

# Mechanisms of pollution-induced airway disease: *in vitro* studies in the upper and lower airways

Devalia JL, Bayram H, Rusznak C, Calderón M, Sapsford RJ, Abdelaziz MA, Wang J, Davies RJ. Mechanisms of pollution-induced airway disease: *in vitro* studies in the upper and lower airways. Allergy 1997; 52 (suppl 38): 45–51. © Munksgaard 1997.

Evidence from both epidemiological and laboratory-based studies suggests that increased exposure to liquid petroleum and gas-derived air pollutants [nitrogen dioxide (NO<sub>2</sub>), ozone, and respirable particulate matter] may play a role in the clinical manifestation of both allergic and non-allergic airway disease. The mechanisms and cell types involved in pollutant-mediated effects in the airways, however, are not clear. *In vitro* studies have suggested that human fibroblasts, B-lymphocytes, alveolar macrophages, and epithelial cells/cell lines may be involved. Studies of fibroblasts and macrophages have demonstrated that exposure to ozone results in decreased cell viability and increased release of pro-inflammatory mediators from macrophages. Similarly, studies of B-lymphocytes have demonstrated that exposure to diesel exhaust particles (DEP) enhances the synthesis of immunoglobulin E by these cells. The airway epithelial cells have received the greatest attention in mechanistic studies of air pollution-induced airway disease and suggest that these cells are likely to play a pivotal role in the pathogenesis of airways disease. Various studies have demonstrated that exposure of nasal or bronchial epithelial cells to NO<sub>2</sub>, ozone, and DEP results in significant synthesis and release of pro-inflammatory mediators, including eicosanoids, cytokines, and adhesion molecules. Additionally, evidence suggests that epithelial cells of atopic individuals release significantly greater amounts of cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-6 (IL-6), IL-8, and regulated on activation, normal T-cell expressed and secreted (RANTES), on exposure to NO<sub>2</sub> and ozone. Studies investigating the biological relevance of epithelial cell-derived pro-inflammatory mediators have shown that these enhance eosinophil chemotaxis and eosinophil adherence to endothelial cells, suggesting that pollution-induced inflammation of the airways is likely to be influenced by modulation of epithelial synthesis and release of these mediators.

**J. L. Devalia, H. Bayram,  
C. Rusznak, M. Calderón,  
R. J. Sapsford, M. A. Abdelaziz,  
J. Wang, R. J. Davies**

Academic Department of Respiratory Medicine,  
St Bartholomew's and the Royal London School  
of Medicine and Dentistry, The London Chest  
Hospital, London, UK

Key words: airway epithelial cells; alveolar macrophages; diesel exhaust particles; nitrogen dioxide; ozone.

Dr Jagdish L. Devalia  
Academic Department of Respiratory Medicine  
St Bartholomew's and the Royal London School  
of Medicine and Dentistry  
The London Chest Hospital  
Bonner Road, London E2 9JX, UK

## Introduction

Epidemiological evidence suggests that air pollution resulting from increased use of liquid petroleum and gas in the transport and manufacturing industries and domestic settings, has progressively increased in the last decade. Indeed, levels of the predominant pollutants, including nitrogen dioxide (NO<sub>2</sub>), ozone, and respirable particulates (PM<sub>10</sub>), have occasionally exceeded the European and WHO safety guidelines in London and other major cities in the UK (1–4).

Although epidemiological studies, particularly

from developed countries, suggest that the increased prevalence of allergic disease, such as asthma, may be associated with air pollution resulting from the increased use of liquid petroleum fuel, the findings from these studies are difficult to interpret due to the confounding effects of cigarette smoke, exposure to allergens, meteorological conditions, and socioeconomic factors (5). Additionally, these studies investigated the effects of the major pollutants individually, without taking into account the potential additive and/or synergistic effects of combinations of pollutants, which is of greater relevance.

However, several studies have demonstrated a clear association between exposure to environmental pollutants and exacerbation of respiratory disease (1–4). Studies have shown that symptoms of rhinitis, chronic cough, phlegm, decreased morning peak flow, emergency room visits, and hospitalization due to asthma, are associated with increased exposure to  $\text{NO}_2$  in asthmatic adults and children. Similarly, increased levels of ozone have also been shown to adversely affect lung function in children, and are associated with increased hospital admissions. Studies of respirable particulate matter have demonstrated that higher levels of  $\text{PM}_{10}$  are associated with worsening peak flow, inhaler usage, respiratory symptoms, and emergency room visits in asthmatic children and adults. More recently, in a cohort of over half a million adults residing in 151 metropolitan areas in the USA between 1982–1989, Pope et al. (6) demonstrated that cardiopulmonary and lung cancer mortality was associated with fine particulate air pollution.

Laboratory-based studies have confirmed the findings of the epidemiological studies and have shown that inhalation of specific pollutants, either individually or in combination, adversely affects lung function and bronchial responsiveness in asthmatics. Although studies of  $\text{NO}_2$  exposure have demonstrated inconsistent effects on lung function and non-specific bronchial responsiveness (3), numerous studies have been in agreement with respect to significant effects of ozone in increasing bronchial responsiveness, causing lung function impairment, and inducing cough in asthmatic and non-asthmatic individuals (1). Investigations focusing on sulphur dioxide ( $\text{SO}_2$ ) inhalation, on the other hand, have shown that although this gas also leads to bronchoconstriction in both healthy and asthmatic subjects, the response may vary within individuals, and asthmatics are more sensitive to the effects of this pollutant (2). More recently, we and others demonstrated that pre-exposure of asthmatics and allergic rhinitics for 1–6 h to either ozone,  $\text{NO}_2$ , or a combination of  $\text{NO}_2$  and  $\text{SO}_2$ , increases the airway responsiveness of these individuals to subsequently inhaled allergen (7–10), and this effect persists over a period of 24–48 h (11). Studies of the pathological effects of exposure to pollutants in asthmatics, allergic rhinitics, and healthy individuals show that pollutants such as diesel exhaust,  $\text{NO}_2$ , and ozone lead to epithelial dysfunction and/or damage and induce an acute inflammatory response in the upper and lower airways (12–19). More recently, studies of healthy individuals exposed to purified diesel exhaust particulates (DEP), shown to be an important component of the vehicle exhaust-derived fraction of air pollution, have demon-

strated that this resulted in a four- to five-fold increase in the amount of immunoglobulin E (IgE), but not IgG, IgA or IgM, in the nasal lavage fluid of these subjects, 4 days after challenge (20).

### Putative mechanisms underlying pollution-induced changes in airway function

Although there is little doubt that exposure to liquid petroleum and gas-derived pollutants can enhance the airway response of asthmatics and allergic rhinitics to inhaled allergens, as well as enhancing airway damage and inflammation, the precise mechanisms underlying these effects are not fully understood. Consequently, studies have generally concentrated on the effect of pollutants on specific types of airway cells, including human fibroblasts, B-lymphocytes, alveolar macrophages, and epithelial cells/cell lines *in vitro*.

#### Fibroblasts and B-cells

Human fibroblasts and B-lymphocytes have been employed as model systems in mechanistic investigations of air pollutants, but comparatively few studies on these cell types have been documented. Mayer and Branscheid (21) investigated the effect of ozone exposure on lung fibroblasts, established as primary explant cultures from lung biopsies of healthy individuals. Although exposure for 20 h to 500 ppb ozone caused significant cell death and synthesis of hyaluronan, the mortality of the fibroblasts was increased to an even greater extent by exposure of the cells to 2.5 ppm ozone for a much shorter period of time (4 h). In addition, fibroblasts from different individuals exhibited marked differences in sensitivity to ozone exposure (Fig. 1). Thus, although ozone is likely to have

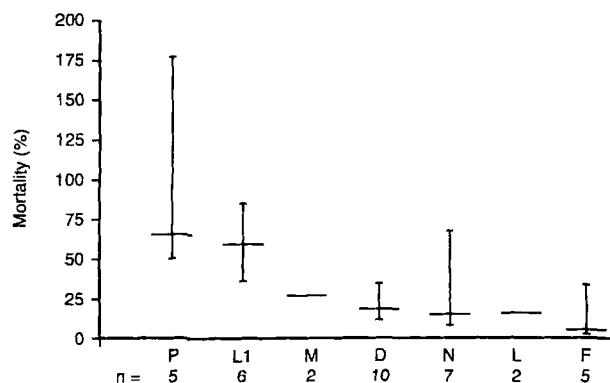


Fig. 1. Variation in resistance to 0.5 ppm ozone for 20 h of fibroblast cultures grown from different individuals. Results are expressed as median and 95% confidence interval (n=number of experiments with single fibroblast lines from each individual). Modified from Mayer and Branscheid (21), with kind permission.

reproducible cytotoxic effects on human lung fibroblasts, the thresholds of cytotoxicity are unlikely to be reproducible because of differences in sensitivity of these cells between different individuals. Kozumbo et al. (22) studied the effect of indirect exposure of fibroblasts to ozone by incubating these cells in a medium containing an arachidonic acid (AA) preparation which had previously been degraded by exposure for 1 h to 400 ppb ozone. The ozonized AA led to DNA single strand breaks, which could be blocked by incubation of the ozonized AA with catalase, but not superoxide dismutase, suggesting that ozone-induced generation of hydrogen peroxide was likely to be involved in this detrimental effect.

Takenaka et al. (23) investigated the effects of exposure to DEP on IgE production from human peripheral blood mononuclear cells or purified tonsillar B-cells, and suggested that its effects are mediated, at least in part, by the polyaromatic hydrocarbons (PAHs), such as phenanthrene, present in DEP. PAH-DEP-induced IgE production from these cell types was enhanced by between 20% and 360% over a period of 10–14 days when the cells were treated simultaneously with both interleukin-4 (IL-4) and CD40. IgE synthesis from either cell type could be prevented by pretreatment with anti-IL-4 or anti-CD40 monoclonal antibody. The authors suggested that PAHs are likely to operate by modifying ongoing transcriptional programs related to IgE production, rather than by inducing such programs *de novo* (23).

### *Alveolar macrophages*

Alveolar macrophages and macrophage-like cells have received greater attention than fibroblasts and B-lymphocytes in studies examining the mechanisms underlying the effects of pollutants, due to both the prevalence of these cells in the human air-space and their contribution in the maintenance of homeostasis and inflammation in the airways. Studies of the putative role of alveolar macrophages in pollution-induced airway disease have concentrated mainly on the effects of ozone, and demonstrated that exposure to this agent leads to synthesis of a variety of pro-inflammatory mediators. Samet et al. utilized dHL60 cells, a human macrophage-like cell line, to investigate the effect of ozone on cell viability, as assessed by release of  $^{51}\text{Cr}$  from pre-labelled cells, and the release of platelet activating factor (PAF) (24). Compared with exposure to filtered air, exposure to all concentrations of ozone between 0.05–1.0 ppm significantly increased the release of PAF after only 15 min, with maximal effect (more than 3-fold increase) after exposure for 2 h to 1.0 ppm

ozone. Although exposure to the lower concentrations did not significantly alter the release of  $^{51}\text{Cr}$ , this was significantly increased after exposure of the cells for 2 h to 0.3–1.0 ppm ozone, compared with exposure to air. More recently, in collaboration with Devlin et al. (25), this group exposed alveolar macrophages, isolated and cultured from bronchoalveolar lavage (BAL) of healthy non-smokers, to 0.1 ppm ozone for 30–60 min. They demonstrated that ozone significantly decreased the viability of these cells by >50%, as indicated by trypan blue exclusion, but it did not affect the release of IL-6, IL-8, or fibronectin from these cells, when compared with exposure to filtered air. Similarly, Arsalane et al. (26) investigated the effect of exposure for 1 h to 0.1–1.0 ppm ozone on macrophages cultured from BAL of smokers. Contrary to the findings of Devlin et al., ozone did not induce any significant cell injury, as indicated by no changes in either the release of lactate dehydrogenase (LDH) from these cells or the adenosine triphosphate content of the cells, at any concentration of ozone studied. However, at 0.4 ppm ozone there was a significant increase in the release of IL-1 $\beta$ , IL-6, IL-8, and tumour necrosis factor- $\alpha$  (TNF $\alpha$ ). Analysis of mRNA in these cultures demonstrated that the expression of cytokine mRNA was also significantly enhanced after exposure to 0.4 ppm ozone compared with exposure to filtered air.

### *Epithelial cells*

The airway epithelial cells, not surprisingly, have been the most widely investigated cells in mechanistic studies of air pollution-induced airway disease, due to their spatial arrangement and predominance in the airways. There is increasing evidence that airway epithelial cells may play a pivotal role in the pathogenesis of airways disease, since they can express and synthesize a variety of inflammatory cytokines and adhesion molecules, including IL-1 $\beta$ , IL-6, IL-8, IL-16, TNF $\alpha$ , granulocyte-macrophage colony stimulating factor (GM-CSF), regulated on activation, normal T-cell expressed and secreted (RANTES), and intercellular adhesion molecule-1 (ICAM-1), which influence the activity of eosinophils and lymphocytes (27–29).

In accordance with the finding of an increased production of PAF by the dHL60 macrophage-like human cell line, Samet et al. (24) demonstrated that, compared with exposure to air, exposure for 1 h to 0.05–1.0 ppm ozone also significantly increased the release of PAF from both primary and cell line (BEAS S6) cultures of human bronchial epithelial cells. Additionally, although the

ozone-induced release of PAF was significantly increased from both the apical and basolateral compartments of the cultures, no significant difference was found with respect to the vectorial release of PAF. Studies of cell viability following exposure to ozone demonstrated that, unlike the dHL60 cells, the BEAS S6 cells were more sensitive to the effects of ozone, in that a lower concentration (0.1 ppm ozone) induced a significant release of  $^{51}\text{Cr}$  from these cells. Devlin et al. (25) have also used the BEAS S6 cell line to investigate the effects of ozone exposure on cell membrane integrity and inflammatory mediators. They demonstrated that exposure for 1 h to 0.1–1.0 ppm ozone led to a dose-dependent toxic effect, as indicated by release of LDH from these cells. At all concentrations of ozone investigated, exposure for 1–4 h also led to a significant release of IL-6, IL-8, and fibronectin, 1–4 h after exposure. In contrast, Dumler et al. (30) investigated the effect of ozone exposure on epithelial cells cultured from human nasal turbinate tissues, and demonstrated that nasal cells are also sensitive to this pollutant, as indicated by a significantly increased release of LDH from these cells, compared with exposure to filtered air. More recently, this group studied the effect of exposure to ozone on the release of AA products from the BEAS S6 cell line, and showed that exposure for 1 h to 0.25–1.0 ppm ozone led to a significant release of leukotriene  $\text{B}_4$  ( $\text{LTB}_4$ ),  $\text{LTC}_4$ ,  $\text{LTD}_4$ , thromboxane  $\text{B}_2$  and prostaglandin  $\text{E}_2$  (31). Studies from our laboratory have also revealed that exposure of human bronchial epithelial cells to 400–800 ppb  $\text{NO}_2$  *in vitro*, led to increased epithelial permeability and cell damage, decreased ciliary activity, and release of pro-inflammatory mediators, including  $\text{LTC}_4$ , GM-CSF,  $\text{TNF}\alpha$  and IL-8 (32, 33). More recently, we have demonstrated that exposure of these cells for 6 h to 0–500 ppb ozone induced significant release of IL-8, GM-CSF,  $\text{TNF}\alpha$ , and soluble ICAM-1 (sICAM-1) at concentrations of 10–50 ppb ozone, and also led to significant epithelial cell damage at concentrations above 100 ppb ozone (34). Furthermore, the ozone-induced release of GM-CSF,  $\text{TNF}\alpha$  and sICAM-1 could be blocked by treating the cells with  $10^{-5}$  M nedocromil sodium, and release of IL-8 could be blocked by treatment with glutathione, a naturally occurring intracellular antioxidant (Fig. 2). The effect of DEP (mean aerodynamic diameter  $0.4\text{ }\mu\text{m}$ ) on cell function of cultured human bronchial epithelial cells has also been recently examined in our laboratory. We found that this pollutant significantly attenuated the ciliary beat frequency (CBF) of these cells, in a dose- and time-dependent manner (35), and led to a significant increase in the release of IL-8, GM-

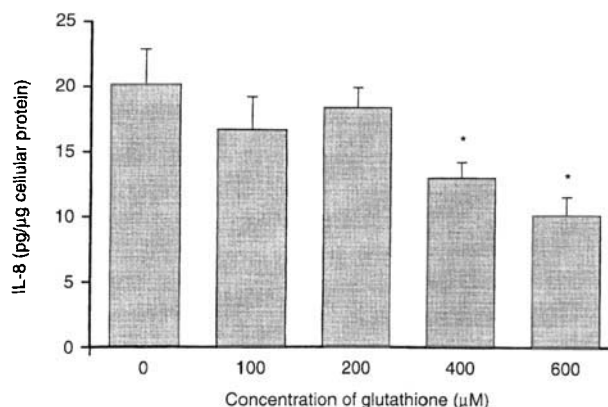


Fig. 2. Effect of 0–600  $\mu\text{M}$  glutathione on 50 ppb ozone-induced release of IL-8 from human bronchial epithelial cells *in vitro*. Results are expressed as mean  $\pm$  SEM ( $n=8$ ; \* $P<0.05$  vs 0  $\mu\text{M}$  glutathione).

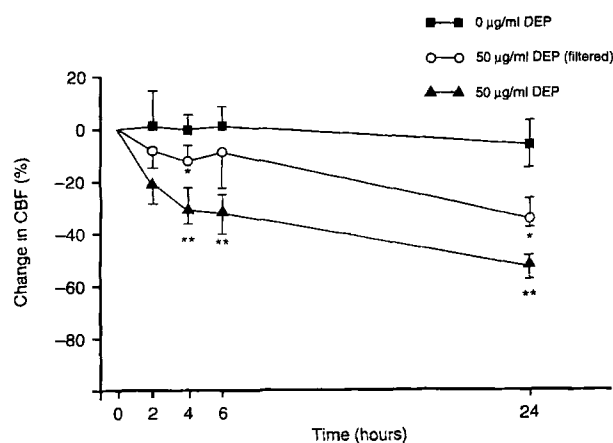


Fig. 3. Effect of exposure for 24 h to filtered or unfiltered solutions of diesel exhaust particulates (DEP) on ciliary beat frequency (CBF) of human bronchial epithelial cells *in vitro*. Results are expressed as median and interquartile values ( $n=6$ ; \* $P<0.05$ , \*\* $P<0.001$  vs 0  $\mu\text{g/ml}$  DEP).

CSF, and sICAM-1 after 24 h. Similarly, incubation of cultures for 24 h at  $37^\circ\text{C}$  in a 5%  $\text{CO}_2$  in air atmosphere in medium which had been prepared by filtering a suspension of 50  $\mu\text{g}$  DEP/ml culture medium through a micropore filter also significantly decreased the CBF of the cells (Fig. 3), and increased the release of IL-8 and sICAM-1 (Fig. 4). These results suggest that compounds adsorbed onto the carbonaceous core of the DEP are likely to be detrimental in the airways.

There is some evidence to support the concept that there may be differences in the epithelial cells of atopic asthmatic and non-atopic non-asthmatic individuals, since the cells of asthmatics produce significantly greater amounts of GM-CSF, macrophage inflammatory protein-1 $\alpha$ , IL-6 and IL-8. Recent studies of epithelial cells cultured from nasal biopsy specimens from atopic rhinitis,

atopic non-rhinitics (patients with atopic eczema), and non-atopic non-rhinitic subjects, in our laboratory, demonstrated that the cells from atopic rhinitic and atopic non-rhinitic subjects generated significantly larger amounts of GM-CSF, TNF $\alpha$ , RANTES, and IL-8, compared with epithelial cells from non-atopic non-rhinitic individuals (36). Further studies of epithelial cells from allergic rhinitics, cultured from tissues obtained during the pollen season, have shown that these cultures release significantly greater quantities of IL-8 and RANTES following exposure for 6 h to either 400 ppb NO<sub>2</sub> or 50–100 ppb ozone, compared with exposure to air (Fig. 5) (37).

Despite increasing evidence for the role of airway epithelial cells in the generation of pro-inflammatory cytokines, there is comparatively little information on the biological relevance of air pollutant-induced release of cytokines in airway inflammation. We have investigated the effect of conditioned medium from bronchial epithelial cell cultures exposed for 6 h to air or 50 ppb ozone

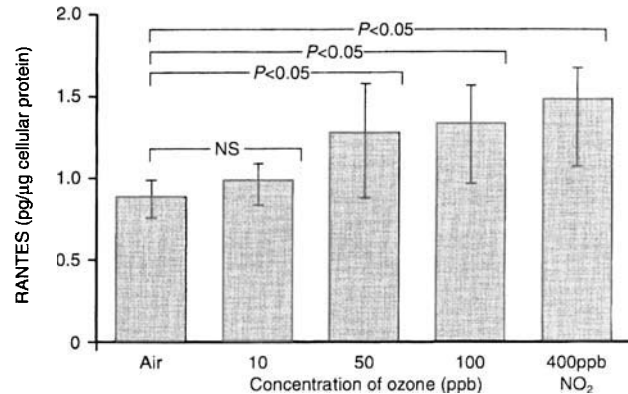


Fig. 5. Effect of exposure for 6 h to 0–100 ppb ozone and 400 ppb NO<sub>2</sub> on release of RANTES from cultured nasal epithelial cells of patients with seasonal allergic rhinitis. Results are shown as median and interquartile values ( $n=9$ ).

on eosinophil chemotaxis and adherence to human endothelial cells. It was demonstrated that the conditioned medium from cultures exposed to ozone significantly increased both eosinophil chemotaxis and adherence to human endothelial cells, when compared with conditioned medium from cells exposed to air. These results suggest that pollution-induced inflammation of the airways may be a consequence of increased synthesis and/or release of epithelial cell-derived mediators which influence the activity of inflammatory cells.

## Conclusions

Taken together, the findings of both epidemiological and laboratory-based studies provide evidence to suggest that exposure to air pollutants generated from petrol and diesel burning engines are likely to precipitate attacks of asthma and rhinitis. Although it is clear that inhalant pollutants can affect the activity of several cell types, including fibroblasts, B-lymphocytes, alveolar macrophages, and airway epithelial cells, the latter are likely to be the cell type most affected, due to their spatial positioning and vital role as the first line of cell defence in the airways. The pollutants may exert their effects at the epithelial barrier directly by:

- (i) Decreasing ciliary activity, increasing epithelial damage and increasing epithelial permeability. This would lead to decreased clearance and increased accessibility of airborne allergens and irritants to the sub-epithelial tissue, where they may react with and activate inflammatory cells, such as mast cells.
- (ii) Depleting naturally occurring anti-oxidants, such as glutathione, which are important in the maintenance of epithelial cell membrane integrity.

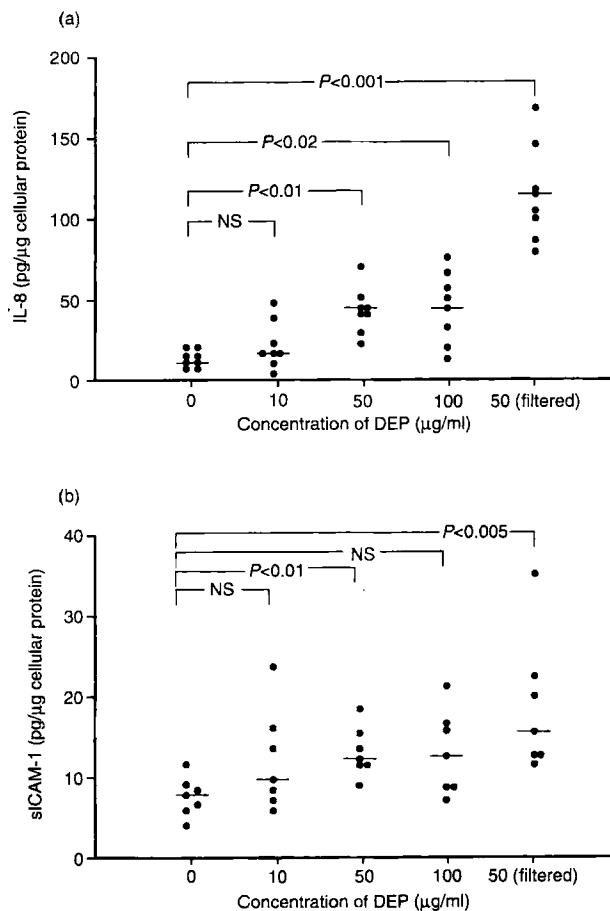


Fig. 4. Effect of exposure for 24 h to 0–100  $\mu\text{g/ml}$  diesel exhaust particulates (DEP) on release of (a) IL-8, and (b) sICAM-1 from human bronchial epithelial cells *in vitro*. Results are shown as median and individual values.

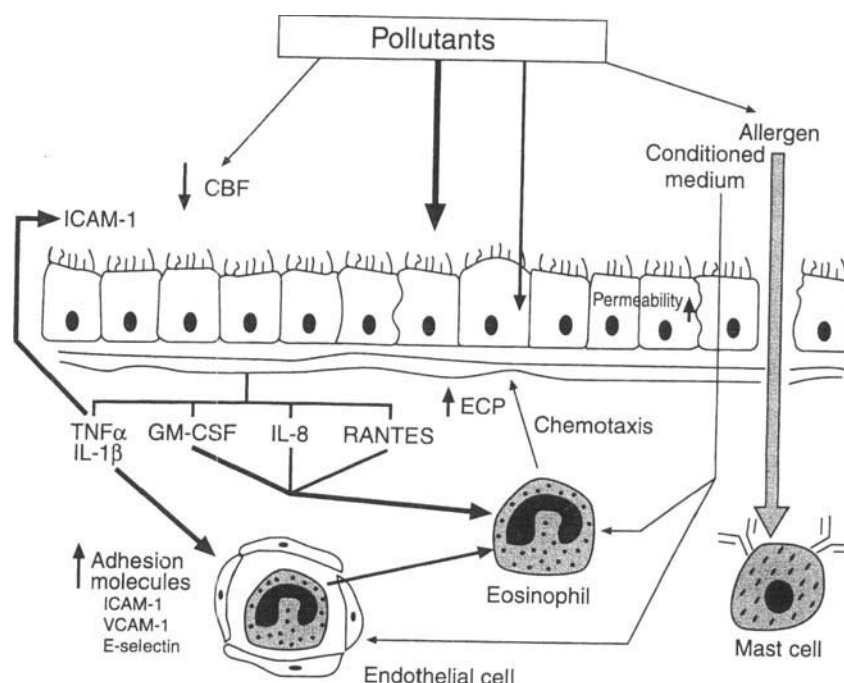


Fig. 6. Schematic view of the putative role of airway epithelium in the development of pollution-induced airway disease.

- (iii) Increasing the release of pro-inflammatory cytokines and cell adhesion molecules, which orchestrate the detrimental functions of inflammatory cells such as eosinophils, mast cells and lymphocytes (Fig. 6).

## References

- Department of Health Advisory Group on the Medical Aspects of Air Pollution Episodes: First Report – Ozone. London: HMSO, 1991.
- Department of Health Advisory Group on the Medical Aspects of Air Pollution Episodes: Second Report – Sulphur dioxide, acid aerosols and particulates. London: HMSO, 1992.
- Department of Health Advisory Group on the Medical Aspects of Air Pollution Episodes: Third Report – Oxides of nitrogen. London: HMSO, 1993.
- Department of Environment Expert Panel on Air Quality Standards: Report – Particles. London: HMSO, 1995.
- Pauli G, Kopferschmitt MC, Spirlet F, Charpin D. Air pollutants and allergic sensitisation. In: Chanez P, Bousquet J, Michel FB, Godard P, editors. From genetics to quality of life. Gottingen: Hogrefe and Huber, 1996:80–9.
- Pope CA 3rd, Thun MJ, Namboodiri MM et al. Particulate air pollution as a predictor of mortality in a prospective study of US adults. *Am J Respir Crit Care Med* 1995; 151:669–74.
- Molfino NA, Wright SC, Katz I et al. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. *Lancet* 1991;338:199–203.
- Jörres R, Nowak D, Magnussen H. Effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. *Am J Respir Crit Care Med* 1996;153:56–64.
- Tunnicliffe WS, Burge PS, Ayres JG. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet* 1994;344: 1733–6.
- Devalia JL, Rusznak C, Herdman MJ, Trigg CJ, Tarraf H, Davies RJ. Effect of nitrogen dioxide and sulphur dioxide on the airway response of mild asthmatic patients to allergen inhalation. *Lancet* 1994;344:1668–71.
- Rusznak C, Devalia JL, Davies RJ. The airway response of asthmatic subjects to inhaled allergen after exposure to pollutants. *Thorax* 1996;51:1105–8.
- Salvi SS, Blomberg A, Rudell B et al. Acute inflammatory response in the airways and peripheral blood after exposure to diesel exhaust in healthy human volunteers. *Eur Respir J* 1996;9(Suppl. 23):415s (abstract).
- Bascom R, Naclerio RM, Fitzgerald TK, Kagay-Sobotka A, Proud D. Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects. *Am Rev Respir Dis* 1990;142:594–601.
- Aris RM, Christian D, Hearne PQ, Kerr K, Finkbeiner WE, Balmes JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. *Am Rev Respir Dis* 1993;148:1363–72.
- Basha MA, Gross KB, Gwizdala CJ, Haidar AH, Popovich J Jr. Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filter purified air. *Chest* 1994;106:1757–65.
- Sandström T, Sternberg N, Eklund A et al. Inflammatory cell response in bronchoalveolar lavage fluid after nitrogen dioxide exposure of healthy subjects: a dose-response study. *Eur Respir J* 1991;3:332–9.
- Helleday R, Hubermann D, Blomberg A, Sterjberg A, Sandström T. Nitrogen dioxide exposure impairs the frequency of the mucociliary activity in healthy subjects. *Eur Respir J* 1995;8:1664–8.
- Peden DB, Setzer RW Jr, Devlin RB. Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of

- perennially allergic asthmatics. *Am J Respir Crit Care Med* 1995;151:1336–45.
19. Wang J, Devalia JL, Duddle JM, Hamilton SA, Davies RJ. The effect of exposure for six hours to nitrogen dioxide (NO<sub>2</sub>) on early phase nasal response to allergen challenge in patients with a history of seasonal allergic rhinitis. *J Allergy Clin Immunol* 1995;96:669–76.
20. Diaz-Sanchez D, Dotson AR, Takenaka H, Saxon A. Diesel exhaust particles induce local IgE production in vivo and alter the pattern of IgE messenger RNA isoforms. *J Clin Invest* 1994;94:1417–25.
21. Mayer D, Branscheid D. Exposure of human lung fibroblasts to ozone: cell mortality and hyaluronan metabolism. *J Toxicol Environ Health* 1992;35:235–46.
22. Kozumbo WJ, Hanley NM, Agarwal S, Thomas MJ, Madden MC. Products of ozonized arachidonic acid potentiate formation of DNA single strand breaks in cultured human lung cells. *Environ Mol Mutagen* 1996;27:185–95.
23. Takenaka H, Zhang K, Diaz-Sanchez D, Tsien A, Saxon A. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: direct effects on B-cell IgE production. *J Allergy Clin Immunol* 1995;95:103–15.
24. Samet JM, Noah TL, Devlin RB et al. Effect of ozone on platelet activating factor production in phorbol-differentiated HL60 cells, a human bronchial epithelial cell line (BEAS S6) and primary human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1992;7:514–22.
25. Devlin RB, McKinnon KP, Noah T, Becker S, Koren HS. Ozone-induced release of cytokines and fibronectin by alveolar macrophages and airway epithelial cells. *Am J Physiol* 1994;266:612–9.
26. Arsalane K, Gosset P, Vanhee D et al. Ozone stimulates synthesis of inflammatory cytokines by alveolar macrophages in vitro. *Am J Respir Cell Mol Biol* 1995;13:60–8.
27. Devalia JL, Davies RJ. Airway epithelial cells and mediators of inflammation. *Respir Med* 1993;87:405–8.
28. Wang JH, Devalia JL, Xia C, Sapsford RJ, Davies RJ. Expression of RANTES by human bronchial epithelial cells in vitro and in vivo and the effect of corticosteroids. *Am J Respir Cell Mol Biol* 1996;14:27–35.
29. Laberge S, Ernst P, Ghaffar O et al. Increase in interleukin-16 (IL-16) expression in baseline asthma. *J Allergy Clin Immunol* 1996;97(1):313 (abstract).
30. Dumler K, Hanley QS, Baker C, Lutche DL, Altman LC, Koenig JQ. The effects of ozone exposure on lactate dehydrogenase release from human and primate respiratory epithelial cells. *Toxicol Lett* 1994;70:203–9.
31. McKinnon KP, Madden MC, Noah TL, Devlin RB. In vitro ozone exposure increases release of arachidonic acid products from a human bronchial epithelial cell line. *Toxicol Appl Pharmacol* 1993;118:215–23.
32. Devalia JL, Sapsford RJ, Cundell DR, Rusznak C, Campbell AM, Davies RJ. Human bronchial epithelial cell dysfunction following exposure to nitrogen dioxide, in vitro. *Eur Respir J* 1993;6:1308–16.
33. Devalia JL, Campbell AM, Sapsford RJ et al. Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells in vitro. *Am J Respir Cell Mol Biol* 1993;9:271–9.
34. Rusznak C, Devalia JL, Sapsford RJ, Davies RJ. Ozone-induced mediator release from human bronchial epithelial cells in vitro and the influence of nedocromil sodium. *Eur Respir J* 1996;9:2298–305.
35. Bayram H, Devalia JL, Sapsford RJ, Ohtoshi T, Davies RJ. Effect of diesel exhaust particulates on the ciliary beat frequency of human bronchial epithelial cells, in vitro. *Eur Respir J* 1995;8:43s (abstract).
36. Calderón MA, Devalia JL, Prior AJ, Sapsford RJ, Davies RJ. A comparison of cytokine profiles generated by epithelial cells cultured from nasal biopsies of atopic rhinitic, atopic non-rhinitic and non-atopic non-rhinitic subjects. *J Allergy Clin Immunol* 1997 (in press).
37. Calderón MA, Devalia JL, Prior AJ, Davies RJ. Effect of 6 h exposure to 50 ppb ozone (O<sub>3</sub>) on release of IL-8 and RANTES from nasal epithelial cells (NEC) from atopic rhinitics and the influence of fluticasone propionate (FP), in vitro. *J Allergy Clin Immunol* 1996;97(1):352 (abstract).