

Review article

Allergen/irritant interaction – its role in sensitization and allergic disease

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Recently, several studies have shown that allergic diseases such as asthma, rhinitis, and eczema have become more common over the last 50 years. Burr et al. (1) have conducted two surveys 15 years apart in 12-year-old schoolchildren in south Wales (UK), and demonstrated that the number of children suffering from asthma, eczema, and hay fever increased significantly from 6% to 12%, 5% to 16%, and 9% to 15%, respectively, during this period. Similarly, three separate surveys conducted by Russell and colleagues (2, 3), in schoolchildren in Aberdeen (UK) over a 30-year period (1964–94), indicated that the prevalence of respiratory symptoms and atopy was increased, as demonstrated by increased diagnosis of asthma (from 4.1% in 1964 to 10.2% in 1989 to 19.5% in 1994), hay fever (from 3.2% in 1964 to 11.9% in 1989 to 12.9% in 1994), and eczema (from 5.3% in 1964 to 12% in 1989 to 17.7% in 1994). The most recent data indicate that this increase has been sustained since 1994. Analysis of the prevalence of allergic airway disease in 1996 has demonstrated that while the prevalence of asthma has risen to 21% among 12–13-year-old schoolchildren, the prevalence of allergic rhinitis has increased even further to 30% (4).

Association between environmental factors and allergic airway disease

Epidemiologic studies have demonstrated that levels of air pollutants, including ozone (O_3), nitrogen dioxide (NO_2), and respirable particulates (PM_{10}), resulting from increased use of liquid petroleum and gas in the transport and manufacturing industries and domestic settings, have progressively increased in the last decade and have occasionally exceeded the European and World Health Organization (WHO) safety guidelines in several major cities in the UK (5–8). Some studies have demonstrated that the increase in NO_2 and O_3 is associated with symptoms of rhinitis, chronic cough, phlegm, decreased morning peak flow, emergency room visits, and increased hospitalization due to asthma in asthmatic adults and children, and that the effects of these pollutants may predominate after 1–2 days (5, 8). Similarly, studies of respirable particulate matter have demonstrated that increased levels of PM_{10} are also associated with worsening peak flow, increased inhaler usage, respiratory symptoms, emergency room visits, and, more recently, cardiopulmonary and lung cancer mortality rates (9).

Although epidemiologic studies, particularly from the developed countries, suggest that the increase in the prevalence of allergic disease, such as asthma, may be associated with air pollution resulting from increased use of liquid petroleum fuel (5–8), the findings from these studies have been difficult to interpret due to the confounding effects of cigarette smoke, exposure to allergens, meteorologic conditions, and socioeconomic factors (10). Additionally, these studies have investigated the effects of only the major pollutants individually, without taking into account the potential additive and/or synergistic effects of combinations of the pollutants which are most relevant.

However, studies from Japan have demonstrated that the incidence of rhinoconjunctivitis in residents living alongside old cedar tree-lined main roads with constant heavy traffic was much higher than that in residents living in cedar forest but with less traffic, although the cedar pollen counts were similar in both areas (11). These studies suggest that the disparity in the incidence of rhinoconjunctivitis in the different areas may be a result of vehicle-exhaust pollution, which was the predominant factor in areas with high incidence. Early studies from Germany, particularly by von Mutius et al., demonstrated that allergic conditions such as hay fever were more common in West German cities than in East German cities, where chronic bronchitis was more prevalent, and suggested that this was probably a consequence of the different types of air pollutants predominating in West and East Germany (12). Recent studies from Germany have demonstrated that while the prevalence of atopic diseases has not altered significantly in Hamburg (West Germany), there has been a significant increase in the prevalence of these diseases in Erfurt (East Germany), suggesting that there is a converging tendency of self-reported asthma and asthma symptoms (13). Overall, however, the differences in the respiratory conditions have largely disappeared since the unification of East and West Germany, and it has been suggested that this is probably the result of the change both in air-pollution patterns and living habits in the former East Germany (14).

More recent studies suggest that indoor environment is probably an important factor in the increased incidence of allergic disease, since we spend as much as 90% of our time indoors (15). von Mutius et al. have investigated the association between skin test reactivity and methods of heating and cooking in the homes of over 5000 school-children in West Germany, and demonstrated that the prevalence of atopy and hay fever was significantly higher in children living in homes with gas ovens, oil furnaces, and central heating than in

children living in homes where coal or wood was used for heating or cooking, indicating the possible deleterious effects of gas- and oil-derived air pollutants (16). Similarly, Luczynska et al. have investigated skin sensitivity to a panel of indoor and outdoor allergens in 10–11-year-old school-children living in Tower Hamlets and Eltham, two areas of London segregated on the basis of their socioeconomic status and traffic pollution, and demonstrated that the prevalence of skin sensitivity to house-dust mite, grass pollen, and cockroach allergen was higher in Tower Hamlets, the more deprived and polluted of the two areas (17).

Studies by Wallis and colleagues have suggested that certain meteorologic factors may also play a role in the pathogenesis of allergic airways diseases such as asthma (18, 19). These authors have investigated the clinical and immunologic characteristics of 640 patients attending the accident and emergency departments of several hospitals for treatment of acute asthma attacks or other airways disease after a thunderstorm on 24 June 1994 in London, and demonstrated that 283 of the 640 patients (44%), who were not known to be asthmatic, experienced asthma attacks. Additionally, 12 of the 15 patients in whom serum specific IgE was measured demonstrated a very high RAST score of 4–6 against grass pollen mix. Knox has speculated that epidemics of asthma following thunderstorms are likely to be due to release into the atmosphere of pollen allergens, which are associated with respirable starch granules (0.5–2.5 μm in diameter), after osmotic rupture of the pollen grains in the rainfall (20).

Laboratory studies of effects of exposure to air pollutants on lung function

Laboratory-based studies have shown inconsistent effects of NO_2 exposure on lung function and nonspecific bronchial responsiveness. In one study, NO_2 at low concentration (400 ppb) did not affect lung function in normal subjects but caused bronchoconstriction and increased bronchial responsiveness in asthmatics (21). In contrast, numerous studies have agreed in finding significant effects of O_3 in both asthmatics and nonasthmatics, who appear to be equally sensitive to the effects of this pollutant gas (5). Studies of O_3 inhalation have also demonstrated that this agent produces concentration- and exposure time-related changes in symptoms and lung function, including an increase in airway resistance, a reduction in lung volumes, and an increase in airway responsiveness to bronchoconstrictor agents (5). In a recent study, Seal et al. (22) investigated the lowest O_3 concentrations and the minimum exposure time required to cause

significant changes in either pulmonary function or respiratory symptoms in healthy male volunteers exposed to 0–240 ppb O_3 for 0–6.8 h. Although cough was induced at a minimum concentration of 180 ppb O_3 , significant adverse changes in FEV_1 were induced at a minimum concentration of 240 ppb O_3 . Furthermore, a minimum exposure time of 6.8 h to O_3 was required to see these changes. Other studies have demonstrated that there is rapid adaptation to continuing exposure to ozone (23).

Studies involving inhalation of sulphur dioxide (SO_2) have demonstrated that this gas also leads to bronchoconstriction in both normal healthy and asthmatic subjects, and that deep breathing and intermittent exercise may potentiate this effect (6). Although the response to SO_2 inhalation is variable in subjects, concentrations of SO_2 that have little or no effect on normal, healthy subjects can produce marked symptomatic bronchoconstriction in patients with asthma.

Some studies have demonstrated that the effects of SO_2 on lung function are potentiated by prior exposure to either NO_2 (24) or O_3 (25), and therefore suggest that exposure to combinations of pollutants is likely to be more harmful than exposure to individual pollutants.

Influence of air pollution on airway responsiveness to inhaled allergen

Recent studies have indicated that exposure to pollutants such as O_3 , NO_2 , and the combination of NO_2 and SO_2 may increase the airway responsiveness of asthmatics to inhaled allergen. Molfino et al. (26) demonstrated that prior exposure for 1 h to 120 ppb O_3 increased the bronchial response of mild asthmatics to inhaled ragweed allergen. These authors found that the mean provocation dose of allergen required to decrease the forced expiratory volume in 1 s (FEV_1) by 15% ($PC_{15}FEV_1$) was significantly reduced to approximately half the provocation dose of allergen required when the subjects were pre-exposed to O_3 , compared with pre-exposure to air. Although this was the first study to suggest that the interaction between an air pollutant and allergens may be of much importance in the exacerbation of airway disease in susceptible subjects, others have not been able to confirm the findings of Molfino et al. with a similar study protocol.

Ball et al. (27) have exposed 15 asthmatics at rest for 1 h to either air or 0.12 ppm O_3 , in a randomized manner, and evaluated the effect of this exposure on the airway response of these subjects to inhaled allergen. Contrary to the findings of Molfino et al., these authors demonstrated that pre-exposure to O_3 did not significantly decrease the concentration

of allergen required to elicit a 15% decrement in FEV_1 (PC_{15}), compared with pre-exposure to air. These authors have suggested that, although the difference in their findings from those of Molfino et al. may be a consequence of methodologic differences and differences in the subject groups used, it is more likely to be a consequence of repeated exposure to allergen, resulting from insufficient randomization, before exposure to air and O_3 . Similarly, preliminary investigations by Chen et al. (28) have demonstrated that exposure for 1 h to a slightly increased concentration of 0.2 ppm O_3 did not significantly alter the airway response to inhaled allergen in exercising allergic asthmatics.

However, more recent studies by Jörres et al. (29) have demonstrated that prior exposure for a longer period and to a higher concentration of O_3 did indeed increase the airway responsiveness to inhaled allergen/methacholine in allergic subjects. These authors exposed groups of intermittently exercising allergic asthmatics, allergic rhinitics without asthma, and healthy subjects for 3 h to 250 ppb O_3 or filtered air, in a randomized manner, followed by challenge with doubling concentrations of methacholine or allergen, until the FEV_1 dropped by 20% from baseline. Exposure to O_3 itself significantly decreased the lung function in all groups of subjects, as indicated by decreased baseline FEV_1 , compared with exposure to filtered air. Pre-exposure for 3 h to 250 ppb O_3 also increased the bronchial responsiveness to allergen in both the asthmatic and rhinitic subjects, as indicated by significant decreases in the amounts of allergen required to reduce the mean FEV_1 by 20% from baseline, compared with pre-exposure for 3 h to filtered air. Furthermore, pre-exposure to O_3 significantly increased the airway responsiveness to methacholine in the asthmatic patients, but not the patients with rhinitis nor the healthy subjects (29).

Employing a slightly different experimental format, Tunnicliffe et al. (30) have investigated the effect of randomized exposure at rest for 1 h to either air, 100 ppb NO_2 , or 400 ppb NO_2 , followed by challenge with a predetermined dose of house-dust-mite (HDM) allergen required to produce a 15% fall in FEV_1 , in mild asthmatics. Although exposure to 400 ppb NO_2 did not significantly alter the baseline FEV_1 in these subjects, it did significantly increase the airway response to inhaled allergen during both the immediate and late phase, when compared with exposure to air.

We have investigated the effect of randomized exposure for 6 h to either air, 400 ppb NO_2 , 200 ppb SO_2 , or a combination of the two pollutants, followed by inhalation of increasing concentrations of *Dermatophagoides pteronyssinus* allergen, on lung function and airway responsiveness in nonexercising,

mildly asthmatic patient volunteers (31). Compared with exposure to air, exposure to NO₂, SO₂, or the combination of the two pollutants did not significantly alter either FEV₁ or forced vital capacity (FVC). Although exposure to NO₂ and SO₂ decreased the mean allergen concentration required to cause a 20% fall in FEV₁ (PD₂₀FEV₁) by 41.2% and 32.2%, respectively, these were not significantly different, compared with exposure to air. In contrast, exposure to the combination of the two pollutants significantly decreased the mean allergen PD₂₀FEV₁ by 60.5%, compared with exposure to air.

In view of the epidemiologic evidence that the effects of air pollutants may lag by 1–2 days, we investigated whether such a lag effect may be reproducible under strictly controlled laboratory conditions, studying the time course over which the airway response-enhancing effects of pollutants may persist (32). Nonexercising, mildly asthmatic patient volunteers were exposed in randomized manner for 6 h to either air or 400 ppb NO₂ plus 200 ppb SO₂. After exposure to air, the subjects underwent challenge immediately with *D. pteronyssinus* allergen as above. After exposure to 400 ppb NO₂ plus 200 ppb SO₂, they underwent randomized allergen challenge either immediately, or 24 or 48 h later. Exposure to the combination of NO₂ and SO₂ significantly decreased the mean allergen PD₂₀FEV₁ by 37.0%, 63.0%, and 49.0% immediately and 24 and 48 h after exposure, respectively, compared with exposure to air. Additionally, the mean allergen PD₂₀FEV₁ at 24 h after exposure was significantly lower than that immediately after exposure to the pollutant mixture, suggesting that the enhanced airway response to inhaled allergen in asthmatic subjects resulting from exposure to pollutants was likely to lag over a period of 24–48 h and to be maximal 24 h after exposure.

Putative mechanisms underlying increased airway responsiveness to inhaled allergen after exposure to pollutants

Although there is only circumstantial evidence to suggest a link between an increase in the prevalence of allergic airway disease and an increase in air pollution, there is little doubt that exposure to certain air pollutants enhances the airway response to inhaled allergens in susceptible subjects. However, the mechanisms underlying these effects are not fully understood.

Some studies have suggested that air pollutants may promote sensitization and subsequent development of allergic disease, by modulating the allergenicity of airborne allergens. Behrendt et al. have demonstrated that pollen collected from roadsides

with heavy traffic and other areas with high levels of air pollution were covered with large numbers of airborne particulates ($\leq 5 \mu\text{m}$ in size), and that incubation of pollen for 2–5 h in aqueous solutions prepared from these particulates led to morphologic alterations in pollen and extravasation of allergens with altered antigenicity (33). Similarly, Thomas et al. have studied the effect of exposure for 4 h to 50–200 ppb NO₂ on viability, germination, and protein release from freshly collected birch, rye, alder, and hazel pollens, and demonstrated that exposure to 100 ppb NO₂ adversely affected the ability of all species of pollen to germinate (34). The detrimental effect on germination was most pronounced for alder pollen (reduction of 55%). The viability of alder and hazel pollens was also reduced, and the release of protein from the various pollens was enhanced maximally by exposure to 100 ppb NO₂. More recently, Knox et al. have investigated the interaction between respirable diesel exhaust particles (DEP; 30–60 nm diameter) and the purified ryegrass pollen allergens Lol p 1 and Lol p 5, and demonstrated that Lol p 1 binds strongly to the DEP (35). Collectively, these studies suggest that air pollutants may modulate the allergenicity of the pollen allergens and also act as environmental triggers for exacerbation of allergic airway disease, particularly during episodes of increased air pollution in the pollen season.

Human and animal studies have suggested that pollutant exposure may increase the synthesis of IgE (36–39). A study of 363 healthy, nonatopic children under the age of 12 years demonstrated that the degree of air pollution in the areas where these children lived was significantly correlated with an increase in their mean total serum IgE levels (40). Diaz-Sanchez et al. have investigated the effects of nasal challenge with 0.30 mg DEP on the local immune response of healthy volunteers, and have shown that this led to a four- to fivefold increase in the amounts of IgE, but not IgG, IgA, or IgM, measured in the nasal lavage (NAL) of these subjects, 4 days after challenge (41). Additionally, these authors demonstrated that the number of IgE-secreting cells and the levels of epsilon mRNA coding for specific IgE proteins were increased approximately 20–25-fold after challenge with DEP.

Recently, these authors investigated the effect of DEP on the synthesis of cytokines known to influence the production of IgE and lead to allergic reactions in the nasal mucosa of subjects allergic to ragweed allergen (42). These studies have demonstrated that the levels of mRNA for interleukins (IL)-2, -4, -5, -6, -10, and -13 and interferon-gamma (IFN- γ) were increased and readily detectable in

the nasal mucosal cells of these subjects 18 h after intranasal challenge with DEP (42). Interestingly, the enhancement in cytokine mRNA after challenge with DEP was considerably greater than that noted after allergen challenge alone, and the levels of mRNAs for IL-4, -5, -6, -10, and -13 were increased even further when allergen and DEP challenges were applied simultaneously. In contrast, the levels of IL-2 were unchanged, while the levels of IFN- γ mRNA were decreased, suggesting that DEP upregulated the activity of TH2-like lymphocytes. More recently, these authors have investigated the ability of DEP to act as an adjuvant to antigen in ragweed-sensitive subjects undergoing nasal provocation challenges with either DEP, the ragweed allergen Amb a 1, or a combination of DEP and Amb a 1 (43). Nasal washes were performed 18 h, as well as 4 and 8 days, after challenge and analysed for total and ragweed-specific IgE. Challenge with ragweed allergen led to an increase in both total and ragweed-specific IgE in the nasal lavage fluid. Although challenge with DEP also led to an increase in total, but not ragweed-specific, IgE, a combined challenge with DEP and ragweed allergen increased the levels of ragweed-specific IgE 16-fold, compared to levels seen after ragweed challenge alone.

In vitro studies by the same group of authors have demonstrated that the effects of DEP may, at least partly, be a result of the polyaromatic hydrocarbons (PAHs) present on the DEP (44, 45). Takenaka et al. have investigated the effect of PAHs extracted from DEP on IgE production in human peripheral blood mononuclear cells (PBMCs) or purified tonsillar B cells. These authors demonstrated that, although PAH extract itself did not induce *de novo* IgE synthesis by either cell type pretreated with either anti-IL-4 or anti-CD40 monoclonal antibody (mAb), the extract enhanced the IgE production of these cells by 20–360% when the cells were treated simultaneously with IL-4 and CD40, suggesting that PAHs were possibly acting by modifying on-going transcriptional programs related to IgE production, rather than inducing such programs *de novo* (45). Similarly, animal studies have demonstrated that specific IgE to ovalbumin and platinum salts is increased compared to air when the animals are exposed to O₃ (37, 38) or DEP (39).

Sato et al. have investigated the effects of airborne particulates collected from residential and industrialized areas of São Paulo, Brazil, on mutagenicity in *Salmonella* (46). These authors demonstrated that particulates collected from residential areas of São Paulo, where vehicle emissions are the major pollution source, were much more mutagenic to the *Salmonella* than the particulates collected

from the industrialized areas of São Paulo. Similarly, Osornio-Vargas et al. have investigated the effect of PM₁₀ samples collected from different parts of Mexico City on the viability of human lung fibroblast cultures *in vitro* (47). Preliminary data from these studies have demonstrated that the PM₁₀ collected from the more industrialized northern part of Mexico City contained more Cu, V, Zn, and Pb and were more cytotoxic than PM₁₀ collected from the less industrialized but more traffic-congested areas of central and southern Mexico City. These authors suggest that the increased cytotoxicity of PM₁₀ from northern Mexico City was probably a result of the increased transition metal load of these PM₁₀.

Several studies have suggested that pollution-induced airway epithelial damage and impaired mucociliary clearance may allow easier penetration and access of inhaled allergens to cells of the immune system. Studies investigating the pathophysiologic effects resulting from inhalation of O₃ have demonstrated that this agent leads to epithelial damage and an increased inflammatory response in the upper and lower airways, as indicated by leakage of lactate dehydrogenase, albumin, and total protein, and increase in neutrophils, eosinophils, mononuclear cells, fibronectin, α -1-antitrypsin, IL-6 and -8, granulocyte/monocyte colony-stimulating factor (GM-CSF), and prostaglandin E₂, in NAL, proximal airway lavage (PAL), and bronchoalveolar lavage (BAL) (48–50). Basha et al. have demonstrated that, although there were no significant differences in spirometric values and symptom scores between asthmatics and healthy volunteers, after exposure for 6 h to 200 ppb O₃, there were significant increases in IL-6, IL-8, and neutrophil numbers in BAL fluid obtained 18 h after exposure in asthmatics only (51). These studies suggest that O₃ may preferentially increase the production of cytokines and inflammatory cells in asthmatics, possibly leading to an acute exacerbation at a later stage.

Sandström et al. have investigated the effects of NO₂ inhalation in healthy, nonsmoking, and lightly exercising subjects and demonstrated that this agent increases the numbers of lymphocytes, lysozyme-positive alveolar macrophages, and mast cells in BAL (52). More recently, this group demonstrated that exposure for 20 min to 1.5–3.5 ppm NO₂ significantly reduced the mucociliary activity in healthy, nonsmoking volunteers (53). Peden et al. have investigated the effect of prior exposure for 2 h to either air or 400 ppb O₃ on subsequent allergen-induced changes in the nasal mucosa of perennially allergic asthmatic patients, and demonstrated that exposure to O₃ significantly increased the allergen-induced release of eosinophil cationic

protein (ECP) in the NAL of these subjects, without significantly affecting the numbers of eosinophils, 4 h after allergen challenge (54). These results suggest that exposure to O_3 may "prime" the eosinophils to subsequent activation by inhaled allergen. Similarly, we have exposed seasonal allergic rhinitics for 6 h to either 400 ppb NO_2 or air with or without allergen challenge following 30 min after exposure, and have evaluated the changes in nasal airway resistance (NAR) and presence of inflammatory mediators in NAL (55). Although exposure to NO_2 alone did not significantly alter the NAR or increase the concentration of ECP, tryptase, or myeloperoxidase in NAL, exposure to NO_2 before allergen challenge significantly increased the concentration of ECP, but not tryptase or myeloperoxidase, compared with exposure to air. Our findings agree with those of Peden et al. (54), and suggest that acute exposure to NO_2 may also "prime" the eosinophils for subsequent activation by allergen in seasonal allergic rhinitics.

To investigate whether treatment with steroids can alter the inflammatory response in the nasal airways under these conditions, we have studied seasonal allergic rhinitics randomized to receive either topical fluticasone propionate (FP) aqueous nasal spray 200 μ g once daily or matched placebo for 4 weeks in a double-blind, crossover design (56). Analysis of ECP in lavage samples of these subjects, demonstrated that this was significantly increased after exposure to NO_2 plus allergen when the subjects were treated with placebo. In contrast, there was a much smaller effect of exposure to NO_2 plus allergen challenge on ECP levels when these subjects were treated with FP. The difference in changes of ECP levels between placebo and FP treatments was significant, suggesting that FP may downgrade NO_2 plus allergen-induced eosinophil activation in allergic rhinitics.

More recently, we have investigated the effect of prior exposure to O_3 on allergen-induced inflammatory cell changes in the lower airways of mild asthmatics randomized to receive either inhaled FP 500 μ g b.d. or matched placebo for 4 weeks (57). After treatment, subjects in both groups underwent randomized exposure for 1 h to either air or 120 ppb O_3 , followed by bronchoscopy and saline challenge in the right upper lobe anterior segmental orifice and allergen challenge in the right middle lobe medial segmental orifice. Bronchoscopy was repeated after 24 h, and bronchial biopsies were obtained from the challenge sites. After a 4-week washout period, treatment with FP or placebo was repeated, and biopsy samples were obtained after prior exposure for 1 h to the alternate atmosphere of air or 120 ppb O_3 . Immunohistochemical analysis of the biopsy tissue

demonstrated that prior exposure for 1 h to O_3 did not significantly increase the numbers of neutrophils, or EG1- or EG2-staining cells, when compared with exposure to air. However, allergen challenge after exposure to either air or ozone significantly increased the numbers of EG2-staining cells in the placebo-treated group, an effect that was markedly reduced in patients receiving FP.

Although there are no equivalent inhalation studies investigating the effects of respirable particulates in man, preliminary studies in rats and mice have demonstrated that exposure by inhalation for 30 min to concentrations of 10^5 to 5×10^5 ultrafine particles/cm³ can lead to

- 1) acute pulmonary inflammation
- 2) severe haemorrhagic pulmonary oedema with increased numbers of PMNs in lung lavage samples collected within 4 h of exposure
- 3) significantly increased concentrations of IL-1 α , IL-6, tumour necrosis factor- α (TNF- α), inducible nitric oxide synthase, and manganese superoxide dismutase, in the lung lavage samples (58).

Additionally, these studies demonstrated that the ultrafine particles could be translocated to epithelial, interstitial, and endothelial sites showing marked cell membrane injuries and cell necrosis. It has been proposed that the ultrafine particles are inhaled as singlet particles and are deposited in the alveolar region of the lung, where, rather than being phagocytized by alveolar macrophages, they penetrate into and interact with the alveolar epithelial, interstitial, and endothelial cells. This leads to the release of proinflammatory mediators and subsequent infiltration by large numbers of activated PMNs, which contribute further to oxidative lung injury.

Putative role of airway epithelial cells in the development of pollution-induced airway disease

There is increasing evidence that airway epithelial cells play a pivotal role in the pathogenesis of allergic airways disease, since they can express and synthesize various inflammatory cytokines and adhesion molecules, including IL-1 β , IL-6, IL-8, IL-16, GM-CSF, TNF- α , regulated on activation, normal T-cell-expressed and -secreted (RANTES), and intercellular adhesion molecule-1 (ICAM-1). All of these influence the activity of eosinophils and lymphocytes, which play important roles in the allergic reaction (59–63). In combination, RANTES, IL-8, and GM-CSF are chemoattractant for eosinophils, and GM-CSF plays an important part in delaying the apoptosis of eosinophils. Studies from

our laboratory have recently shown that exposure of human bronchial epithelial cells to 400–800 ppb NO_2 , *in vitro*, leads to increased epithelial permeability and damage of these cells, decreased ciliary activity, and release of proinflammatory mediators, including leukotriene (LT) $_4$, GM-CSF, TNF- α , and IL-8 (64, 65). Similarly, Rusznak et al. have demonstrated that exposure of these cells for 6 h to ambient concentrations of 10–50 ppb O_3 , well below the WHO safety guidelines, induced significant release of IL-8, GM-CSF, TNF- α , and soluble (s)ICAM-1, and that the release of GM-CSF, TNF- α , and sICAM-1 could be blocked by treatment of the cells with 10^{-5} M nedocromil sodium (66). Additionally, these authors demonstrated that exposure to O_3 led to significant epithelial cell damage at concentrations above 100 ppb O_3 . More recently, Luchtel et al. have investigated the effect of exposure for 3 h to 300 ppb O_3 on the synthesis of extracellular matrix molecules in monkey nasal epithelial cell cultures, and demonstrated that the mRNA levels for tenascin and decorin were increased 1.4- and 1.6-fold, respectively, 24 h after exposure to O_3 , compared with exposure to air (67). These studies suggest that air pollutants may influence epithelial integrity also by modulating the expression and synthesis of cell structural proteins.

More recently, we have hypothesized that air pollutants, such as DEP and cigarette smoke, facilitate the development of atopy by compromising the barrier function of the epithelium. This would lead to

- 1) decreased clearance of allergens as a result of impaired ciliary activity
- 2) increased access of the allergen to the intra- and subepithelially resident immunocompetent cells, such as Langerhans' cells and T and B cells, due to increased permeability of the epithelium.

It is likely that increased interaction between the allergen and the cells of the immune system leads to synthesis of specific proinflammatory mediators and allergen-specific IgE, and this effect leads, in turn, to sensitization of the airways. To test this hypothesis, we have investigated the effect of incubation with 10–100 $\mu\text{g}/\text{ml}$ DEP (mean aerodynamic diameter 0.4 μm) on cultured human bronchial epithelial cells, and demonstrated that this pollutant significantly attenuated the ciliary beat frequency of these cells and increased the release of IL-8, GM-CSF, and sICAM-1 from these cells (68). Similarly, we have also investigated the effect of prior exposure for 20 min to cigarette smoke, in the absence or presence of 300 ng/ml Der p, on the permeability of confluent human bronchial epithelial cell cultures and the passage of

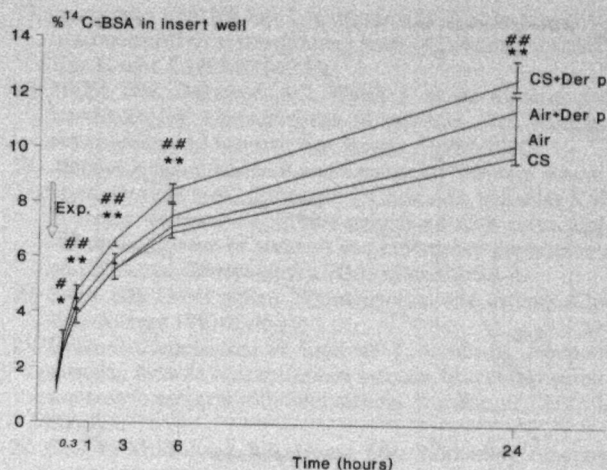


Fig. 1. Effect of 20-min exposure to cigarette smoke (CS), in absence or presence of 300 ng/ml Der p, on passage of ^{14}C -BSA (bovine serum albumin) across confluent human bronchial epithelial cell cultures over period of 24 h ($n=12$ at each time point; * $P<0.005$, ** $P<0.001$, CS + Der p vs air + Der p; # $P<0.005$, ## $P<0.001$, CS + Der p vs CS).

Der p across these epithelial cultures (69). These studies demonstrated that, although exposure to cigarette smoke alone did not significantly alter the permeability of the epithelial cell cultures, when compared with exposure to air, it did significantly enhance the Der p-induced permeability of the epithelial cell cultures (Fig. 1). Additionally, the transepithelial migration of the Der p itself was significantly increased by prior exposure to cigarette smoke (Fig. 2), suggesting that, although short-term exposure to cigarette smoke may not increase nonspecific epithelial permeability, it may render the epithelium more susceptible to the adverse effects of allergens such as Der p.

However, recent studies have demonstrated that there are differences in the ability of epithelial cells of atopic and nonatopic subjects to synthesize different amounts and and/or profiles of proinflammatory cytokines, and these studies suggest that genetic predisposition and manifestation of the symptoms of allergic airway disease in atopic subjects may, at least in part, be a consequence of these differences.

Studies of epithelial cells cultured from nasal tissue of nonatopic, nonrhinitic subjects, patients with allergic rhinitis, and patients with nasal polyps have demonstrated that the epithelial cells from rhinitis and subjects with nasal polyps synthesize significantly greater quantities of GM-CSF and IL-8 than the cells of healthy, nonatopic, nonrhinitic subjects (70, 71). More recently, we have demonstrated that epithelial cells cultured from nasal biopsies of atopic nonrhinitic and atopic rhinitic patients release significantly greater amounts of

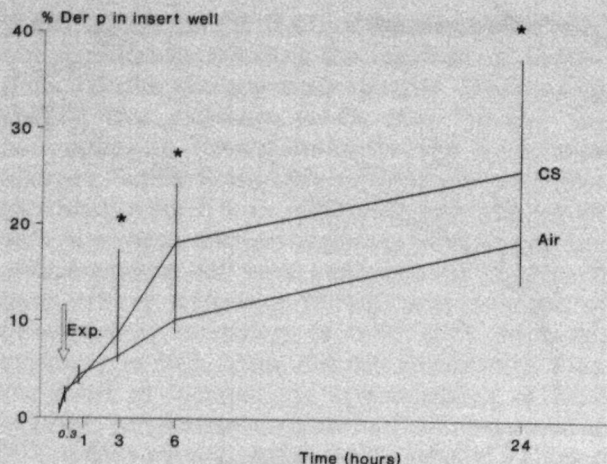


Fig. 2. Effect of 20-min exposure to cigarette smoke (CS) on passage of Der p across confluent human bronchial epithelial cell cultures over period of 24 h ($n=12$ at each time point, $*P<0.05$, CS + Der p vs air + Der p).

IL-8, GM-CSF, and TNF- α than the epithelial cells from nonatopic, nonrhinitic volunteers (72), and that this release of cytokines, particularly RANTES, is significantly enhanced during the pollen season (73). Additionally, our studies have demonstrated that the cells of atopic rhinitics are also significantly more susceptible to the effects of pollutants, such as O_3 and NO_2 , compared with exposure to air, during the pollen season, than cells of these subjects outside the pollen season (Fig. 3).

Similarly, studies of bronchial epithelial cells have demonstrated that the cells of asthmatic patients synthesize greater quantities of IL-1 β , IL-16, GM-CSF, and MCP-1 than the cells of nonasthmatic subjects (73–76), *in vivo*, and that the expression of GM-CSF and IL-16 correlates well with the numbers of activated eosinophils (77) and CD4 $^+$ cells (76), respectively. Preliminary studies of epithelial cells cultured from bronchial biopsies of well-characterized groups of asthmatic and nonasthmatic subjects in our laboratory have also demonstrated that the bronchial epithelial cells of atopic asthmatics release significantly greater amounts of constitutive IL-8, GM-CSF, RANTES, and sICAM-1 than the cells of nonatopic nonasthmatics (78). As we found for the nasal epithelial cells of atopic rhinitic and nonatopic, nonrhinitic subjects, the cells of atopic asthmatics also release significantly more RANTES, and are also more susceptible after exposure to O_3 and NO_2 , compared with exposure to air, than the cells of nonatopic nonasthmatics (Fig. 4).

Collectively, these studies suggest that genetic predisposition to and manifestation of the symptoms of allergic airway disease in atopic subjects may, at least in part, be a consequence of increased expression, synthesis, and release of specific pro-

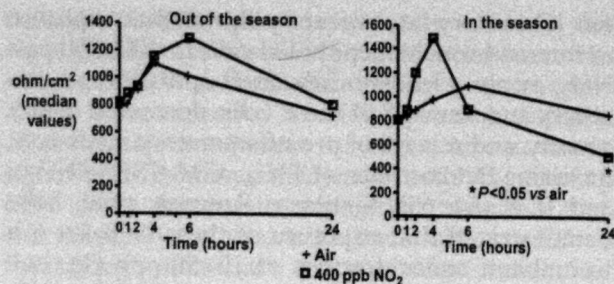


Fig. 3. Effect of exposure for 6 h to NO_2 on electrical resistance of nasal epithelial cells cultured from patients with atopic rhinitis, outside and during pollen season ($n=10$ at each time point).

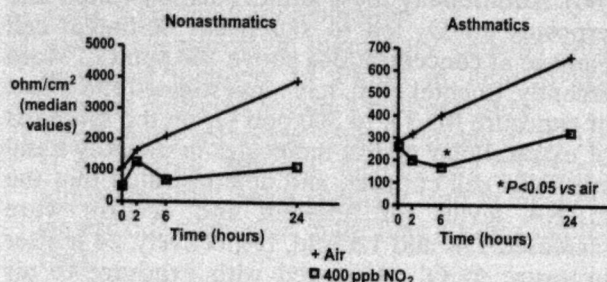


Fig. 4. Effect of exposure for 6 h to NO_2 on electrical resistance of bronchial epithelial cells cultured from nonatopic, nonasthmatic subjects and atopic asthmatic patients ($n=8$ at each time point).

inflammatory mediators from their airway epithelial cells, both constitutively and after exposure to external factors such as allergens and pollutants.

Summary

Although epidemiologic evidence suggests that there may be a link between increased air pollution resulting from excessive use of liquid petroleum and an increase in exacerbations of allergic airway disease, there is little direct evidence to substantiate this. Recent, laboratory-based studies have demonstrated that exposure to pollutants such as O_3 and NO_2 with or without SO_2 can enhance the airway response of particularly susceptible subjects, such as asthmatics and rhinitics, to inhaled allergen. Although such studies cannot simulate exactly the meteorologic/atmospheric conditions under which natural exposure to pollutants may occur, and which possibly lead to different responses in the airways of asthmatics and rhinitics, they do provide an invaluable means of elucidating the putative mechanisms underlying these changes. Studies investigating the effects of pollutants at the cellular and subcellular levels have suggested that various cells are likely to be involved and will interact with one another to determine the overall inflammation and airway hyperresponsiveness observed in allergic

airways disease. At the molecular level, the cell-cell and irritant/allergen-cell interactions lead to the synthesis of proinflammatory mediators, including cytokines and cell-adhesion molecules, which modulate the activity and function of the inflammatory cells (eosinophils, mast cells, and neutrophils) and immunocompetent cells (such as Langerhans' cells, T cells, and B cells). In turn, these cells, in combination, lead to an overall increase in sensitization and incidence of allergic disease.

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