

REVIEW

Airway inflammatory and antioxidant responses to oxidative and particulate air pollutants – experimental exposure studies in humans

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Introduction

In recent years, it has been shown that air pollutants such as nitrogen dioxide (NO₂), ozone (O₃) and diesel exhaust increase airway symptoms in healthy subjects as well as in individuals with asthma, allergy and chronic obstructive pulmonary disease (COPD). There is evidence that airway diseases such as asthma and allergy are increasing in terms of both prevalence and incidence in many western countries [1]. Current evidence also suggests that asthmatics are more sensitive to the effects of air pollutants [1–3].

Concentrations of NO₂ are often much higher in indoor air compared with ambient air and are thus of primary importance in particular industrial environments, while ozone is mainly an outdoor problem during the summer. Diesel exhaust is one of the most important sources of particulate matter pollution. These pollutants have previously been shown to induce inflammatory changes in the human airways, but more detailed information has been lacking. Very little is known about their effects in the lung tissue *per se* and the importance of lung epithelial lining fluid (ELF) antioxidants in the defence against their oxidative properties.

There is no clear evidence of a safe threshold level and many studies observe that health effects increase with pollution levels, often with a near dose–response relationship [4]. Furthermore, it has been shown that area sampling data may be of limited value in the investigation of the biological effects of exposure to pollution. Personal sampling produces two to 10 times higher values on average than corresponding measurements of area concentration [5]. As a result, personal exposure assessments may be crucial in determining the health effects attributable to different concentrations of air pollutants.

Epidemiological studies alone cannot provide an adequate

insight into the mechanisms responsible for the adverse human health effects of exposure to ambient or workroom concentrations of air pollutants. Although experimental exposure studies using bronchoscopy with bronchoalveolar lavage (BAL) have certain limitations, they have given the opportunity to assess the airway effects of air pollutants in man and are needed to obtain basic information which may contribute to the understanding of the mechanisms of the toxicity of air pollutants. In this review, studies addressing the inflammatory and antioxidant responses in the airways to these air pollutants will be discussed along with proposed mechanisms contributing to air pollutant-induced airway inflammation.

Antioxidants

Recently, it has been suggested that antioxidants such as glutathione (GSH), uric acid, ascorbate and α -tocopherol present in ELF may protect the airways from oxidant injury induced by exposure to air pollutants [6]. The antioxidants act as sacrificial substrates scavenging oxidant pollutants from the airways and thereby preventing oxidation of macromolecules such as lipids, proteins and carbohydrates. Increased GSH concentrations in the BAL fluid have been reported in a number of conditions thought to be mediated by oxidative stress, including cigarette smoking [7], adult respiratory distress syndrome (ARDS) [8] and asthma [9]. There is also considerable evidence linking COPD with increased oxidative stress [10].

Uric acid and ascorbate represent major substrates for ozone. Studies of BAL fluid from human subjects have shown that uric acid and ascorbate are consumed in an air pollutant concentration and time-dependent manner. The rate of consumption of ascorbate in ELF obtained from BAL is greater than that of GSH, suggesting that ascorbate is a very important component of the protective antioxidant screen present in the ELF [11,12]. Recently, Housley and collaborators reported a gender difference in the concentration of uric acid in human nasal lavage, in that women displayed lower nasal levels of uric acid than men [13].

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Factors leading to decreased levels of uric acid may thus be related to an individual sensitivity to inhaled oxidative air pollutants. In contrast to the water-soluble antioxidants ascorbic acid and uric acid, α -tocopherol is lipid-soluble and is believed to act as a chain-breaking antioxidant, scavenging peroxy radicals and thus preventing the propagation of lipid peroxidation processes [14] (Fig. 1).

Nitrogen dioxide

The major source of NO_2 in the atmosphere is the combustion of fossil fuels for heating and power generation and motor vehicles [15]. Urban levels of NO_2 vary according to the time of day, season, meteorological conditions and human activities and are systematically characterized by two daily peaks occurring during the morning and afternoon rush-hours, when the traffic is most intense. Since indoor concentrations are considerably higher than outdoor levels, indoor NO_2 is the major contributor to personal exposure [15], especially in certain industries and in homes with gas stoves. Here the 24-h averages may reach 0.5 p.p.m. and peak concentrations may be as high as 1 p.p.m. [16].

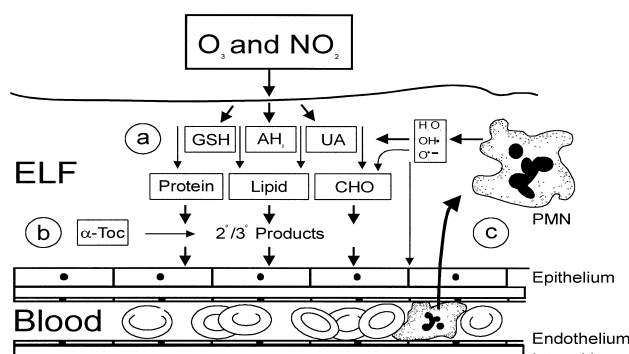


Fig. 1. Possible mechanisms of O_3 – NO_2 -induced injury of the lung. Schematic representation of the interactions between O_3 , NO_2 and the components of the ELF. (a) O_3 and NO_2 will react with all hydrocarbons but the intrinsic reaction rates between them and the water-soluble antioxidants, GSH, ascorbate (AH_2) and uric acid (UA) are particularly rapid. The antioxidants act as sacrificial substrates scavenging O_3 and NO_2 and thereby preventing oxidation of macromolecules such as lipids, proteins and carbohydrates (CHO). When the antioxidant defences are overwhelmed by the oxidative burden, the oxidative air pollutants then react with these macromolecules with the resultant generation of secondary and tertiary products, many of which are toxic. (b) Vitamin E (α -tocopherol; α -Toc) provides a second tier of defence by scavenging peroxy radicals and preventing the propagation of free radical reactions. (c) Exposure to both O_3 and NO_2 results in airway inflammation with activated neutrophils (PMNs) marginating into the ELF, providing an additional source of reactive oxygen species, which further impinge upon the oxidative burden experienced within this microenvironment

The dose response and time kinetics of the NO_2 -induced airway inflammation have been investigated in non-smoking healthy subjects. Dose-dependent increases in mast cells and lymphocytes were found in BAL fluid after a single exposure to 2.25–5.5 p.p.m. of NO_2 and the airway inflammation resolved within 72 h after exposure [17,18]. However, at NO_2 concentrations of below 2 p.p.m., no changes in cell numbers have been found [19,20]. Markers of inflammation such as albumin, fibronectin, hyaluronan, angiotensin-converting enzyme (ACE), β_2 -microglobulin, lactate dehydrogenase, total protein, leukotriene B₄ (LTB₄), prostaglandin E₂ (PGE₂), thromboxane A₂ (TxA₂) and tumour necrosis factor α (TNF α) have all been assayed and found to be unaffected by NO_2 exposure [17,19].

In a recent study, exposure to 2 p.p.m. of NO_2 for 4 h induced an early (1.5 h after exposure) increase in interleukin (IL) -8 in a small volume lavage, bronchial wash followed by a 2.5-fold increase in neutrophils at 6 h. In contrast to the bronchial wash findings, no signs of inflammatory cell recruitment or upregulation of the expression of vascular endothelial adhesion molecules were seen in endobronchial mucosal biopsies from more proximal airways. Other novel findings here were decreases in total protein, albumin, immunoglobulin (Ig) A and soluble ICAM-1 in bronchial wash [21]. It was suggested that the increase in neutrophils was most likely due to the enhanced IL-8 secretion, but the mechanism behind the increase in IL-8 and its cellular origin, are still unclear. It is plausible that increased oxidant stress during the exposure to NO_2 is involved. The neutrophilic inflammation in the bronchial wash, but not signs of inflammatory cell recruitment in the biopsies, suggest that the major transit of inflammatory cells in the airways following a single exposure to NO_2 is restricted to the smaller airways and probably the terminal bronchioles.

The kinetics of the NO_2 -induced antioxidant reactions were examined in a study in which healthy subjects were exposed to 2 p.p.m. of NO_2 for 4 h and assigned to bronchoscopy at three different time points, 1.5, 6 or 24 h after each exposure. In both bronchial wash and BAL, exposure to NO_2 resulted in rapid losses of ascorbate and uric acid with concentrations returning to baseline levels at 6 and 24 h, respectively. In contrast, GSH levels increased at both 1.5 and 6 h in bronchial wash after exposure to NO_2 and subsequently returned to control levels by 24 h. The data suggest that antioxidants present in the lung ELF react with, and hence modulate the impact of, NO_2 on the airways [22].

The effects of repeated exposure to NO_2 have been investigated in four studies. Rubinstein *et al.* exposed healthy volunteers to 0.60 p.p.m. of NO_2 for 2 h on 4 separate days within a 6-day period. The only significant change was a slight increase in the percentage of NK-cells [23]. In contrast, repeated exposure to 1.5 and 4 p.p.m. of

NO₂ for 20 min every second day produced a different cell response in BAL after six exposures. The amounts of lavaged B cells and NK cells decreased and the CD4⁺/CD8⁺ cell ratio was altered, but no effect on lymphocyte or mast cell numbers was found [24,25]. Recently, the effects of repeated exposure to NO₂ on lung function, airway antioxidant status, inflammatory cell and mediator response were examined in healthy subjects exposed to 2 p.p.m. of NO₂ for 4 h on 4 consecutive days. Data from this study revealed significant decrements in FEV₁ and FVC after the first exposure to NO₂ but that these attenuated with repeated exposures. Repeated NO₂ exposure also resulted in a decrease in neutrophil numbers in the bronchial epithelium. Signs of a neutrophilic airway inflammation together with an increase in myeloperoxidase was found in the bronchial wash indicative of both migration and activation of neutrophils in the airways. Antioxidant status was unchanged after the fourth NO₂ exposure [26]. Taken together, these data suggest that sequential exposure to NO₂ results in a neutrophilic airway inflammation in the absence of significant persistent changes in pulmonary function and depletion of antioxidants.

Ozone

Ozone is a secondary air pollutant which is mainly produced in the troposphere by a series of sunlight-driven reactions involving nitric oxides (NO_x) and volatile organic compounds (VOCs) arising largely from mobile and stationary combustion sources [27,28]. Ozone is regarded as an outdoor problem during the summer. During hot summers, ground-level concentrations of ozone may exceed 0.2 p.p.m. in Central Europe and other areas [28].

Since 1986, the airway effects of exposure to ozone have been investigated with BAL in healthy human subjects. It is well known that exposure to ozone concentrations of 0.4 and 0.6 p.p.m. for 2 h induces a pronounced inflammatory response in the airways characterized by increases in neutrophils, protein, albumin and PGE₂ [29,30].

The time-dependent changes in markers associated with the airway inflammation following ozone exposure have been addressed in a study in which bronchoscopy was carried out 1 or 18 h after exposure to 0.4 p.p.m. of O₃ for 2 h during heavy exercise [31]. The results revealed early increases in neutrophils, IL-6 and PGE₂. In contrast, fibronectin was higher at 18 h compared with the 1-h measurement, while total protein was increased to the same degree at both time points. Schelegle *et al.* exposed healthy subjects to 0.3 p.p.m. of O₃ for 1 h during heavy exercise and bronchoscopy was performed at 1, 6 and 24 h after exposure in different individuals. The results revealed an increase in the percentage of neutrophils in the proximal lavage sample at all three time points, but showed that the acute airway neutrophilia appeared to peak between 6 and 24 h [32].

While these earlier studies evaluated the effects of quite high ozone concentrations on the human airways, Devlin and coworkers investigated healthy subjects using exposure to ambient ozone levels (0.08 and 0.10 p.p.m.) for 6.6 h. On the basis of these data, it was concluded that exposure to a O₃ level as low as 0.08 p.p.m. was sufficient to initiate an inflammatory airway response [33].

The first human study to use bronchial biopsies to study the ozone-induced inflammation in the proximal airways revealed increased neutrophil numbers in the bronchial mucosa 18 h after exposure to 0.4 p.p.m. of O₃ for 4 h [34]. In another study, it was demonstrated that the vascular endothelial adhesion molecule ICAM-1 was significantly upregulated in the endothelium 18 h after exposure to 0.2 p.p.m. of O₃ for 4 h, together with non-significant trends for increased neutrophil and eosinophil infiltration in the bronchial mucosa [35]. Our research group has recently performed studies using immunohistochemistry on endobronchial mucosal biopsies to determine the airway inflammatory response following exposure to O₃. In the first investigation, subjects were exposed to 0.12 p.p.m. of O₃ for 2 h and bronchoscopy was performed 1.5 h after exposure. At this very early time point after exposure to an ambient concentration of O₃, no signs of inflammatory cell recruitment were found in either the lavage or the bronchial mucosa. However, a significant upregulation of the adhesion molecule P-selectin was demonstrated in vascular endothelium [36]. The upregulation of P-selectin early after ozone exposure suggests one of the earliest events taking place in the inflammatory response, such as 'rolling' of the neutrophils on the vessel wall before transendothelial migration. The time-kinetics of the airway inflammation following exposure to ozone were addressed in two studies in which the subjects were exposed to a higher ozone concentration (0.20 p.p.m.) with bronchoscopy at 1.5 or 6 h after the end of the exposures. At the early time point, the upregulation of P-selectin and ICAM-1 was shown in the vascular endothelium along with an increase in submucosal mast cells, but in the absence of any neutrophil recruitment [37]. While P-selectin is involved in the early phase of leucocyte endothelial adhesion, ICAM-1 is considered important during firmer leucocyte adhesion to the endothelial surface and subsequent migration from the vasculature [38]. At 6 h, neutrophil numbers had increased in the airway mucosa as well as in bronchial wash and BAL. P-selectin and ICAM-1 were still upregulated and mast cell numbers were increased in the submucosa [39]. These data concluded that exposure to ozone in ambient concentrations induced a neutrophil-dominated airway inflammation detectable in both endobronchial mucosal biopsies and airway lavage. The neutrophil influx was preceded by an upregulation of the expression of P-selectin and ICAM-1 in the vascular

endothelium suggesting that the endothelial adhesion molecules could be of critical importance in the development of airway inflammation after ozone exposure. However, on the basis of results from other studies [34,40–43], it has been suggested that cytokines such as IL-8 and GRO- α may also play an important role in mediating the inflammatory response following ozone exposure.

As ozone is a powerful oxidant it has been hypothesized that ozone would affect the antioxidant defence present in the ELF. It was shown that no effects on the determined antioxidants were seen 1.5 h after exposure to 0.12 p.p.m. of ozone [44]. In contrast, following exposure to 0.20 p.p.m. increases in ELF GSH was found at 1.5 h. At 6 h, GSH levels did not differ compared with air exposure, while an increase in oxidized glutathione (GSSG) was found. Furthermore, ascorbate was depleted 6 h after the ozone exposure [39]. These data support the hypothesis that ozone causes an oxidative stress in the airways and that antioxidants may be important in modulating the impact of O₃ on the airways.

Some studies have addressed the question of whether there is a relationship between the changes in lung function and indices of airway inflammation measured as a neutrophil increase after ozone. Schelegle *et al.* reported a trend towards an inverse relationship between the maximum FEV₁ decrements and the peak neutrophilia after O₃ exposure [32]. This trend was later confirmed by Aris and coworkers who found a significant inverse relationship between each subject's FEV₁ decrement to O₃ and BAL fluid neutrophil percentage [34]. In two recent studies, it has also been suggested that the O₃-induced respiratory symptoms and airway inflammatory markers were not related to the magnitude of decrements in FEV₁ and FVC [37,40]. On the basis of these studies, it can thus be concluded that there is no evidence of a relationship between the intensity of the inflammatory response and the functional decrement after exposure to O₃.

Very recently, the effects of repeated daily exposure to 0.2 p.p.m. of ozone for 4 h on 4 consecutive days were demonstrated. It was shown that the number of neutrophils, IL-6 and fibronectin decreased after 4 days' exposure compared with a single day's exposure to ozone. These data suggested that there is an attenuation of the O₃-induced inflammatory responses with repeated daily exposures [45].

More interestingly, the effects of experimental exposure to O₃ have been evaluated in asthmatics [41,46]. The results showed that asthmatic subjects may not have greater sensitivity to ozone in terms of lower respiratory symptoms and lung function responses, but they could be at risk of developing a more severe O₃-induced airway inflammation than normal subjects. These findings may contribute to the explanation of the increased asthma morbidity associated with ozone pollution episodes reported in epidemiological

studies, but further studies are needed to more thoroughly address the mechanisms behind the differences between healthy and asthmatic subjects in their response to ozone.

Diesel exhaust

Diesel exhaust is a major contributor to atmospheric particulate matter pollution all over the world. Water and carbon dioxide (CO₂) are the end points of optimal combustion but, due to incomplete combustion, liquid and solid particles and gases are generated. Diesel exhaust consists of a complex mixture of gases such as carbon monoxide (CO), nitric oxides (NO, NO₂), sulphur dioxide (SO₂), hydrocarbons, ash, transition metals and carbon particles [47]. Measurements from Scandinavian countries have shown that the average 24-h particulate matter concentrations vary between 30 and 150 $\mu\text{g}/\text{m}^3$ total suspended particles, i.e. particles in air less than 50 μm . However, in certain industrial areas, concentrations of 1500 $\mu\text{g}/\text{m}^3$ have been measured [48]. In Stockholm, 1-h mean value levels of 300–500 $\mu\text{g}/\text{m}^3$ have been found at kerbsides and maximum levels of almost 600 $\mu\text{g}/\text{m}^3$ or more have been reached in a tunnel in the central part of the city [48].

The physical and chemical nature of the particles, their distribution in the respiratory tract and the biological events occurring in response to the particles determine their biological effects. Particle deposition in the respiratory tract is dependent on both particle size and the pattern of breathing. Most inhaled particles with an aerodynamic diameter of more than 5 μm are deposited in the upper or larger airways, whereas smaller particles are more prone to be deposited in the smaller airways and the alveoli [49,50].

When the diesel exhaust particles are generated by combustion, the size distribution in terms of numbers of particles is predominantly very small, with two peaks at around 30 and 100 nm [51]. Recently, concern has been voiced regarding the health effects of environmental exposure to ultrafine particles (diameter < 0.05–0.10 μm). These particles are always present in the urban atmosphere in very large numbers ($> 0.5 \times 10^5$ particles/cm³) and can be highly reactive [52]. The ultrafine particles have a relatively short life, condensing into larger (but still very small) ones and they are able to provoke a different alveolar reaction from that induced by larger particles. The ultrafine particles may penetrate through the epithelium and vascular walls and be transported by the blood to distal organs where pro-inflammatory events may occur. This could be of importance not only in respiratory illness but also by provoking acute changes in blood coagulability and in the heart [53,54].

The effects of exposure to diesel exhaust have been evaluated in human studies using bronchoscopy with BAL. In one study, healthy volunteers were exposed to

diesel exhaust, keeping the NO₂ concentration at 1.5 p.p.m. BAL 18 h after diesel exhaust exposure revealed a significant decrease in the total number of metachromatic cells (mast cells) in the bronchial portion and a significant increase in neutrophils in the bronchoalveolar portion. An increase in the CD4⁺/CD8⁺ ratio was found in the bronchoalveolar portion, along with a reduced phagocytosis rate by alveolar macrophages *in vitro* [55].

A recent study has investigated whether a particle trap on the tail-pipe of an idling diesel engine would reduce the diesel exhaust-induced effects on symptoms and lung function caused by exposure to diesel exhaust without the particle trap. Although the particle trap reduced the number of particles by 46%, no significant changes were found in the symptoms and lung function compared with the situation after exposure to diesel exhaust without the particle trap [56]. Neither were any significant changes detected in neutrophil numbers with the particle trap compared with the numbers without it [57]. On the basis of these data, it cannot be excluded that components of the vapour phase, including highly irritating organic compounds such as aldehydes could also be related to the acute effects.

In a recent study, healthy subjects were exposed to diesel exhaust at a particulate concentration of 300 µg/m³ for just 1 h and evaluated with immunohistochemistry on bronchial biopsies processed in glycomethacrylate (GMA) [58]. Increases in neutrophils, mast cells, CD3⁺, CD4⁺ and CD8⁺ T lymphocytes in the airway mucosa along with the upregulation of ICAM-1 and VCAM-1 in the vascular endothelium and the numbers of LFA-1⁺ cells (the ligand for ICAM-1) were found in the bronchial tissue 6 h after the exposure to diesel exhaust (Figs 2,3). In bronchial wash, diesel exhaust exposure induced increases in the number of neutrophils whereas B lymphocytes and the concentration of methyl histamine were increased in BAL. The findings in this study reinforce the view that diesel exhaust is capable of causing a marked airway inflammatory response involving a variety of cells. Studies are now needed to evaluate if there is a threshold for these inflammatory effects and if subjects with asthma are more sensitive to exposure to diesel exhaust than healthy subjects.

In vivo exposure to diesel exhaust particles (DEPs) has resulted in a non-TH1, non-TH2 increase in cytokine mRNA expression in cells recovered from the nasal mucosa [59], but a TH2 response was elicited when DEPs were combined with allergen [60]. So far, no published data are available on the effects of diesel exhaust exposure prior to allergen challenge in the lower airways. Previous studies involving nasal challenges with DEPs have suggested that DEPs can enhance B-cell differentiation and IgE production [61]. In summary, the increases in mast cells, T cells, B cells and VCAM-1 demonstrated after diesel exhaust exposure in

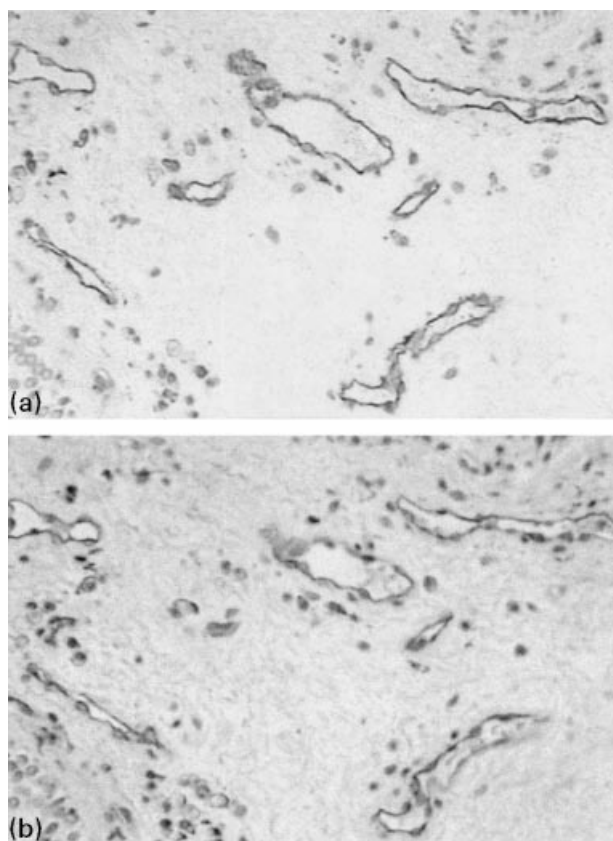


Fig. 2. Immunohistochemical staining of bronchial mucosa after exposure to diesel exhaust (sequential sections). (a) Vascular endothelium stained with the endothelial antibody (EN4). (b) Expression of the vascular endothelial adhesion molecule ICAM-1

healthy subjects might be of some relevance in conditions such as asthma and allergy.

Summary

In this review it has been demonstrated that exposure to air pollutants in workroom and ambient concentrations are important inducers of airway inflammation in healthy subjects. It is suggested that antioxidants present in the ELF may play a role in modulating the impact of oxidative air pollutants on the airways. The upregulation of vascular adhesion molecules and secretion of pro-inflammatory cytokines may be of importance in mediating the inflammatory response in the airways after exposure to air pollutants. Future studies are needed to study lower concentrations and, if possible, determine threshold levels for the noxious effects of the air pollutants. Furthermore, epidemiological and clinical data suggest that people with asthma, allergy and other respiratory illnesses appear to represent more sensitive groups with respect to air pollution.

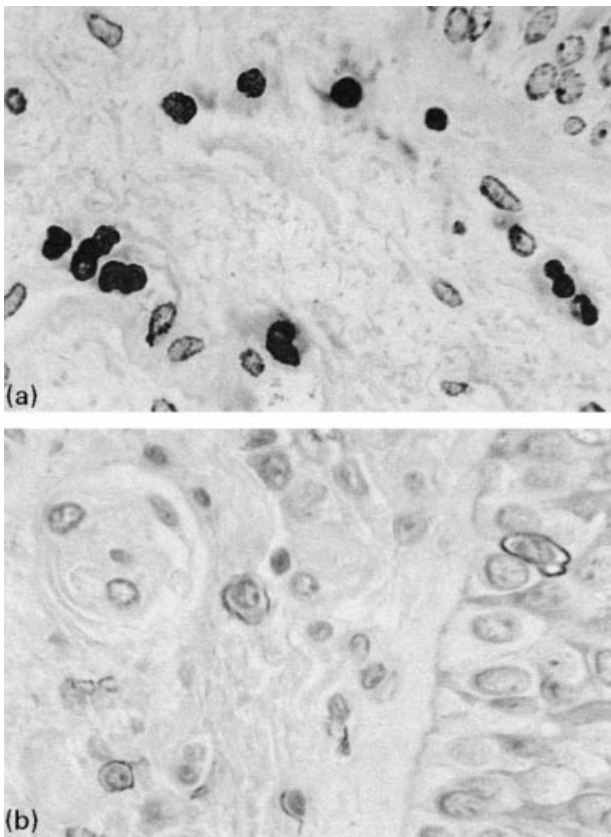


Fig. 3. Immunohistochemical staining of bronchial epithelium and submucosa. (a) Neutrophils with cytoplasmic staining for neutrophil elastase (b). CD8-positive lymphocytes with ring staining

Thus it is of importance to investigate the mechanisms behind the inflammatory changes in the airways following exposure to air pollutants both in healthy and in sensitive groups in more detail.

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