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Impact of environmental factors on lung defences

D. Olivieri and E. Scoditti

ABSTRACT: The lungs are one of the most important organs exposed to environmental agents. The lungs have the ability to protect themselves by both immunological and nonimmunological mechanisms. An individual's susceptibility to the impact of environmental agents will determine their adverse effects.

This article focuses on air pollution, in particular ozone (O_3), nitrogen dioxide (NO_2), particulate matter (PM) and sulphur dioxide (SO_2). O_3 inhalation first modifies the ciliary cells. O_3 is an extremely strong oxidant, since it increases the permeability of epithelial cells and decreases mucociliary clearance. NO_2 is a less potent and less reactive oxidant pollutant and impairs the function of epithelial cells and alveolar macrophages. PM induces oxidative stress in macrophages and epithelial cells, and increases tumour necrosis factor-β, interleukin (IL)-6, interferon-γ, transforming growth factor-β and nuclear factor-κB. Diesel exhaust particulate, contained in PM, increases IL-8 production. High concentrations of SO_2 increase the production of reactive oxygen species in the lungs.

In conclusion, air pollution certainly interferes with aspecific and specific lung defences, thus facilitating the development of pulmonary diseases, such as exacerbation of chronic obstructive pulmonary disease, allergies and asthma.

KEYWORDS: Air pollution, environmental factors, lung defences, mucociliary clearance, ozone, particulate matter

AFFILIATIONS
Dept of Clinical Sciences, Section of Respiratory Disease, University of Parma, Italy.

CORRESPONDENCE
D. Olivieri
Clinica Pneumologica
Ospedale Rasori
Via Rasori, 10
43100 Parma
Italy
Fax: 39 0521292615
E-mail: dario.olivieri@unipr.it

The lung is one of the most important organs exposed to environmental agents [1]. Airway injury is caused by multiple factors that vary both in their nature and effect [2]. In this article ''air pollution'', a term encompassing a wide range of chemical and biological components in the outdoor and indoor atmosphere, including environmental tobacco smoke (ETS), is examined [3]. Outdoor air pollutants that are hazardous for the respiratory tract are gaseous chemicals (nitrogen dioxide (NO_2), ozone (O_3), sulphur dioxide (SO_2)), inhaled particulate matter (PM) and aeroallergens, such as those derived from fungal spores and allergenic pollen. Indoor air contains a range of noxious substances derived from a multitude of sources. Outdoor pollutants can also affect indoor air. Present in the home are ETS, NO_2, formaldehyde (HCHO) and other volatile organic compounds (VOCs), as well as PM, SO_2, O_3 and allergens derived from a diverse range of plants, animals and insects.

The ability of the lungs to protect themselves by both immunological and nonimmunological mechanisms and the individual's susceptibility to their impact will determine the outcome in terms of their adverse effects [4]. This paper focuses on NO_2, O_3 and PM in the atmosphere of urban areas, and SO_2 in industrial areas, because of their particular action on lung defences (table 1).

OZONE

O_3 is generated at ground level by a photochemical reaction between ultraviolet radiation and atmospheric mixtures of NO_2 and hydrocarbons derived from vehicle emissions. O_3 levels depend on NO_2 emitted by cars and particularly on sunny weather that transforms NO_2 into O_3. O_3 is the most important factor for summer smog, because it accounts for up to 90% of total oxidant levels in cities that enjoy a mild sunny climate.

Short-term O_3 inhalation first modifies the ciliary cells, which seem to be the most sensitive. The clara cells then undergo degranulation and destruction, while the reorganisation of the epithelium takes place over 7 days.

O_3 is a very strong oxidant, which has the ability to overwhelm the natural defences of the lungs. It induces lipid peroxidation and inactivation of biomolecules.

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TABLE 1 Main environmental factors having impact on lung defences

Type	Origin	Respiratory Effect	Lung defences
Ozone	Photochemical reaction from vehicle traffic	Lung function reduction Bronchial hyperresponsiveness Increased prevalence of respiratory symptoms Increased hospitalisation Reduced exercise tolerance	Antioxidant system Permeability of epithelial cells Inflammatory cytokines Mucociliary clearance
Nitrogen dioxide	Gas combustion Vehicle traffic	Lung function reduction Bronchial hyperresponsiveness Increased prevalence of respiratory symptoms Reduced exercise tolerance	Antioxidant system Inflammatory cytokines
Particulate matter	Vehicle traffic Industrial activity	Lung function reduction Increased prevalence of respiratory symptoms Increased mortality from cardiorespiratory disease	Antioxidant system Inflammatory cytokines Mucociliary clearance
Diesel exhaust		Bronchoconstrition Bronchial hyperresponsiveness Airway inflammation	Antioxidant system Inflammatory cytokines Macrophage phagocytosis Sensitisation
Sulphur dioxide	Fuel combustion from Industry Vehicle traffic	Lung function reduction Increased prevalence of respiratory symptoms Increased mortality from respiratory disease	Antioxidant system

O_3 potentially leads to the formation of secondary and tertiary reactive products [5], producing an immediate dose-dependant increase in intracellular reactive O_2 species. Moreover, O_3 activates stress signalling pathways in epithelial cells [6] and interacts with nuclear factor (NF)-κB [7]. The antioxidants, ascorbic acid, uric acid and glutathione, are the first lines of defence in the epithelial lining fluid.

O_3 also increases the permeability of epithelial cells, favouring the entry of inhaled allergens and toxins and a subsequent release of inflammatory cytokines (interleukin (IL)-1, IL-6, IL-8 and tumour necrosis factor (TNF)). In an animal study, mucociliary clearance times decreased as O_3 increased [8], causing susceptibility to bacterial respiratory infections. Furthermore, O_3 induces decrements in pulmonary function, increasing airway responsiveness and resistance and altering lung volumes and flow.

Recently, the effect of ambient O_3 concentrations on human health has been heavily studied. There is now overwhelming evidence showing associations between the levels of this pollutant and adverse respiratory effects, such as decrements in lung function, aggravation of pre-existing respiratory disease, increase in hospital admission and premature respiratory deaths [9, 10].

Several studies suggest that O_3 increases asthma morbidity by enhancing airway inflammation, as demonstrated by the increase of IL-6, IL-8, granulocyte macrophage colony stimulating factor (GM-CSF) and fibronectin in bronchoalveolar lavage fluid (BALF) [11].

There are important individual differences in responsiveness to inhaled O_3, as seen in mice [12, 13] and human subjects [14]. In particular, genes for TNF-α, glutathione peroxidase and manganese superoxide dismutase, emphasising the importance of lung antioxidants in protecting against O_3 exposure [15], and the effects of a diet supplemented with antioxidants [16], have been studied.

Several studies show the effects of O_3 on mortality. During the warm season, an increase in the 1-h O_3 concentration by 10 $\mu g \cdot m^{-3}$ is associated with an increase of 0.33% in the total number of deaths daily, 0.45% in the number of cardiovascular deaths, and 1.13% in the number of respiratory deaths [17].

NITROGEN DIOXIDE

NO_2 derives from automobile exhaust and by the burning of fossil fuels. In conjunction with sunlight and hydrocarbons, it produces O_3. NO_2, like O_3, is an oxidant pollutant but it is less potent and less reactive. The inflammatory response also differs, because there is the recruitment of T lymphocytes and macrophages [18]. Indoor NO_2 is a major pollutant, especially in homes with gas stoves and kerosene space heaters. It has been associated with lower respiratory tract infections [19] and environmental air pollution, even if tobacco smoke is an important contributor to an individual's exposure. In normal subjects, inhalation of NO_2 causes lung inflammation [20], reduces ascorbic and uric acid and increases glutathione, with no significant changes in lung function [21].

NO_2 might contribute, moreover, to exacerbation of respiratory disease because of its capacity to impair the function of epithelial cells and alveolar macrophages [22]. However, findings are inconsistent due to difficulties in separating the effects of NO_2 from those of co-pollutants [23].

Because of widespread indoor exposure, NO_2 has been the focus of much attention in particular countries, such as in the UK where 50% of homes have gas cookers and millions of

people are potentially exposed to indoor NO_2 and its combustion by-products [24, 25]. Highly notable health effects have been demonstrated [26, 27]. In children exposed to a long-term increase of 30 $\mu g \cdot m^{-3}$ of NO_2 (equivalent to having a gas cooker), a 29% increase in their risk of respiratory illness has been demonstrated [28].

PARTICULATE MATTER

Airborne PM is a major component of urban air pollution. PM consists of a mixture of particle components, including traffic and combustion-derived carbon-centred ultrafine particles (<100 nm in diameter), secondary particles (nitrates and sulphates), wind-blown dust of geological origin potentially containing endotoxin, and biological particles (*e.g.* spores, pollen) with their associated allergens. PM that can reach the lower airways is called PM_{10} (the so called thoracic PM) and $PM_{2.5}$, particles with an aerodynamic diameter <2.5 μm (the so called respirable PM).

Human lung parenchyma retains $PM_{2.5}$ [29], while particles >5 and <10 μm reach the proximal airways and are eliminated by mucociliary clearance. PM is a highly toxic material because of its small size rather than its chemical composition, as suggested by the influx of inflammatory leukocytes into the airspaces [30]. PM has a variety of effects on lung defences. Transition metals contained in PM, in particular iron, damage the airways by generating free radicals and inducing oxidative stress [31]. The main cells involved in the initial pro-inflammatory responses to particles are the macrophages and epithelial cells. PM induces oxidative stress only in these cells [32].

The PM_{10} capacity of inducing oxidative stress in the airways has been proven in rats, by intratracheal instillation and subsequent increases in neutrophils in BALF, which is accompanied by decreased glutathione concentrations [33].

The oxidative effect of PM_{10} was confirmed, furthermore, by enhanced levels of NF-κB after mice were exposed to 300 μm^{-3} of $PM_{2.5}$ [34]. This factor translocates to the nucleus where it binds to DNA consensus sequences in the promoters of proinflammatory genes that code for cytokines (GM-CFS, TNF-α) and chemokines (IL-8), thereby causing neutrophil recruitment and tissue damage.

A recent work has shown, *in vivo*, that after instillation of $PM_{2.5}$ through a bronchoscope in healthy subjects there was an increase in radical oxidant generation and cytokine concentrations (IL-6 and TNF-α) in BALF [35]. This study, together with similar ones [36], demonstrates that chronically elevated levels of IL-6 and TNF-α may play a role in triggering allergic sensitisation.

The inflammatory effect of PM has been studied in mice, in which the inhalation of 300 μm^{-3} of $PM_{2.5}$ causes increases in TNF-β, IL-6, interferon-γ and transforming growth factor-β [37]. Furthermore, carbon particles, which are an important constituent of PM_{10}, cause the release of immature neutrophils from the bone marrow [38], underlining the chief role of PM in the pathogenesis of chronic obstructive pulmonary disease (COPD).

Epidemiological evidence indicates a clear relationship between the levels of PM and increased morbidity, including respiratory symptoms, exacerbation of allergies, asthma, reduction in lung function and hospital admissions in patients with COPD. In Europe, the Air Pollution and Health: a European Approach (APHEA–2) studies demonstrated that hospital admissions for asthma and COPD increased by 1.0% per 10 $\mu g \cdot m^{-3}$ PM_{10} [39].

Diesel exhaust particulate (DEP) constitutes a large proportion of the PM in ambient air. In particular, diesel exhaust fumes causes bronchoconstriction, neutrophilic inflammation and dysfunction of alveolar macrophage phagocytosis, together with histamine release from mast cells in healthy individuals [40].

A few studies have shown the effect of DEP inhalation in asthmatic subjects [41]. An increased bronchial hyperresponsiveness and an enhanced allergen response were demonstrated after local diesel exhaust particle instillation in the nose [42].

The increase in the volume of road traffic and in air pollution shows a parallel increase in allergic disease, such as asthma and rhinitis. In fact, after inhaling DEP, the normal defence mechanism of the lung may be overwhelmed by the quantity or the toxicity of those particles. More specifically, the bronchial epithelium and cilia are damaged and the allergens remain on the epithelial surface for long time. Likewise, DEP may bind pollen or other allergens, thereby sensitising the airways to successive allergen exposure. Epidemiological studies have shown a significant association between a PM_{10} exposure and exacerbation of asthma in children and adults [43, 44].

In vivo murine models have shown an increased production in total and specific immunoglogulin (Ig)E after DEP injection [45], but also increased bronchial hyperresponsiveness and airway inflammation [46].

Studies of human exposure to DEP demonstrate acute inflammation in healthy subjects [47], and the DEP has an important pro-inflammatory effect of DEP in the upper airways, as suggested by the levels of cytokines in the nasal mucosa [48]. *In vitro* culture studies have concentrated on the mechanism by which PM_{10} causes airway damage and inflammation.

Apart from the large number of studies suggesting that DEP induces the production of inflammatory mediators and cytokines, the most recent studies have analysed the effects of suspended DEP on IL-8 gene expression [49], which is known to be a potent chemotactic factor for neutrophils, eosinophils and T-lymphocytes, and is undoubtedly an important factor in bronchial hyperresponsiveness. There is evidence that DEP induces oxidative stress in macrophages and bronchial epithelial cells by activation of NF-κB [50].

Other important *in vitro* studies have shown experimental evidence that DEP can cause an increase in the release of T-helper cell type 2 chemokines and IgE production from purified B-cells [51]. In addition, the capacity of DEP to activate p38 mitogen-activated protein kinases, which control the production of IL-8 and RANTES by human epithelial cells, has been shown [52].

SULPHUR DIOXIDE

SO_2 derives primarily from fuel combustion, mainly from industries and vehicle traffic, and is not an important indoor pollutant in terms of health. SO_2 has been shown to produce reactive oxygen species in the lungs, but high concentrations are needed to cause important effects on the antioxidant mechanism. Asthmatics may be more susceptible than normal subjects to SO_2 bronchoconstriction [53].

In contrast to O_3, the bronchoconstriction caused by SO_2 inhalation is immediate, and significant responses on lung function in asthmatic individuals are observed within 2 min [54].

FORMALDEHYDE

HCHO occurs ubiquitously in the environment and is found in cigarette smoke, vehicle exhaust, in fruits, vegetables and other foods, in construction, furniture and cleaning agents. Although some studies have demonstrated the irritant effect of HCHO on the eyes, nose and throat, there is no evidence of any increase in respiratory symptoms or decreased lung function at current domestic levels in adults. One study demonstrates that asthma and bronchitis (but not other respiratory symptoms) are more prevalent among children living in houses with higher HCHO levels [55].

OTHER VOLATILE ORGANIC COMPOUNDS

VOCs encompasses a wide range of hydrophilic or lipophilic substances of neutral, basic or acid character. They derive from building materials, paints, furniture, cleaning agents, resins *etc*. Active and passive cigarette smoking contribute to indoor concentrations. Many VOCs are irritants or narcotics and can cause depression of the central nervous system, but there is no association between VOC levels in the air and respiratory health [56].

PASSIVE SMOKE EXPOSURE

Tobacco smoke contains drops of tar and a mixture of other toxic elements, such as CO, NO, ammonia and proven animal carcinogens, such as polycyclic aromatic hydrocarbons and benzene. ETS has been classified as carcinogenic only in Germany [57]. The real mechanism of action of ETS is not well known, but it is recognised that tobacco smoke is a carcinogen, stimulates wheezing and sputum production, inhibits mucociliary clearance, causes inflammation of the airways and has a negative impact on lung defences [58]. It is difficult to estimate an individual's exposure to ETS because of other airborne contaminants. To assess exposure, it is possible to measure ETS metabolites, such as urinary or salivary cotinine, a metabolite of nicotine, or ETS directly, using questionnaires. A recent review found an association between passive smoke exposure and an increased risk of chronic bronchitis and asthma in young adults [59]. There is evidence of an association between ETS during childhood and respiratory symptoms [60].

INDOOR ALLERGENS

The most important indoor allergens are dust mites, domestic pets, such as cats and dogs, mice, rats, insects, especially cockroaches and fungi. Their allergenic activity depends on the capacity of proteins to interact with IgE bound to fragment crystallisable receptors on basophils and mast cells and to trigger the release of cytokines and other mediators involved in inflammation. Indoor sensitisation is a risk factor for the development of asthma [61]. With increasing allergen exposure, asthma symptoms, bronchial reactivity and lung function all worsen in sensitised patients.

Indoor allergens have been implicated in increasing the severity and prevalence of asthma, but there is no evidence that allergen exposure can be the primary cause of asthma [62]. Several longitudinal studies have shown a strong association between allergen exposure early in life and the high probability of developing asthma [63].

CONCLUSION

The lungs have immunological mechanisms that are generally highly efficient at defending the entire organism. All the environmental factors examined in this article interact with lung defences, some of them stimulating (with increased inflammatory cytokines), while others inhibiting (with an alteration of the oxidant-antioxidant balance, or with a decreased mucociliary clearance), the normal defence mechanisms.

Inflammatory mechanisms are, by definition, defence mechanisms. Thus, airway inflammation means mucus hypersecretion, chemotaxis for inflammatory cells, and release of mediators that are potentially noxious for lung tissues and able to destroy the alveolar attachments and to modify the small airways structures. These changes are often seen in chronic respiratory disease, such as COPD, patients.

Immunological mechanisms are highly specific lung defences that integrate inflammatory processes. However, the immunological mechanism can be modified by environmental factors. Some air pollutants stimulate the release of TNF-β and IL-6. When their levels remain chronically elevated, they can trigger allergic disease. The destruction of the bronchial epithelium and cilia by inhaled environmental factors permits the allergens to remain on the epithelial surface for a prolonged time, hence sensitising the subject. Also, the consequent increase of IgE is important in determining and maintaining bronchial hyperresponsiveness.

In summary, there is rarely a clear cause–effect relationship between environmental factors and lung disease [64] (table 2). However, air pollution certainly interferes with aspecific and specific lung defences, thus facilitating the development of pulmonary diseases.

TABLE 2 Effect on lung defences and association with lung disease

ENVIRONMENTAL FACTOR	LUNG DISEASE
Ozone	Asthma Increased respiratory mortality
Nitrogen dioxide	Exacerbation of respiratory disease
Particulate matter	Exacerbation of COPD Exacerbation of allergies and asthma Increased hospital admission Increased respiratory mortality
Sulphur dioxide	Exacerbation of asthma

COPD: chronic obstructive pulmonary disease.

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