induce an inflammatory response, with an increase in neutrophil count and proteins in bronchoalveolar lavage (BAL) or sputum. This in turn is more intense in asthmatics than in normal individuals, though the lower airways symptoms and changes in lung function are not much greater in asthmatics than in normal subjects [38,39]. Other markers of pulmonary damage induced by ozone have been described, such as lactate dehydrogenase, PGE_2 or IL-8 [40]. Other authors have reported an increase in the number of neutrophils, IL-8 and oncogene-alpha related to growth in the bronchoalveolar lavage of healthy individuals exposed to ozone in the course of physical exercise, though no increase has been seen in inflammatory markers in the bronchial biopsy [41]. It has been described that lipid ozonization could give rise to the release of mediators capable of triggering an inflammatory process [42]. On the other hand, it is believed that the C-fibers, through the release of mediators - including possibly substance P - could be responsible for the effects of ozone [43]. This mechanism would form part of the protective pulmonary response to irritant agents.

Some studies have reported that antioxidant ingestion can protect against the acute effects of ozone upon lung function, but not upon the associated inflammatory response [44-46]. On the other hand, a study in healthy individuals showed that inhalatory budesonide at high doses does not protect against the effects of ozone upon lung function [47]. It likewise did not protect against the effects upon lung function in groups of patients with mild asthma - though the drug did reduce IL-8 levels and the number of eosinophils in the induced sputum samples [48].

3. Particulate matter

The term "particulate matter" refers to the solid particles and/or droplets of variable size found in suspension in the air. The size can vary greatly, from particles measuring less than 0.001 μm to pollen and spores measuring 2-50 μm, and large particles of up to 1000 μm. PM can be primary (when emitted directly into the atmosphere from anthropogenic or natural sources) or secondary (when formed in the atmosphere as a result of oxidation and other chemical reactions from sulfur dioxide, nitrogen oxides and volatile organic compounds). The secondary particles are fundamentally of anthropogenic origin. In most European countries, industrialization and intense traffic have caused anthropogenic sources to predominate, particularly in the urban setting. These sources are similar throughout the continent. The most important sources are motor vehicles, energy production plants, combustion processes (industrial and residential), loading and unloading processes, mining, fire of human origin and - in certain concrete locations - construction and stone quarry work. The main natural sources are marine spray and the wind-induced resuspension of soil particles. In the Mediterranean basin and in the Atlantic archipelagos (Canary Islands, Azores), dust from the Sahara and emissions of volcanic origin can constitute important sources of particles [49].

Basically, PM is composed of a black carbon core upon which a series of chemical and physical components deposit. Size and chemical composition are regarded as the principal characteristics of PM. In consonance, PM_{10} is defined as the mass of particles with an aerodynamic diameter of less than 10 μm per unit volume ($\mu g/m^3$) - these being regarded as the breathable particles. The particles of larger size reach the greater-diameter airways, while the smaller particles (particularly $PM_{2.5}$ and $PM_{1.0}$, with diameters of under 2.5 and 1.0 μm, respectively) are able to reach the alveolar region. It is common to designate $PM_{2.5}$ as the fine fraction, and $PM_{2.5-10}$ as the large fraction [50]. The fine fraction contains most of the acid and mutagenic activity, and accounts for most of the mass. Recently, the effects of ultrafine natural particles are being taken into consideration (<0.1 μm) [51,52], along with those of nanoparticles (<0.1 μm) - taken to represent those of industrial origin [53]. However, the detection of $PM_{0.1}$ is still in the very early stages.

3.1. Effects of PM. The World Health Organization (WHO) has established that there is a causal relationship between PM exposure and health effects - though no causal correlation to any concrete PM component can be established. Nevertheless, it can be affirmed that the fine fraction ($PM_{2.5}$) is more deleterious than the larger particle fractions. The main characteristics accounting for PM toxicity are the metal contents, the presence of aromatic polycyclic hydrocarbons and other organic components, endotoxin content, and an either small (under 2.5 μm) or very small particle size (under 0.1 μm) [35].

One of the most significant studies on the effect of PM upon respiratory illnesses was carried out between 1985 and 1988 by Pope, in the Utah valley [54]. This area was characterized by the presence of a steel laminating plant that constituted the principal source of PM_{10}, and which was closed down from

August to September 1987, as the result of a labor strike. This made it possible to compare the admissions due to lung diseases - including asthma - with the levels of such particles. The study concluded that PM_{10} levels were markedly associated with hospital admissions, particularly in the case of children and patients with asthma or bronchitis. These effects have since been confirmed in different parts of the world by other studies [55-62].

3.2. Mechanisms of action. It can be considered that the effects of PM depend on the action exerted by the central core on one hand, and by the chemicals transported by the particles on the other - including aromatic polycyclic hydrocarbons and quinolones, and transition metals such as chromium, vanadium, nickel, copper, cobalt and iron [63]. Overall, the particles as such possess intrinsic adjuvant activity capable of increasing antibody response, including IgE, with the induction of inflammation [64,65] - while the associated substances exert different effects upon immune allergic responses [66-68]. In this context, Granum and Løvik consider that the particles as such behave as a general "motor" for the production of antibodies, while the adhered chemical substances, and possibly other factors such as load, structure and size, are able to direct the effect towards either a Th1 or Th2 response [69].

In animal models it has been seen that PM_{10} possess free radical activity and can cause epithelial damage and pulmonary inflammation [62,70]. They are reportedly able to induce the release of IL-8, MIP-2 and IL-6 in human alveolar cells [71]. In the allergological setting, it has been reported that particulate matter can act as an adjuvant and increase specific IgE production in animal models [72].

3.3. Diesel emission particles (DEPs). As has been commented above, motor vehicles are one of the most important sources of polluting agents, including PM. The most important source in relation to motor vehicles corresponds to diesel engines. In this sense, diesel engines can emit up to 100 times more particles than gasoline engines. Furthermore, the number of diesel-driven vehicles has increased considerably, due to their increased efficacy and durability, and the lower cost of diesel fuel. Diesel exhaust particles are composed of a central core to which hundreds of chemical compounds and transition metals adhere. These in turn are responsible for the adjuvant and proinflammatory properties of DEPs. Most DEPs are classified as fine (2.5-0.1 μm) or ultrafine particles (<0.1 μm). The smaller particles are considered to have a relatively greater surface, and therefore may contribute a larger number of chemical agents - resulting in a more marked biological effect [73].

Since precise determination of environmental DEP exposure *in vivo* in humans has been complicated by a lack of biomarkers, very few studies have analyzed the effects of diesel vehicle exhaust considered isolatedly [74]. As a result, exposure to moving traffic has been used as an approximation. Such exposure has been related to respiratory symptoms, asthma, and allergic disorders in different populations [75-79]. In a very recent study conducted in a large sample of children, diminished lung function has been recorded in children living closer to intense traffic than in those living at a distance from such traffic - regardless of air quality [80]. Studies have been made of the effects of DEPs upon many types of cells, and in healthy subjects (reviewed in depth in [81]). Basically, DEPs are able to act upon many cells - inducing the expression of different inflammatory markers (Table 4). In healthy individuals, DEP inhalation increases the number of inflammatory cells in the airways and raises circulating neutrophil and platelet counts, and histamine. In turn, the expression of certain cytokines, chemokines and adhesion molecules also increases, while macrophage function decreases and respiratory tract resistance increases. In patients with asthma, DEPs have been reported to increase nonspecific bronchial hyper-responsiveness, airways resistance, and IL-6 in sputum [82]. In another interesting study, Holgate et al. performed bronchial provocation with DEPs in healthy subjects and in asthmatics - both groups showing a discrete increase in airways resistance [83]. In healthy individuals they reported an increase in neutrophils in bronchoalveolar lavage, with IL-8 elevation in the latter and also in the biopsy. However, the asthmatics showed no change in the parameters, with the exception of increased IL-10 expression in the biopsy. Studies have also been made of DEP effects upon allergic responses. Thus, it has been seen that DEPs are able to act as adjuvants in the mucosal membranes, in the context of a *de novo* IgE-mediated response, and thus increase allergic sensitization [84]. Furthermore, nasal provocation with DEPs and Ambrosia can induce an isotype change towards IgE, with a rise in the local levels of this immunoglobulin [85]. Nasal provocation with DEPs can increase the expression of Th2 cytokines [86]. It also has been seen that in application to basophils, DEPs can induce histamine and IL-4 release, independently of the administration of an allergen [87]. On the other hand, in a study of patients with dust mite allergy, the intranasal administration of DEPs prior to provocation with the allergen induced higher symptoms scores, required a fifth of the amount of allergen, and induced a three-fold higher histamine release than when provocation was limited to the allergen alone [88]. DEPs have been shown to be able to induce proinflammatory mediator release by human bronchial epithelial cells [89]. Moreover, these cells have been seen to behave differently in asthmatic patients versus healthy individuals, in terms of the amount and types of mediators released, and of sensitivity to DEPs [90].

Genetic aspects

Although the responses to the different environmental pollutants are reproducible, there is marked interindividual variability. This has been related to the intervention of possible genetic factors. These effects have been investigated in animal models fundamentally in relation to ozone [91]. Few studies have been conducted in humans. In this context, Wintentor et al. reported an association between a certain polymorphism of the promoter region of the TNFA gene and the response to SO_2 in asthmatic patients - though no such association was observed with other genes such as IL4RA, B2ADR, CC16 or LTA [92]. In turn, Yang et al. observed an association between the same TNFA gene polymorphism and ozone response [93]. Polymorphisms in "antioxidant" genes have also been evaluated. Specifically, in healthy volunteers exposed to ozone during physical exercise, the maximum risk genotypes of the genes NQO1 (nicotinamide adenine dinucleotide (phosphate)

Table 4. Direct effects of diesel particles (DEPs) or of their extracts upon different cells (taken from [81])

Nasal and bronchial epithelial and endothelial cells	Increased chemokine and cytokine expression (IL-8, eotaxin, RANTES, GM-CSF, IL-6) Increased receptor H_1 expression Increased ICAM-1 expression
Eosinophils	Increased adherence to nasal epithelial cells Induction of degranulation
Mast cells	Increased IgE induced histamine release Increased production of cytokines IL-4 and IL-6
Basophils	Induction of histamine release in absence of IgE Increased production of IL-4
Peripheral blood mononuclear cells	Induction of chemokine production (IL-8, RANTES) In combination with allergen, increases in IL-8, RANTES and TNF-α
B cells	Increased IgE production after stimulation with IL-4 and anti-CD40
Monocytes - macrophages	Modulation of cytokine production (inhibition of the production of IL-12p40) Inhibition of PGE_2 release

reduced quinone oxidoreductase) and GSTM1 (glutathione-S-transferase µ1) - which imply excessive free radical production - have been correlated to a greater reduction in lung function, and to an increase in inflammatory markers [94,95]. In a study conducted in children in Mexico city, continuously exposed to high ozone levels, certain genotypes of the aforementioned genes were seen to entail a lesser risk of developing asthma [96]. In another placebo controlled study conducted among 155 children in the same city, involving the administration of vitamin E and C and antioxidants, it was seen that children nulligenic for a certain polymorphism of the GSTM1 gene presented greater lung function reduction when given placebo. Moreover, these children benefited more from the administration of antioxidants [97]. In a later study, the same authors found that asthmatic children nulligenic for a certain polymorphism of the GSTM1 gene and with Val/Val in GSTP1 presented greater susceptibility towards the development of respiratory symptoms related with ozone exposure [98].

Conclusions

Technical and industrial development has led to environmental pollution problems, with deleterious effects upon health. In the last few decades special attention has focused on pollutants - mostly of anthropogenic origin. Epidemiological studies have revealed a statistical association between the levels of a pollutant or series of pollutants and the exacerbation of certain allergic respiratory diseases, among other processes. These data are reinforced by the findings in animal models, and by the experimental results of *in vitro* and in vivo studies in humans. The main pollutants are nitrogen dioxide, ozone, and suspended particles. Nitrogen dioxide is able to induce respiratory symptoms and bronchial inflammation at high doses, in healthy volunteers. In the case of asthmatics, and although there may be debate on the matter, it seems that exposure to this pollutant can increase the symptoms, bronchial hyper-responsiveness and bronchial inflammation at lower doses. Ozone, which presents a complex production process, is a potent antioxidant that can induce changes in lung function. The most susceptible populations are children and patients with respiratory diseases. Generally, the effects are more notorious with intermittent exposures than as a consequence of continuous exposure. Particulate matter in suspension is composed of a central core to which different chemical compounds adhere. Size is important in relation to the effect of such particles, and in this sense, attention is increasingly centering on the smallest particles - especially those under 2.5 and 0.1 µm in size. Their effects depend both on the central core and on the associated particles. Special mention must be made of diesel emission particles (DEPs). Apart from their effects on lung function, they are able to act as adjuvants in allergic reactions.

Lastly, some polymorphisms have been described - generally related to oxidative pathways - that could imply a protective or deleterious effect in relation to pollutants.

References

1. Bernstein JA, Alexis N, Barnes C, Bernstein IL, Bernstein JA, Nel A, Peden D, Díaz-Sanchez D, Tarlo SM, Williams PB. Health effects of air pollution. J Allergy Clin Immunol. 2004;114:1116-1123.
2. Trasande L, Thurston GD. The role of air pollution in asthma and other pediatric morbidities. J Allergy Clin Immunol. 2005;115:689-699.
3. D'Amato G, Liccardi G, D'Amato M. Environmental risk factors (outdoor air pollution and climatic changes) and increased trend of respiratory allergy. J Investig Allergol Clin Immunol. 2000;10:123-128.
4. WGND. Position Paper on Air Quality. Nitrogen Dioxide. Working Group on Nitrogen Dioxide. 1-75. 1997. Sweden, European Commission, Directorate-General XI.
5. Sunyer J, Spix C, Quenel P, Ponce-de-Leon A, Ponka A, Barumandzadeh T, Touloumi G, Bacharova L, Wojtyniak B, Vonk J, Bisanti L, Schwartz J, Katsouyanni K. Urban air pollution and emergency admissions for asthma in four European cities. the APHEA Project. Thorax. 1997;52:760-765.
6. Castellsague J, Sunyer J, Saez M, Anto JM. Short-term association between air pollution and emergency room visits for asthma in Barcelona. Thorax. 1995;50:1051-1056.
7. Smith BJ, Nitschke M, Pilotto LS, Ruffin RE, Pisaniello DL, Willson KJ. Health effects of daily indoor nitrogen dioxide exposure in people with asthma. Eur Respir J. 2000;16:879-885.
8. Nitschke M, Pilotto LS, Attewell RG, Smith BJ, Pisaniello D, Martin J, Ruffin RE, Hiller JE. A cohort study of indoor nitrogen dioxide and house dust mite exposure in asthmatic children. J Occup Environ Med. 2006;48:462-469.
9. Kwon HJ, Lee SG, Jee YK, Lee SR, Hwang SS: [Effects of personal exposure to nitrogen dioxide on peak expiratory flow in asthmatic patients]. J Prev Med Pub Health. 2007;40:59-63.
10. Schouten JP, Vonk JM, de GA. Short term effects of air pollution on emergency hospital admissions for respiratory disease: results of the APHEA project in two major cities in The Netherlands, 1977-89. J Epidemiol Community Health. 1996;50 Suppl 1: s22-9.:s22-s29.
11. Emenius G, Pershagen G, Berglind N, Kwon HJ, Lewne M, Nordvall SL, Wickman M. NO2, as a marker of air pollution, and recurrent wheezing in childrIn: a nested case-control study within the BAMSE birth cohort. Occup Environ Med. 2003;60:876-881.
12. Tenias JM, Ballester F, Rivera ML. Association between hospital emergency visits for asthma and air pollution in Valencia, Spain. Occup Environ Med. 1998;55:541-547.
13. Galan I, Tobias A, Banegas JR, Aranguez E. Short-term effects of air pollution on daily asthma emergency room admissions. Eur Respir J. 2003;22:802-808.
14. Orehek J, Massari JP, Gayrard P, Grimaud C, Charpin J. Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. J Clin Invest. 1976;57:301-307.
15. Hazucha MJ, Ginsberg JF, McDonnell WF, Haak ED, Jr., Pimmel RL, Salaam SA, House DE, Bromberg PA. Effects of 0.1 ppm nitrogen dioxide on airways of normal and asthmatic subjects. J Appl Physiol. 1983;54:730-739.
16. Jorres R, Magnussen H. Effect of 0.25 ppm nitrogen dioxide on the airway response to methacholine in asymptomatic asthmatic patients. Lung. 1991;169:77-85.
17. Bylin G, Lindvall T, Rehn T, Sundin B. Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. Eur J Respir Dis. 1985;66:205-217.
18. Bylin G, Hedenstierna G, Lindvall T, Sundin B. Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. Eur Respir J. 1988;1:606-612.
19. Avol EL, Linn WS, Peng RC, Valencia G, Little D, Hackney JD. Laboratory study of asthmatic volunteers exposed to nitrogen dioxide and to ambient air pollution. Am Ind Hyg Assoc J. 1988;49:143-149.
20. Strand V, Rak S, Svartengren M, Bylin G. Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. Am J Respir Crit Care Med. 1997;155:881-887.
21. Blomberg A, Krishna MT, Helleday R, Soderberg M, Ledin MC, Kelly FJ, Frew AJ, Holgate ST, Sandstrom T. Persistent airway inflammation but accommodated antioxidant and lung function responses after repeated daily exposure to nitrogen dioxide. Am J Respir Crit Care Med. 1999;159:536-543.
22. Blomberg A, Krishna MT, Bocchino V, Biscione GL, Shute JK, Kelly FJ, Frew AJ, Holgate ST, Sandstrom T. The inflammatory effects of 2 ppm NO2 on the airways of healthy subjects. Am J Respir Crit Care Med. 1997;156:418-424.
23. Ayyagari VN, Januszkiewicz A, Nath J. Pro-inflammatory responses of human bronchial epithelial cells to acute nitrogen dioxide exposure. Toxicology. 2004;197:149-164.
24. Ayyagari VN, Januszkiewicz A, Nath J. Effects of nitrogen dioxide on the expression of intercellular adhesion molecule-1, neutrophil adhesion, and cytotoxicity: studies in human bronchial epithelial cells. Inhal Toxicol. 2007;19:181-194.
25. Pathmanathan S, Krishna MT, Blomberg A, Helleday R, Kelly FJ, Sandstrom T, Holgate ST, Wilson SJ, Frew AJ. Repeated daily exposure to 2 ppm nitrogen dioxide upregulates the expression of IL-5, IL-10. IL-13, and ICAM-1 in the bronchial epithelium of healthy human airways. Occup Environ Med. 2003;60:892-896.
26. Ozone Position Paper; Ad Hoc Working Group on Ozone Directive and Reduction Strategy Development. European Communities. 1-174. 1999.
27. McDonnell WF, Stewart PW, Smith MV, Pan WK, Pan J. Ozone-induced respiratory symptoms: exposure-response models and association with lung function. Eur Respir J. 1999;14:845-853.
28. McDonnell WF. Intersubject variability in human acute ozone responsiveness. Pharmacogenetics. 1991;1:110-113.
29. McDonnell WF, Stewart PW, Andreoni S, Smith MV. Proportion of moderately exercising individuals responding to low-level, multi-hour ozone exposure. Am J Respir Crit Care Med. 1995;152:589-596.
30. Fauroux B, Sampil M, Quenel P, Lemoullec Y. Ozone: a trigger for hospital pediatric asthma emergency room visits. Pediatr Pulmonol. 2000;30:41-46.
31. Tobias A, Campbell MJ, Saez M. Modelling asthma epidemics on the relationship between air pollution and asthma emergency visits in Barcelona, Spain. Eur J Epidemiol. 1999;15:799-803.
32. Stieb DM, Burnett RT, Beveridge RC, Brook JR. Association between ozone and asthma emergency department visits in

Saint John, New Brunswick, Canada. Environ Health Perspect. 1996;104:1354-1360.
33. Weisel CP, Cody RP, Lioy PJ. Relationship between summertime ambient ozone levels and emergency department visits for asthma in central New Jersey. Environ Health Perspect. 1995;103 Suppl 2:97-102:97-102.
34. Cody RP, Weisel CP, Birnbaum G, Lioy PJ. The effect of ozone associated with summertime photochemical smog on the frequency of asthma visits to hospital emergency departments. Environ Res. 1992;58:184-194.
35. WHO. Health Aspects of Air Pollution with Particulate Matter, Ozone and Nitrogen Dioxide. http://www.euro.who.int/document/e79097.pdf 2007.
36. Koren HS, Bromberg PA. Respiratory responses of asthmatics to ozone. Int Arch Allergy Immunol. 1995;107:236-238.
37. Vagaggini B, Taccola M, Cianchetti S, Carnevali S, Bartoli ML, Bacci E, Dente FL, Di FA, Giannini D, Paggiaro PL. Ozone exposure increases eosinophilic airway response induced by previous allergen challenge. Am J Respir Crit Care Med. 2002;166:1073-1077.
38. Nightingale JA, Rogers DF, Barnes PJ. Effect of inhaled ozone on exhaled nitric oxide, pulmonary function, and induced sputum in normal and asthmatic subjects. Thorax. 1999;54:1061-1069.
39. Balmes JR, Aris RM, Chen LL, Scannell C, Tager IB, Finkbeiner W, Christian D, Kelly T, Hearne PQ, Ferrando R, Welch B. Effects of ozone on normal and potentially sensitive human subjects. Part I: Airway inflammation and responsiveness to ozone in normal and asthmatic subjects. Res Rep Health Eff Inst. 1997;1-37.
40. Kinney PL, Nilsen DM, Lippmann M, Brescia M, Gordon T, McGovern T, El-Fawal H, Devlin RB, Rom WN. Biomarkers of lung inflammation in recreational joggers exposed to ozone. Am J Respir Crit Care Med. 1996;154:1430-1435.
41. Krishna MT, Madden J, Teran LM, Biscione GL, Lau LC, Withers NJ, Sandstrom T, Mudway I, Kelly FJ, Walls A, Frew AJ, Holgate ST. Effects of 0.2 ppm ozone on biomarkers of inflammation in bronchoalveolar lavage fluid and bronchial mucosa of healthy subjects. Eur Respir J. 1998;11:1294-1300.
42. Kafoury RM, Pryor WA, Squadrito GL, Salgo MG, Zou X, Friedman M. Induction of inflammatory mediators in human airway epithelial cells by lipid ozonation products. Am J Respir Crit Care Med. 1999;160:1934-1942.
43. Uysal N, Schapira RM. Effects of ozone on lung function and lung diseases. Curr Opin Pulm Med. 2003;9:144-150.
44. Grievink L, Smit HA, Brunekreef B. Anti-oxidants and air pollution in relation to indicators of asthma and COPD: a review of the current evidence. Clin Exp Allergy. 2000;30:1344-1354.
45. Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, Tellez-Rojo MM, Moreno-Macias H, Reyes-Ruiz NI, del Rio-Navarro BE, Ruiz-Navarro MX, Hatch G, Slade R, Hernandez-Avila M. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. Am J Respir Crit Care Med. 2002;166:703-709.
46. Samet JM, Hatch GE, Horstman D, Steck-Scott S, Arab L, Bromberg PA, Levine M, McDonnell WF, Devlin RB. Effect of antioxidant supplementation on ozone-induced lung injury in human subjects. Am J Respir Crit Care Med. 2001;164:819-825.
47. Nightingale JA, Rogers DF, Chung KF, Barnes PJ. No effect of inhaled budesonide on the response to inhaled ozone in normal subjects. Am J Respir Crit Care Med. 2000;161:479-486.
48. Vagaggini B, Taccola M, Conti I, Carnevali S, Cianchetti S, Bartoli ML, Bacci E, Dente FL, Di FA, Giannini D, Paggiaro PL. Budesonide reduces neutrophilic but not functional airway response to ozone in mild asthmatics. Am J Respir Crit Care Med. 2001;164:2172-2176.
49. European Commission. Ambient Air Pollution By Particulate Matter Position Paper. http://www.europa.eu.int.comm/ environment/air/ambient.htm. 1997.
50. CAFE Working Group on PM. Second position paper on particulate matter. http://ec.europa.eu/environment/air/cafe/pdf/working_groups/2nd_position_paper_pm.pdf. 2004.
51. Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005;113:823-839.
52. Kreyling WG, Semmler-Behnke M, Moller W. Ultrafine particle-lung interactions: does size matter? J Aerosol Med. 2006;19:74-83.
53. Gwinn MR, Vallyathan V. Nanoparticles: health effects--pros and cons. Environ Health Perspect. 2006;114:1818-1825.
54. Pope CA, III. Respiratory disease associated with community air pollution and a steel mill, Utah Valley. Am J Public Health. 1989;79:623-628.
55. Bayer-Oglesby L, Grize L, Gassner M, Takken-Sahli K, Sennhauser FH, Neu U, Schindler C, Braun-Fahrlander C. Decline of ambient air pollution levels and improved respiratory health in Swiss children. Environ Health Perspect. 2005;113:1632-1637.
56. Barnett AG, Williams GM, Schwartz J, Neller AH, Best TL, Petroeschevsky AL, Simpson RW. Air pollution and child respiratory health: a case-crossover study in Australia and New Zealand. Am J Respir Crit Care Med. 2005;171:1272-1278.
57. Mar TF, Larson TV, Stier RA, Claiborn C, Koenig JQ. An analysis of the association between respiratory symptoms in subjects with asthma and daily air pollution in Spokane, Washington. Inhal Toxicol. 2004;16:809-815.
58. Biggeri A, Bellini P, Terracini B. [Meta-analysis of the Italian studies on short-term effects of air pollution--MISA 1996-2002]. Epidemiol Prev. 2004;28:4-100.
59. Galan I, Tobias A, Banegas JR, Aranguez E. Short-term effects of air pollution on daily asthma emergency room admissions. Eur Respir J. 2003;22:802-808.
60. Petroeschevsky A, Simpson RW, Thalib L, Rutherford S. Associations between outdoor air pollution and hospital admissions in Brisbane, Australia. Arch Environ Health. 2001;56:37-52.
61. Atkinson RW, Anderson HR, Sunyer J, Ayres J, Baccini M, Vonk JM, Boumghar A, Forastiere F, Forsberg B, Touloumi G, Schwartz J, Katsouyanni K. Acute effects of particulate air pollution on respiratory admissions: results from APHEA 2 project. Air Pollution and Health: a European Approach. Am J Respir Crit Care Med. 2001;164:1860-1866.
62. Romeo E, De SM, Forastiere F, Compagnucci P, Stafoggia M, Bergamaschi A, Perucci CA. [PM 10 exposure and asthma exacerbations in pediatric age: a meta-analysis of panel and time-series studies]. Epidemiol Prev. 2006;30:245-254.
63. Nel A. Atmosphere. Air pollution-related illness: effects of particles. Science. 2005;308:804-806.

64. Lovik M, Hogseth AK, Gaarder PI, Hagemann R, Eide I. Diesel exhaust particles and carbon black have adjuvant activity on the local lymph node response and systemic IgE production to ovalbumin. Toxicology. 1997;121:165-178.
65. Granum B, Gaarder PI, Lovik M. IgE adjuvant effect caused by particles - immediate and delayed effects. Toxicology. 2001; 156:149-159.
66. Bommel H, Li-Weber M, Serfling E, Duschl A. The environmental pollutant pyrene induces the production of IL-4. J Allergy Clin Immunol. 2000;105:796-802.
67. Tsien A, Díaz-Sanchez D, Ma J, Saxon A. The organic component of diesel exhaust particles and phenanthrene, a major polyaromatic hydrocarbon constituent, enhances IgE production by IgE-secreting EBV-transformed human B cells in vitro. Toxicol Appl Pharmacol. 1997;142:256-263.
68. Fahy O, Tsicopoulos A, Hammad H, Pestel J, Tonnel AB, Wallaert B. Effects of diesel organic extracts on chemokine production by peripheral blood mononuclear cells. J Allergy Clin Immunol.1999;103:1115-1124.
69. Granum B, Lovik M. The Effect of Particles on Allergic Immune Responses. Toxicol Sci. 2002;65:7-17.
70. Li XY, Gilmour PS, Donaldson K, MacNee W. Free radical activity and pro-inflammatory effects of particulate air pollution (PM10) in vivo and in vitro. Thorax. 1996;51:1216-1222.
71. Hetland RB, Cassee FR, Refsnes M, Schwarze PE, Lag M, Boere AJ, Dybing E. Release of inflammatory cytokines, cell toxicity and apoptosis in epithelial lung cells after exposure to ambient air particles of different size fractions. Toxicol In Vitro. 2004;18:203-212.
72. Dybing E, Lovdal T, Hetland RB, Lovik M, Schwarze PE. Respiratory allergy adjuvant and inflammatory effects of urban ambient particles. Toxicology. 2004;198:307-314.
73. Oberdorster G, Utell MJ. Ultrafine particles in the urban air: to the respiratory tract--and beyond? Environ Health Perspect. 2002;110:A440-A441.
74. Gluck U, Schutz R, Gebbers JO. Cytopathology of the nasal mucosa in chronic exposure to diesel engine emission: a five-year survey of Swiss customs officers. Environ Health Perspect. 2003;111:925-929.
75. Brauer M, Hoek G, Van VP, Meliefste K, Fischer PH, Wijga A, Koopman LP, Neijens HJ, Gerritsen J, Kerkhof M, Heinrich J, Bellander T, Brunekreef B. Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children. Am J Respir Crit Care Med. 2002;166:1092-1098.
76. Morgenstern V, Zutavern A, Cyrys J, Brockow I, Gehring U, Koletzko S, Bauer CP, Reinhardt D, Wichmann HE, Heinrich J. Respiratory health and individual estimated exposure to traffic-related air pollutants in a cohort of young children. Occup Environ Med. 2007;64:8-16.
77. Brauer M, Hoek G, Smit HA, de Jongste JC, Gerritsen J, Postma DS, Kerkhof M, Brunekreef B. Air pollution and development of asthma, allergy and infections in a birth cohort. Eur Respir J. 2007;29:879-888.
78. Kim JJ, Smorodinsky S, Lipsett M, Singer BC, Hodgson AT, Ostro B. Traffic-related air pollution near busy roads: the East Bay Children's Respiratory Health Study. Am J Respir Crit Care Med. 2004;170:520-526.
79. Nicolai T, Carr D, Weiland SK, Duhme H, von EO, Wagner C, von ME. Urban traffic and pollutant exposure related to respiratory outcomes and atopy in a large sample of children. Eur Respir J. 2003;21:956-963.
80. Gauderman WJ, Vora H, McConnell R, Berhane K, Gilliland F, Thomas D, Lurmann F, Avol E, Kunzli N, Jerrett M, Peters J. Effect of exposure to traffic on lung development from 10 to 18 years of age: a cohort study. Lancet. 2007;369:571-577.
81. Riedl M, Díaz-Sanchez D. Biology of diesel exhaust effects on respiratory function. J Allergy Clin Immunol. 2005;115:221-228.
82. Nordenhall C, Pourazar J, Ledin MC, Levin JO, Sandstrom T, Adelroth E. Diesel exhaust enhances airway responsiveness in asthmatic subjects. Eur Respir J. 2001;17:909-915.
83. Holgate ST, Sandstrom T, Frew AJ, Stenfors N, Nordenhall C, Salvi S, Blomberg A, Helleday R, Soderberg M. Health effects of acute exposure to air pollution. Part I: Healthy and asthmatic subjects exposed to diesel exhaust. Res Rep Health Eff Inst. 2003;1-30.
84. Díaz-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. 1999;104:1183-1188.
85. Fujieda S, Díaz-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. 1998;19:507-512.
86. Díaz-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. 1996;98:114-123.
87. Devouassoux G, Saxon A, Metcalfe DD, Prussin C, Colomb MG, Brambilla C, Díaz-Sanchez D. Chemical constituents of diesel exhaust particles induce IL-4 production and histamine release by human basophils. J Allergy Clin Immunol. 2002;109:847-853.
88. Díaz-Sanchez D, Penichet-Garcia M, Saxon A. Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity. J Allergy Clin Immunol. 2000;106:1140-1146.
89. Bayram H, Devalia JL, Sapsford RJ, Ohtoshi T, Miyabara Y, Sagai M, Davies RJ. The Effect of Diesel Exhaust Particles on Cell Function and Release of Inflammatory Mediators from Human Bronchial Epithelial Cells In Vitro. Am J Respir Cell Mol Biol. 1998;18:441-448.
90. Bayram H, Devalia JL, Khair OA, Abdelaziz MM, Sapsford RJ, Sagai M, Davies RJ. Comparison of ciliary activity and inflammatory mediator release from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients and the effect of diesel exhaust particles in vitro. J Allergy Clin Immunol. 1998;102:771-782.
91. Kleeberger SR. Genetic aspects of pulmonary responses to inhaled pollutants. Exp Toxicol Pathol. 2005;57 Suppl. 1:147-53.:147-153.
92. Winterton DL, Kaufman J, Keener CV, Quigley S, Farin FM, Williams PV, Koenig JQ. Genetic polymorphisms as biomarkers of sensitivity to inhaled sulfur dioxide in subjects with asthma. Ann Allergy Asthma Immunol. 2001;86:232-238.

93. Yang IA, Holz O, Jorres RA, Magnussen H, Barton SJ, Rodriguez S, Cakebread JA, Holloway JW, Holgate ST. Association of Tumor Necrosis Factor- Polymorphisms and Ozone-induced Change in Lung Function. Am J Respir Crit Care Med. 2005;171:171-176.
94. Corradi M, Alinovi R, Goldoni M, Vettori M, Folesani G, Mozzoni P, Cavazzini S, Bergamaschi E, Rossi L, Mutti A. Biomarkers of oxidative stress after controlled human exposure to ozone. Toxicol Lett. 2002;134:219-225.

Ignacio Dávila

Servicio de Inmunoalergia
Hospital Universitario de Salamanca
Paseo de San Vicente 58-162
37007 Salamanca, Spain
E-mail: idg@usal.es