

The impact of pollution on allergic disease

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Evidence suggests that allergic disease is becoming more common, particularly in industrialized societies. Two studies of schoolchildren from Aberdeen, Scotland aged 8–13 years were undertaken in 1964 and 1989 using identical questionnaires, and found that the reported prevalence of asthma had risen from 4.1% to 10.2% during this period, hay fever from 3.2% to 11.9% and eczema from 5.3% to 12%. Indication that air pollution may contribute to this increase has come from several studies. In Japan, allergic rhinoconjunctivitis was found to be more prevalent in individuals living near motorways than in cedar forests. Severe asthma also occurs more commonly than mild asthma in children living in polluted areas. Exercise-induced asthma and the use of asthma medication were twice as high in a town near two power stations compared with a non-polluted town. A recent study in Finland showed that admissions to hospital with severe asthma correlated with atmospheric levels of nitrogen dioxide. Deterioration in peak flow recordings in asthmatics and exacerbations of symptoms in hay fever sufferers correlate with ambient levels of ozone. Elucidation of the mechanisms by which exposure to air pollutants may influence the frequency of allergic disease or exacerbate symptoms has come from *in vitro* and *in vivo* experiments in animals and man. Animals exposed to ozone, sulphur dioxide, nitrogen dioxide and particles from diesel exhaust, together with allergens, show more ready development of allergic sensitization compared with those exposed to allergen alone. The dose of allergen necessary to produce a 20% fall in FEV₁ (forced expiratory volume in 1 second) in mild asthmatics is reduced by previous exposure to ozone or a mixture of nitrogen dioxide and sulphur dioxide. It has also been suggested that the allergic potency of pollen grains may be increased when they are coated in pollutants. Bronchial and nasal lavage studies have shown that air pollutants can induce an influx of inflammatory cells and proinflammatory cytokines into the respiratory tract. Studies on human epithelial cells cultured to confluence *in vitro* have indicated that exposure to nitrogen dioxide can decrease ciliary beat frequency which would, in theory, reduce allergen clearance and increase the risk of sensitization. Further, exposure of these cells to either ozone or nitrogen dioxide induces the release of inflammatory mediators, such as leukotriene C₄, and proinflammatory cytokines, including granulocyte-macrophage colony-stimulating factor, tumour necrosis factor- α and interleukin-8. This effect can be attenuated by the use of anti-inflammatory drugs.

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Introduction

Before the enforcement of Clean Air Acts in several countries, episodes of severe air pollution, particularly by sulphur dioxide released from the burning of carboniferous products, were associated with increased death rates from respiratory disease. Dramatic episodes of severe air pollution, such as those that occurred in the Meuse Valley (Belgium) in 1931, Donora (Pennsylvania, USA)

in 1948 and London (United Kingdom) in 1952 have demonstrated that air pollution can lead to substantial morbidity and mortality from respiratory disease (1–6). In the last decade, however, it has become clear that new forms of air pollution may have equally damaging effects on the lungs (7). Such pollution results from:

- burning of liquid petroleum gas or kerosene
- high urban concentrations of vehicle exhaust

- fumes containing hydrocarbons, nitrogen dioxide, ozone, lead and particle matters
- changes in domestic heating methods.

Monitoring of ground level ozone in the United Kingdom has demonstrated that, in rural and industrialized areas, the normal background values of 30–40 ppb rise during sunny weather and often exceed the World Health Organization's guidelines for maximal exposure (70–100 ppb for 1 h or 50–60 ppb for 8 h) (8). Similarly, monitoring of nitrogen dioxide (9) and sulphur dioxide (10) have demonstrated that the World Health Organization's recommended maximal exposure over 1 h (mean 247 ppb nitrogen dioxide and 122 ppb sulphur dioxide) have been exceeded on many occasions, particularly in heavy traffic conditions and in industrialized areas.

Increase in the prevalence of allergic disease

Epidemiological studies

Epidemiological studies suggest that allergic diseases, such as asthma, rhinitis and eczema, have become more common over the last 50 years, particularly in children and young adults. Burr et al. (11) conducted two surveys 15 years apart in 12-year-old schoolchildren in South Wales. Children underwent peak expiratory flow (PEF) measurement and exercise provocation test. Questionnaires concerning the occurrence of symptoms, such as wheezing, breathlessness, asthma, eczema and hay fever, were completed by parents. Increases in the prevalence of these conditions over the 15 years of the study were 6% to 12% for asthma, 5% to 16% for eczema and 9% to 15% for hay fever. A similar study by Burney and colleagues (12) investigated the change in the prevalence of asthma in another school population in England between 1973 and 1986. Comparison of results demonstrated a significant increase in asthma in both boys and girls.

Ninan and Russell (13) determined the prevalence of respiratory symptoms and atopy in Aberdeen schoolchildren by analysis of questionnaires completed in 1964 and 1989. The reported diagnosis of asthma rose from 4.1% to 10.2%, and the prevalence of hay fever increased from 3.2% to 11.9%. Eczema also became more common (5.3% to 12%). The same phenomena were observed in several other countries. Aberg (14) analyzed computer records of examinations of Swedish conscripts undertaken between 1971 and 1981 and found that asthma had become more common, increasing from 1.9% in 1971 to 2.8% in 1981. The prevalence of allergic rhinitis had followed a simi-

lar pattern, increasing from 4.4% (1971) to 8.4% (1981). In Finland, Haahtela and colleagues (15) analysed records of military conscripts over longer time spans, between 1926 and 1961, and 1966 and 1989. There was little change in the prevalence of asthma during the first period (0.02% in 1926 and 0.08% in 1961). From 1961, however, the prevalence began to rise, increasing steadily to 0.29% in 1966 and to 1.79% in 1989. This represents a 22-fold increase in asthma prevalence since 1961. These epidemiological studies clearly demonstrate that the prevalence of atopy-related diseases has increased, and further suggests that higher diagnosis rates for asthma, hay fever and eczema are not simply due to changes in diagnostic fashion or improvements in detection, but are likely to reflect a real increase in the prevalence of respiratory symptoms and atopy over the past decades.

Increases correlate with pollution

It has long been suspected that air pollution plays a role in the manifestation of airway diseases, and more recent epidemiological studies have confirmed this suspicion. Usetti and colleagues (16) examined an asthma outbreak in Barcelona in 1983, and found clear correlation between the concentration of oxides of nitrogen and hospital admission for asthma. The German unification provided an excellent opportunity to study genetically similar populations living in different environments. In the study by von Mutius et al. (17), the prevalence of asthma and allergic disorders among children in two cities in Germany were investigated. While bronchitis was found to be more common in Leipzig (Eastern Germany), asthma and hay fever had a higher prevalence in Munich. Industrialized areas of East Germany are characterized by high concentrations of sulphur dioxide and particulate matter compared to West German cities, such as Munich where these pollutants are not a serious problem. On the other hand, levels of nitrogen dioxide were much higher in Munich than in Leipzig – probably due to the greater number of motor vehicles. This suggests that exposure to nitrogen dioxide rather than sulphur dioxide and particulate matter is associated with a higher prevalence of allergic diseases. Indeed, a very recent study of schoolchildren in Munich by Wjst et al. (18) revealed a significant correlation between increased numbers of cars passing daily through the school district and decreases in PEF and maximum expiratory flow (MEF) at 75% and 50% of forced vital capacity, respectively. Recurrent dyspnoea also correlated with traffic levels.

One of the most dramatic increases in allergic disease has taken place in Japan. Although cases

of pollinosis were hardly recognized before the Second World War, it has recently become a common allergic disease. Ishizaki and colleagues studied the prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area (19). They found that people living along intercity main roads lined with old cedar trees and with heavy car traffic all day had a high incidence of cedar pollinosis (13.2%), compared with individuals living in cedar forest areas with less intense traffic (5.1%). Pollen counts in both areas were identical.

Henry and colleagues (20) recently published their survey and longitudinal follow-up study on the prevalence of asthma in Australian primary schoolchildren living near power stations. The prevalence of asthma in the study town close to power stations was double the prevalence in the control town which was free from major sources of air pollution. Bronchial reactivity was also found to be greater in children living in the polluted area.

Particulate air pollution has recently received a great deal of attention. In Seattle, Schwartz and colleagues (21) recorded the daily emergency room visits for asthma and measured the level of respirable particulate matter of mean median diameter ≤ 10 microns (PM_{10}). A significant association was found between visits and PM_{10} exposure on the previous day in those aged under 65 years. The mean of the previous 4 days exposure levels of PM_{10} , however, proved an even better predictor of an asthmatic attack. Interestingly, daily PM_{10} concentrations never exceeded 70% of the current quality standards during the study period, suggesting that revision of current safety levels for particulate matter air pollution may be necessary.

Does air pollution lead to sensitization?

Elucidation of the mechanisms by which exposure to air pollutants may influence the frequency of allergic disease or exacerbate symptoms has come from *in vitro* and *in vivo* experiments in animals and humans. Kainka-Stanicke and colleagues (22) have suggested that air pollutants may promote sensitization and the subsequent development of allergic disease by binding to pollen or other inhaled allergens and modulating the allergic response. Alternatively, air pollutants may cause functional and morphological changes in the epithelium which normally acts as a physico-chemical barrier against inhaled substances. Epithelial injury would provide allergens with easier access to cells of the immune system. Additionally, dysfunction or loss of cilia would result in impairment of mucociliary clearance, and would allow allergens to remain on the epithelial surface. This would

increase diffusion of proteins into the cell rich sub-epithelial layer.

Human studies

In vivo studies Molino and colleagues (23) recently demonstrated that exposure to 120 ppb of ozone for 1 h increased the airway response to inhaled allergens in resting asthmatic subjects without changes in baseline airway function. They found that the mean dose of allergen needed to produce an early response after breathing ozone was approximately half the dose required when air preceded the allergen challenge. Similar studies investigating the effects of nitrogen dioxide, sulphur dioxide and ozone on the airway response of mild asthmatics to house dust mite allergens are currently being carried out in our department. Our preliminary studies exposed individuals with mild asthma to a combination of 400 ppb nitrogen dioxide and 200 ppb sulphur dioxide for 6 h in an environmental chamber. This led to an immediate increase in the airway response to inhaled allergen when compared to that occurring after exposure to the individual gases at the same concentrations (24) (Fig. 1).

Nasal and bronchoalveolar lavage have been studied to assess cellular and biochemical changes in both the upper and lower respiratory tract. In the study by Bascom and colleagues (25), asymptomatic subjects with a history of allergic rhinitis were randomly exposed at rest to either clean air or 500 ppb ozone for 4 h. After exposure, patients underwent nasal challenge with ragweed or grass antigen followed by nasal lavage. Analysis of the lavage fluid revealed a seven-fold increase in the number of neutrophils, a 20-fold increase in eosinophils and a ten-fold increase in mononuclear cells when patients were exposed to ozone. Devlin and colleagues (26) investigated the effect of prolonged exposure of non-smoking males to low con-

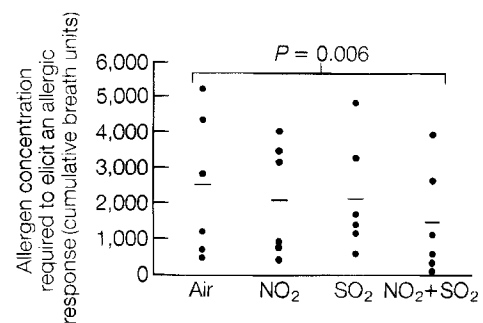


Fig. 1. The effect of a 6 h exposure to 200 ppb sulphur dioxide and/or 400 ppb nitrogen dioxide on inhaled allergen response in mild asthmatic subjects. The level of significance was determined after log transformation of the data. NO₂, nitrogen dioxide, SO₂, sulphur dioxide.

centrations of ozone. Volunteers were randomly exposed to clean filtered air, or to 80 ppb or 100 ppb ozone for 6.6 h during moderate exercise, followed by bronchoalveolar lavage (BAL) 18 h later. BAL fluid from patients exposed to 100 ppb ozone showed a significant increase in neutrophils, protein, prostaglandin E₂, fibronectin, interleukin-6 and lactate dehydrogenase compared to that from the same volunteers exposed to air.

Animal studies

Osebold and colleagues (27) demonstrated that exposure of mice to very high levels of ozone (500–800 ppb) led to immunological changes in the lung which included increases in secretory IgA and IgA-containing cells in bronchus-associated lymphoid tissue. Further studies by the same group (28), in which mice were exposed to ozone (500–800 ppb) or sulphur dioxide (370 ppb) prior to immunization with ovalbumin, revealed the immune response (anaphylaxis) was exacerbated by the pollutants compared to air. Synergism between ozone and sulphur dioxide was also observed. Primate studies (29) have investigated exposure to ozone at 1,000 ppb together with platinum salt. Results showed an increase in the incidence of positive skin tests to platinum, and increased bronchial responsiveness to both inhaled methacholine and platinum when compared to exposure to either allergen separately. Ozone levels in the United Kingdom and the World Health Organization's guidelines are given in Table 1.

The relationship between granulocyte migration and tracheobronchial epithelial injury in Rhesus monkeys following short-term ozone exposure was investigated by Hyde and colleagues (30). After an 8 h exposure to either filtered air or 960 ppb ozone, the animals were sacrificed and necropsies were carried out 1, 12, 24, 72 and 168 hours post-exposure. Trachea, bronchi and respiratory bronchioles from each monkey were examined by electron microscopy. Neutrophil influx into lung tissue and BAL were maximal at 12 h post-exposure and returned to baseline by 24 h. In contrast, the level of total neutrophils in BAL was significantly elevated 24 h after exposure, and returned to baseline by 72 h. Morphological investigations showed epi-

thelial necrosis at 1 and 12 hours in the trachea and bronchioles, which continued to be observed in the bronchi in significant numbers 24 h post-exposure. Eosinophils, however, were maximally increased in the bronchial epithelium and interstitium 24 h post-exposure, at a time when epithelial necrosis was maximal.

The steady increase in the number of diesel powered vehicles has meant that the level of airborne diesel exhaust particles (DEP) continues to rise. Miyamoto and colleagues (31) investigated the effect of these particles on IgE antibody production, and found that production was enhanced when experimental animals were immunized either intraperitoneally or intranasally with ovalbumin and DEP in comparison to ovalbumin alone.

In vitro studies

Despite large numbers of investigations in both animals and humans, the precise mechanisms underlying the effects of exposure to nitrogen dioxide or ozone have not been elucidated. As it has been demonstrated that the respiratory epithelium is the site most affected following exposure to these agents (32, 33), interest has focused on the role of airway epithelial cells in the development or modulation of allergic airway diseases. This group has developed an *in vitro* technique for culturing human bronchial cells and nasal epithelial cells (34). Using this *in vitro* model system, we have investigated the effect of a 20 minute exposure to different concentrations of nitrogen dioxide on the movement of ¹⁴C labelled bovine serum albumin across the bronchial epithelial cell monolayers, and

Table 1. Ozone levels in the United Kingdom and the World Health Organization's guidelines

Background	World Health Organization guidelines	Highest hourly recordings
Up to 60 ppb (Normal=20–30 ppb)	1 hour: 76–100 ppb 8 hours: 50–60 ppb	1989: 135 ppb 1990: 161 ppb

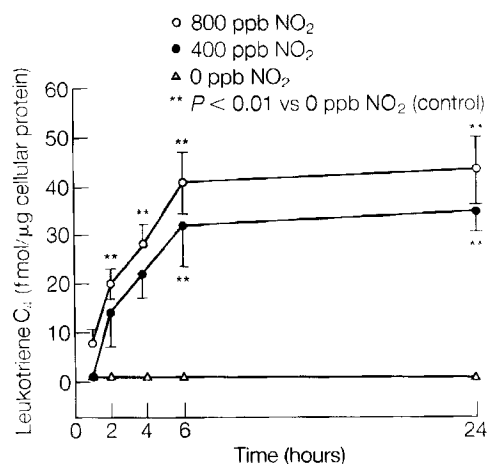


Fig. 2. Generation of leukotriene C₄ by human bronchial epithelial cells *in vitro*, following a 20 minute exposure to nitrogen dioxide and further incubation for up to 24 h at 37°C in 5% CO₂ in air, analysed by reverse-phase high performance liquid chromatography. Results are expressed as mean ± SEM (n=6 at each time point). NO₂, nitrogen dioxide.

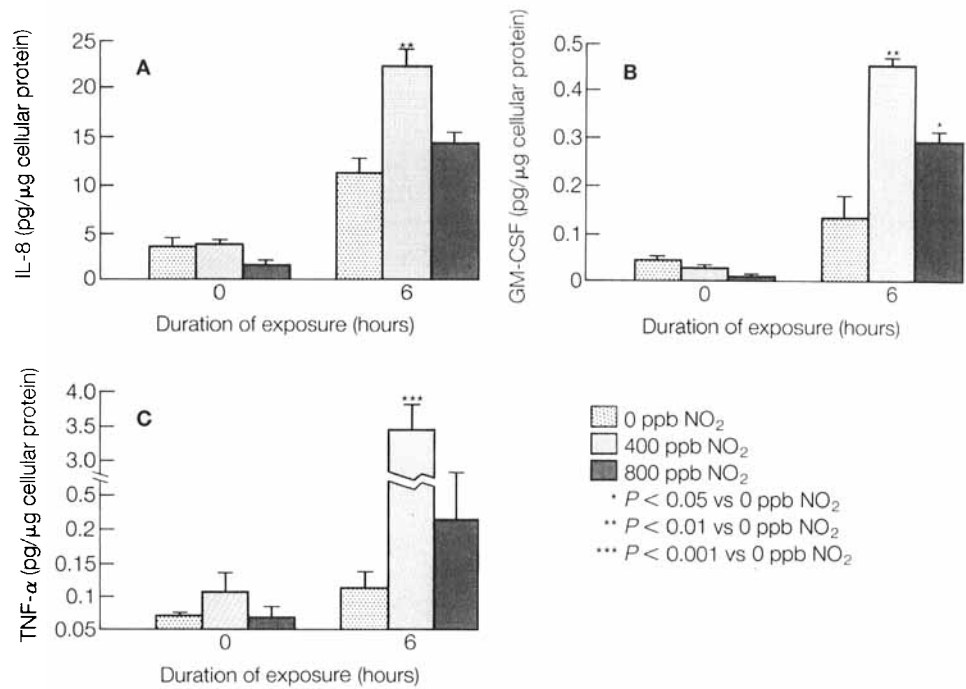


Fig. 3. Effect of a 6 h exposure to nitrogen dioxide on the production of (A) interleukin-8 (IL-8), (B) granulocyte-macrophage colony-stimulating factor (GM-CSF) and (C) tumour necrosis factor- α (TNF- α) by human bronchial epithelial cells *in vitro*. Results are expressed as mean $\pm$ SEM ($n=6$ at each time point). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs 0 ppb nitrogen dioxide.

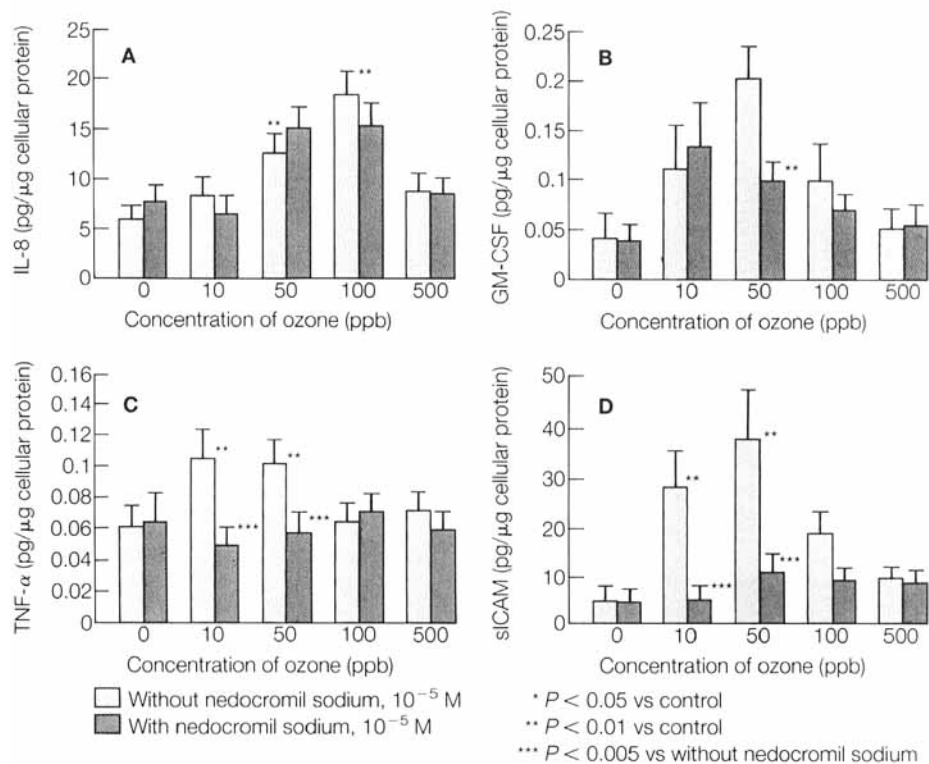


Fig. 4. Effect of a 6 h exposure to ozone on the production of (A) interleukin-8 (IL-8), (B) granulocyte-macrophage colony-stimulating factor (GM-CSF), (C) tumour necrosis factor- α (TNF- α) and (D) soluble intercellular adhesion molecule-1 (sICAM-1) by human bronchial epithelial cells *in vitro*. Results are expressed as mean $\pm$ SEM ($n=6$ each time point).

the metabolism of arachidonic acid by these cells (35). Our studies have demonstrated that human bronchial epithelial cells are capable of synthesizing several arachidonic acid metabolites known to be involved in allergic reactions, including prostaglandin E₂, 12- and 15-hydroxyeicosatetraenoic acid and leukotriene C₄. Exposure of these cells to both 400 ppb and 800 ppb nitrogen dioxide led to significant generation of these metabolites, predominantly leukotriene C₄ (Fig. 2).

In view of the importance of proinflammatory cytokines in the development of allergic diseases, we also investigated the ability of cultured human bronchial cells to express and synthesize cytokines (36), and the effect of a 6 h exposure to nitrogen dioxide on the *in vitro* synthesis of these cytokines. Bronchial epithelial cells grown to confluence in culture plates were found to be capable of expressing and synthesizing interleukin 1 β , interleukin-8, granulocyte-macrophage colony-stimulating factor

and tumour necrosis factor- α but not interleukin-4 or interferon- γ . Nitrogen dioxide significantly up-regulated the synthesis of interleukin-8, granulocyte-macrophage colony-stimulating factor and tumour necrosis factor- α by these cells, and these effects were dependent on the level of nitrogen dioxide exposure (Fig. 3). Significant cytokine production occurred on exposure of the cells to 400 ppb nitrogen dioxide. This level can be found at the kerb side in heavy traffic conditions, or indoors in households using gas ovens.

The effect of a 6 h exposure to 0–500 ppb ozone on the synthesis of interleukin-8, granulocyte-macrophage colony-stimulating factor, tumour necrosis factor- α and soluble intercellular adhesion molecule-1 by bronchial epithelial cells was also studied (37) (Fig. 4). Ozone induced a dose-dependent increase in the release of all four mediators, particularly at the concentration range of 10–100 ppb.

Taken together, these studies on the effects of nitrogen dioxide and ozone on cultures of human bronchial epithelial cells suggest that pollutants may not only increase the permeability of the bronchial epithelium, but also cause the generation of potent mediators and proinflammatory cytokines which could enhance the airway response to inhaled allergen.

Conclusion

There is now compelling evidence from both epidemiological and direct *in vivo* and *in vitro* exposure studies to suggest that exposure to pollutants from petrol and diesel engines may precipitate attacks of asthma. Pollutants may also be one of several causes of the recognized increase in the prevalence of this disorder. The mechanisms by which pollutants exert this effect are likely to be direct and dose-dependent oxidative actions on epithelial cells leading to changes in permeability, and generation of vasomotor and bronchoconstrictor arachidonic acid metabolites and proinflammatory cytokines.

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