

Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients in vitro

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Background: Although studies have suggested that ozone (O₃) and nitrogen dioxide (NO₂) may play a role in the pathogenesis of asthma, the underlying mechanisms are not clear.

Objective: We aimed to investigate the effects of O₃ and NO₂ on the release of IL-8, GM-CSF, RANTES, and soluble intercellular adhesion molecule 1 (sICAM-1) from human bronchial epithelial cells (HBECs) of nonatopic nonasthmatic subjects (nonasthmatic subjects) and atopic subjects with mild asthma (asthmatic subjects) in vitro.

Methods: We cultured HBECs from bronchial biopsy specimens of nonasthmatic and asthmatic subjects; exposed these for 6 hours to air, 10 to 100 ppb O₃, or 100 to 400 ppb NO₂; and analyzed the release of IL-8, GM-CSF, RANTES, and sICAM-1 after 24 hours' incubation.

Results: There was no significant difference between the constitutive release of IL-8, GM-CSF, and sICAM-1 from HBECs of asthmatic and nonasthmatic subjects. RANTES was detected only in HBECs derived from asthmatic subjects. Exposure of HBECs of asthmatic subjects to both 50 to 100 ppb O₃ and 200 to 400 ppb NO₂ significantly increased the release of IL-8, GM-CSF, RANTES, and sICAM-1 from these cells after 24 hours of incubation. However, 50 to 100 ppb O₃ and 200 to 400 ppb NO₂ led to a significant increase in release of only IL-8 and sICAM-1 from HBECs of nonasthmatic subjects after 24 hours' incubation. A comparison between the pollutant-induced release of mediators demonstrated that 100 ppb O₃-induced release of GM-CSF and sICAM-1 was significantly greater in HBECs of asthmatic subjects (medians, 0.59 and 27.4 pg/μg cellular protein, respectively) than in HBECs of nonasthmatic subjects (medians, 0.27 and 14.4 pg/μg cellular protein, respectively; *P* < .02).

Conclusion: These results suggest that O₃ and NO₂ may modu-

late airway diseases, such as asthma, by increasing the release of inflammatory mediators from bronchial epithelial cells and that the cells of asthmatic subjects may be more susceptible to the adverse effects of these pollutants. (*J Allergy Clin Immunol* 2001;107:287-94.)

Key words: Atopic asthma, ozone, nitrogen dioxide, bronchial epithelial cells, cytokines, soluble intercellular adhesion molecule 1

Studies have reported that there is an association between an increase in the levels of ozone (O₃) and oxides of nitrogen and increases in respiratory morbidity and hospital admissions for asthma in both adults and children.^{1,2} The underlying mechanisms, however, are not clear.

In vivo exposure studies have demonstrated that O₃ leads to significant increases in the levels of inflammatory mediators, including IL-8, IL-6, GM-CSF, and fibronectin in bronchoalveolar lavage (BAL) fluid of healthy volunteers.^{3,4} Recently, studies of asthmatic subjects have reported that exposure to 160 to 200 ppb O₃ for 2 hours led to increased levels of PMNs, eosinophils, lactate dehydrogenase, total protein, myeloperoxidase, fibronectin, and IL-8 in proximal airway lavage and BAL fluid from these subjects and that the percentage of PMNs and protein concentration were increased in asthmatic compared with nonasthmatic subjects.^{5,6} Studies investigating the effects of nitrogen dioxide (NO₂) have also demonstrated that exposure for 4 hours to 2 ppm of this pollutant increases the levels of neutrophils, IL-6, and IL-8 in bronchial wash specimens of nonasthmatic intermittently exercising subjects.⁷ A recent study of asthmatic and nonasthmatic subjects has reported that exposure to 1 ppm NO₂ for 3 hours led to an increase in thromboxane B₂ and PGD₂ in BAL fluid recovered from asthmatic patients but not in BAL fluid from nonasthmatic subjects.⁸

In vitro studies of primary human bronchial epithelial cell (HBEC) cultures obtained from surgical explants have demonstrated that exposure for 6 hours to 50 to 100 ppb O₃ or 400 ppb NO₂ leads to increased release of proinflammatory cytokines and other mediators, including IL-8, TNF-α, GM-CSF, RANTES, and soluble intercellular adhesion molecule 1 (sICAM-1) from these cells.⁹⁻¹¹

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Abbreviations used

BAL:	Bronchoalveolar lavage
DEP:	Diesel exhaust particle
HBEC:	Human bronchial epithelial cell
NO ₂ :	Nitrogen dioxide
O ₃ :	Ozone
SF-1 medium:	Serum-free supplemented medium
sICAM-1:	Soluble intercellular adhesion molecule 1

In addition to the role of proinflammatory cytokines in the inflammatory process,¹² recent evidence suggests that ICAM-1 (both cell-bound and soluble) also plays a role in the pathogenesis of airway inflammation in asthma.^{13,14} Studies have demonstrated that ICAM-1 is actively involved in the recruitment and migration of eosinophils and neutrophils by binding the CD11a/CD18 receptor (leukocyte function antigen 1) on these cells and therefore playing a role in the trafficking of inflammatory cells to the airway epithelium.^{13,14} Indeed, studies of asthmatic subjects have reported increased levels of sICAM-1 both in BAL fluid and serum from these patients 18 hours after allergen challenge.¹⁵

Our recent studies have demonstrated that incubation with diesel exhaust particles (DEPs) increases the release of IL-8, GM-CSF, and sICAM-1 from HBECs cultured from biopsy explants of nonatopic nonasthmatic subjects and atopic subjects with mild asthma and that the increase in asthmatic cells was significantly greater than the release from HBECs of nonasthmatic subjects.¹⁶ To our knowledge, there is no information to date on the effect of exposure to either O₃ or NO₂ on HBEC cultures of asthmatic patients, who are generally thought to be at a greater risk from the adverse respiratory effects of these pollutants.

In the present study we cultured HBECs from well-characterized groups of both nonatopic nonasthmatic subjects (nonasthmatic subjects) and atopic patients with mild asthma (asthmatic subjects) and investigated the effect of O₃ and NO₂ on the release of IL-8, GM-CSF, RANTES, and sICAM-1 from these cells in vitro.

METHODS**Nonatopic nonasthmatic subjects and atopic asthmatic patients**

Twenty-eight volunteers (12 women and 16 men) with a mean age of 29 years (range, 19–45 years) took part in the study. Of these, 13 were nonatopic nonasthmatic subjects (control subjects), and 15 were subjects with atopic mild asthma; there were no significant differences in the age and sex ratios. Atopy was confirmed by positive skin prick reactions (wheals with a mean diameter of >3 mm with associated flare and itching in the absence of any reaction to the negative control) to house dust mite (*Dermatophagoides pteronyssinus*) with or without other allergens, such as grass pollen, *Aspergillus fumigatus*, and dog or cat dander (ALK, Allergolast Laboratorium, Horsholm, Denmark). All asthmatic patients had a resting FEV₁ of greater than 70% predicted for their age and height and a provocative concentration of 2 mg/mL histamine or less, leading to a PC₂₀ FEV₁. Before entry into the study, the patients were

clinically stable and had not required systemic antihistamines, non-steroidal anti-inflammatory agents, β blockers, topical corticosteroids, long acting β_2 -agonists, or oral bronchodilators for 6 weeks or inhaled steroid, nedocromil sodium, or sodium cromoglycate for 6 months. During the study, only inhaled salbutamol or terbutaline, as required, was permitted. None of the volunteers entering into the study were smokers or exsmokers and had not suffered from upper or lower respiratory tract infections within 1 month of the study. The study was approved by the ethics committee of St. Bartholomew's Hospital, and all volunteers provided written informed consent on entry.

Reagents

All reagents were of tissue culture grade and purchased from Sigma Chemical Company (Poole, Dorset, UK), unless stated otherwise.

Bronchial tissue

Bronchial biopsy specimens were obtained from the right segmental and subsegmental carinae of the nonasthmatic subjects and asthmatic patients at the time of bronchoscopy, which was performed under light sedation with midazolam and local anesthesia with lignocaine, according to the American Thoracic Society guidelines.¹⁷ A maximum of 6 specimens of approximately 1 to 2 mm³ in size were obtained from each individual by using a rotatable Olympus FB-19KR cup-biopsy forceps (KeyMed Ltd, Southend-on Sea, UK) and immediately placed in ice-cold medium 199 before further processing for culture of HBECs.

Culture of bronchial epithelial cells

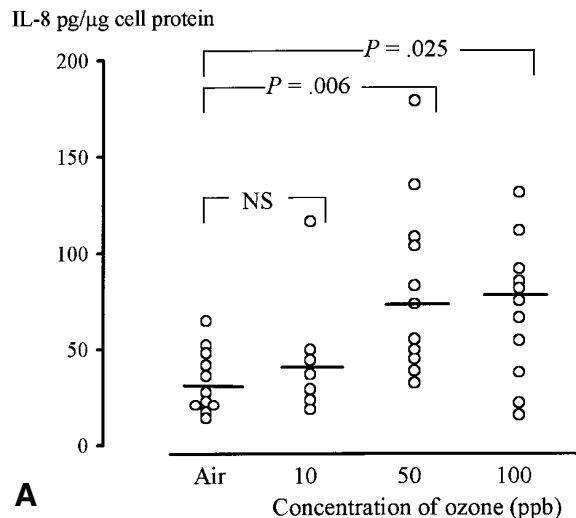
HBECs were cultured by modification of an explant cell culture technique developed by Devalia and colleagues.¹⁸ Each biopsy specimen was observed with a dissecting microscope, and the epithelium was dissected from the underlying tissue. The epithelium was then cut further into smaller sections, approximately 0.5 mm³ in size, and 2 to 3 sections were processed and explanted into untreated 6-cm diameter Falcon "Primaria" plastic culture dishes (Becton Dickinson Ltd, Oxford, UK) and incubated at 37°C in a 5% CO₂ in air atmosphere in 2 mL of freshly prepared and micropore filter-sterilized culture medium, as previously described.¹⁶ These 2- to 3-week old confluent cultures were used for other studies. The tissue explants were then transferred into 9-mm diameter Falcon Cell Culture Inserts, with 0.45- μ m pore size microporous membranes (Becton Dickinson Ltd). Medium (0.2 mL and 0.4 mL) was added into the insert and the insert well, respectively, and treated as for the cultures established in dishes. When cells reached confluence, all explants were removed to allow the cultures to grow to complete confluence before use in further studies.

The purity and identity of the cells were checked in randomly selected cultures and confirmed by using (1) light microscopy, (2) electron microscopy, and (3) indirect immunoperoxidase staining techniques with specific mAbs directed toward cytokeratin and specific ciliated epithelial cell antigens, as described previously.^{16,18} Similarly, randomly selected cultures were analyzed for the presence of contaminating cell types by staining with specific mAbs for T and B lymphocytes, endothelial cells, eosinophils, fibroblasts, mast cells, neutrophils, and macrophages, as previously explained.¹⁶

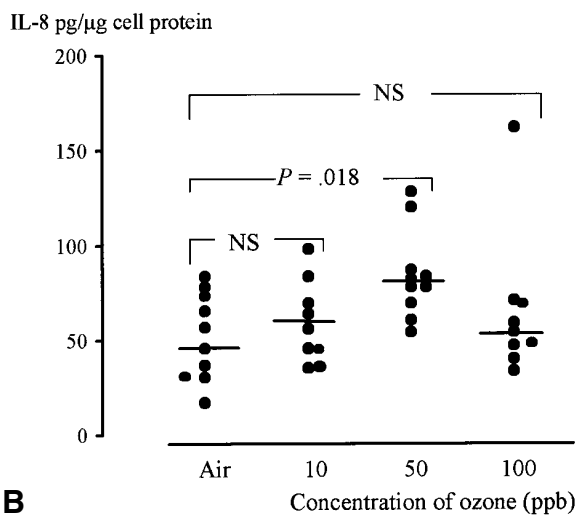
Exposure of bronchial epithelial cell cultures to O₃ and NO₂

HBEC cultures from both nonasthmatic and asthmatic subjects were exposed to O₃ and NO₂, as described previously.^{9,10}

Gas mixtures containing concentrations of 0 to 100 ppb O₃ in air were generated by use of a Dasibi Model 1108 generator-monitor

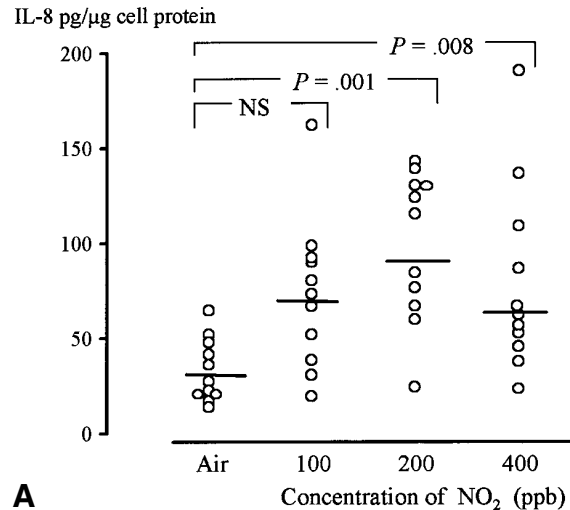


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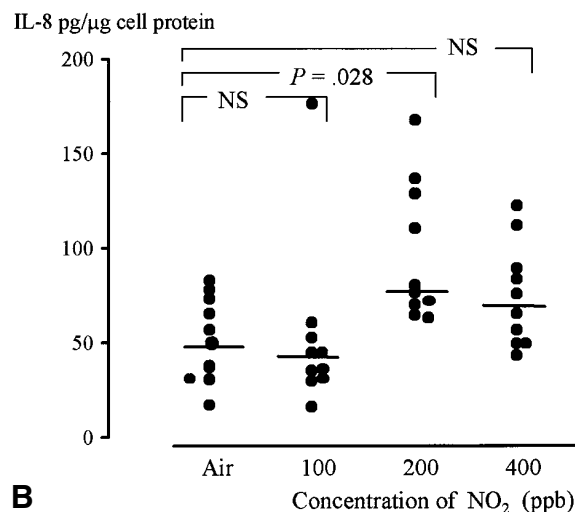


B

FIG 1. Effect of exposure for 6 hours to 10 to 100 ppb O_3 on the release of IL-8 from bronchial epithelial cell cultures of nonatopic nonasthmatic subjects (**A**) and atopic patients with mild asthma (**B**). Results are expressed as median and individual values.



A



B

FIG 2. Effect of exposure for 6 hours to 100 to 400 ppb NO_2 on the release of IL-8 from bronchial epithelial cell cultures of nonatopic nonasthmatic subjects (**A**) and atopic patients with mild asthma (**B**). Results are expressed as median and individual values.

(Quantitec Ltd, Milton Keynes, UK) and supplied at a flow rate of 3 L/min through Teflon tubing connected to a rocking airtight polycarbonate chamber of 5.5-L volume containing the HBEC cultures.

Similarly, gas mixtures containing concentrations of 0 to 400 ppb NO_2 in 5% CO_2 in air were generated by diluting 20 ppm NO_2 in air Spectraseal grade stock gas mix (BOC Special Gases, London, UK) with a 5% CO_2 in air gas mix by using a Quantitec Model QC 101 gas blender (Quantitec Ltd). The concentration of NO_2 in the final gas mix was assessed and confirmed by the use of a CEA Instruments Inc Model TGM 555 NO_2 monitor (Quantitec Ltd) and supplied into the polycarbonate chamber through Teflon tubing at a flow rate of 1.51 min^{-1} .

Direct exposure to the final O_3 or NO_2 gas mixtures was achieved by alternately tilting the chamber to an angle of 10° from horizontal every 90° in the horizontal plane on a Luckham Model 4RT rocking table (Luckham Ltd, Burgess Hill, UK), thereby eliminating gas or mediator diffusion gradients.

Appropriate controls were prepared by exposing confluent cultures to 5% CO_2 in air for 6 hours and treating them exactly as the test cultures.

Effect of O_3 and NO_2 on release of IL-8, GM-CSF, RANTES, and sICAM-1 from bronchial epithelial cells of nonasthmatic subjects and asthmatic patients

Two- to 3-week-old confluent HBEC cultures were used for these experiments. Before investigation, HBECs were grown to confluence, and the explants were removed. The epithelial cells were allowed to grow over the area of the culture insert membrane left barren on removal of the explant and, when completely confluent, were used further. Before exposure to air, O_3 , or NO_2 , all cultures were equilibrated by incubation for 24 hours in serum-free supplemented medium (SF-1 medium), which was prepared by mixing 0.5 mL of

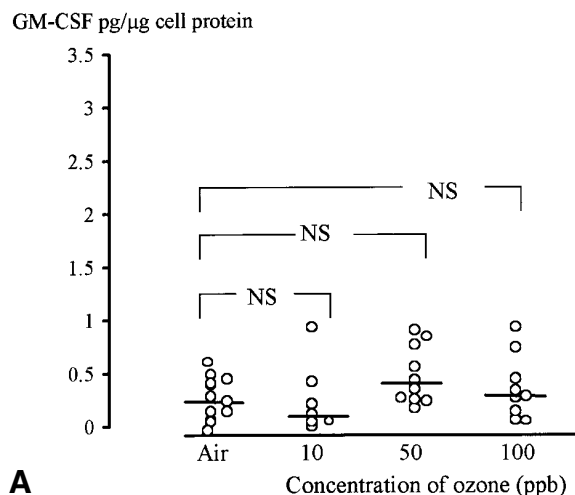
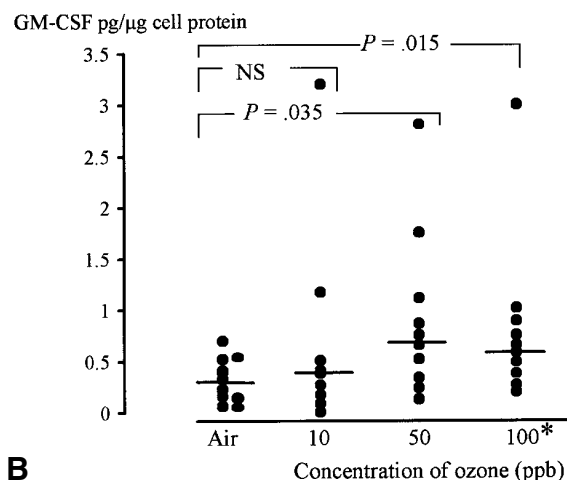
**A****B**

FIG 3. Effect of exposure for 6 hours to 10 to 100 ppb O_3 on the release of GM-CSF from bronchial epithelial cell cultures of nonatopic nonasthmatic subjects (**A**) and atopic patients with mild asthma (**B**). Results are expressed as median and individual values (* $P < .05$ vs nonatopic nonasthmatic subjects).

SF-1 serum-free supplement (Northumbria Biologicals Ltd, Cramlington, UK) and 1.0 mL of antibiotic-antimycotic solution in 100 mL of medium 199. After this initial incubation, the cultures were gently washed 3 times with fresh prewarmed and pregassed SF-1 medium. Sets of at least 6 cultures, each from different individuals, were exposed for 6 hours to either (1) air; (2) 10, 50, and 100 ppb O_3 ; or (3) 100, 200, and 400 ppb NO_2 in the rocking polycarbonate chamber at 37°C in a humidified tissue culture incubator. After exposure for 6 hours to air, O_3 , or NO_2 , the cultures were incubated for a further 18 hours under standard culture conditions before termination of the experiment. At the end of incubation, the medium was collected from the inserts and insert wells and pooled together before storage at $-70^\circ C$ until analysis for IL-8, GM-CSF, RANTES, and sICAM-1 with commercially available ELISA kits (R&D Systems, Abingdon, UK). The insert membrane was detached from the insert and immersed in 250 μL of 1.0 mol/L NaOH before storage at $-20^\circ C$ until analyzed for cellular protein, according to the method of Lowry et al.¹⁹ All results were expressed as picograms of mediator per microgram of cellular protein.

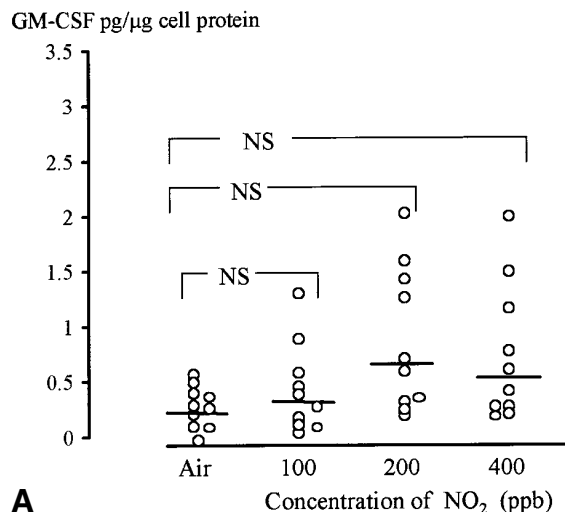
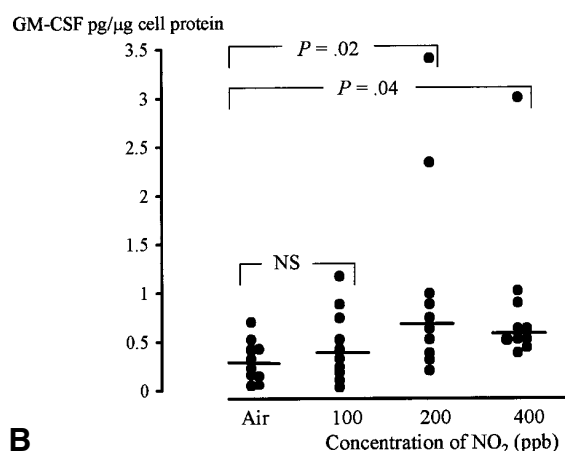
**A****B**

FIG 4. Effect of exposure for 6 hours to 100 to 400 ppb NO_2 on the release of GM-CSF from bronchial epithelial cell cultures of nonatopic nonasthmatic subjects (**A**) and atopic patients with mild asthma (**B**). Results are expressed as median and individual values.

Statistical analysis

All data were tested for skewness and then analyzed further by using nonparametric statistical tests. The Mann-Whitney U test was applied to test for differences in the age of nonasthmatic subjects and atopic asthmatic patients. First, the Kruskal-Wallis one-way ANOVA by ranks test was applied to determine the significance of any differences between all the sets of cultures investigated. This was followed by further analysis with the Mann-Whitney U test to assess the significance of any differences between the individual treatment groups. The Bonferroni correction was used for multiple comparisons, and results were expressed as median and individual values. All statistical tests were performed with a standard computer package (Minitab Data Analysis Software Release 6.1.1, 1987; Minitab Inc, State College, Pa), and all P values of less than .05 were considered to be significant.

RESULTS

The cells reached confluence after 2 to 3 weeks in culture and demonstrated typical polygonal morphology,

which is characteristic of epithelial cells. The epithelial nature of the cells was confirmed by positive staining of the cells for cytokeratin. Staining with specific mAbs against contaminating cell types, including fibroblasts, muscle cells, endothelial cells, macrophages, T and B lymphocytes, mast cells, eosinophils, and neutrophils did not show any indication of these cells.

Effect of exposure to O₃ and NO₂ on the release of inflammatory mediators

IL-8. HBECs of both nonasthmatic and asthmatic subjects released IL-8 constitutively, and there was no significant difference between the 2 groups (Fig 1).

Exposure of HBECs of nonasthmatic subjects to 50 ppb ($P < .01$) and 100 ppb O₃ ($P < .03$) and to 200 ppb ($P < .002$) and 400 ppb NO₂ ($P < .01$) significantly increased release of IL-8 from these cells after 24 hours' incubation compared with exposure to air (Figs 1, A, and 2, A). Furthermore, exposure of HBECs derived from asthmatic subjects to O₃ and NO₂ demonstrated that 50 ppb O₃ ($P < .02$) and 200 ppb NO₂ ($P < .03$) led to a significant increase in the release of IL-8 from these cells after 24 hours' incubation compared with exposure to air (Figs 1, B, and 2, B).

A comparison between O₃- and NO₂-induced release of IL-8 from HBECs of asthmatic and nonasthmatic subjects, however, did not indicate any significant difference.

GM-CSF. HBECs of both nonasthmatic and asthmatic individuals also released GM-CSF constitutively, but as shown for IL-8, the constitutive release of this cytokine was not found to be significantly different from the cells derived from individuals from the 2 study groups (Fig 3).

Exposure to either O₃ (10-100 ppb) or NO₂ (100-400 ppb) did not alter the release of GM-CSF from HBECs of nonasthmatic subjects after 24 hours' incubation compared with exposure to air (Figs 3, A and 4, A). In contrast, O₃ at concentrations of 50 ppb ($P < .05$) and 100 ppb ($P < .02$) and NO₂ at concentrations of 200 ppb ($P < .03$) and 400 ppb ($P < .05$) significantly enhanced the release of GM-CSF from HBECs of asthmatic subjects compared with exposure to air (Figs 3, B, and 4, B).

Analysis of pollutant-induced release of GM-CSF from HBECs of asthmatic and nonasthmatic subjects demonstrated that such release was significantly greater in HBECs of asthmatic subjects after exposure to 100 ppb O₃ ($P < .02$; Fig 3, A and B).

RANTES. HBECs of only asthmatic subjects released RANTES, and exposure to 50 ppb O₃ ($P < .01$) and 200 ppb NO₂ ($P < .01$) significantly increased the release of this cytokine from HBECs of asthmatic subjects compared with exposure to air (Figs 5 and 6).

sICAM-1. HBECs of both nonasthmatic and asthmatic individuals released sICAM-1 constitutively, but as shown for the other mediators, the constitutive release of this adhesion molecule was not significantly different in the cells obtained from the 2 study groups (Fig 7).

Exposure of HBECs of nonasthmatic subjects to 50 ppb O₃ ($P < .03$) and 200 ($P < .003$) and 400 ppb NO₂ ($P < .03$) significantly increased the release of sICAM-1

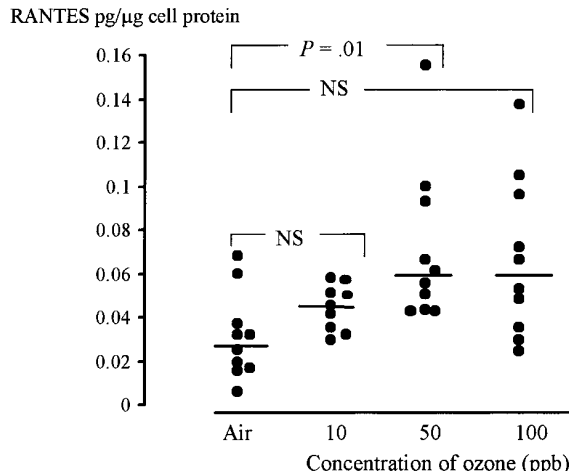


FIG 5. Effect of exposure for 6 hours to 10 to 100 ppb O₃ on the release of RANTES from bronchial epithelial cell cultures of atopic patients with mild asthma. Results are expressed as median and individual values.

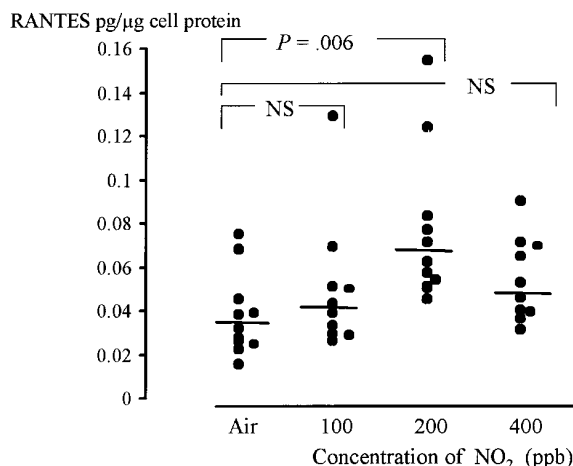


FIG 6. Effect of exposure for 6 hours to 100 to 400 ppb NO₂ on the release of RANTES from bronchial epithelial cell cultures of atopic patients with mild asthma. Results are expressed as median and individual values.

from HBECs of nonasthmatic subjects (Figs 7, A, and 8, A). Similarly, 100 ppb O₃ ($P < .03$) and 200 ppb NO₂ ($P < .02$) led to a significant increase in release of sICAM-1 from HBECs of asthmatic subjects compared with exposure to air (Figs 7, B, and 8, B).

As was the case for GM-CSF, 100 ppb O₃-induced release of sICAM-1 was significantly greater in HBECs of asthmatic subjects ($P < .02$) compared with the cells of nonasthmatic subjects (Figs 7, A and B).

DISCUSSION

In these studies we have confirmed our previous findings that bronchial epithelial cells can be grown as confluent explant cultures from biopsy specimens of well-characterized atopic patients with mild asthma and

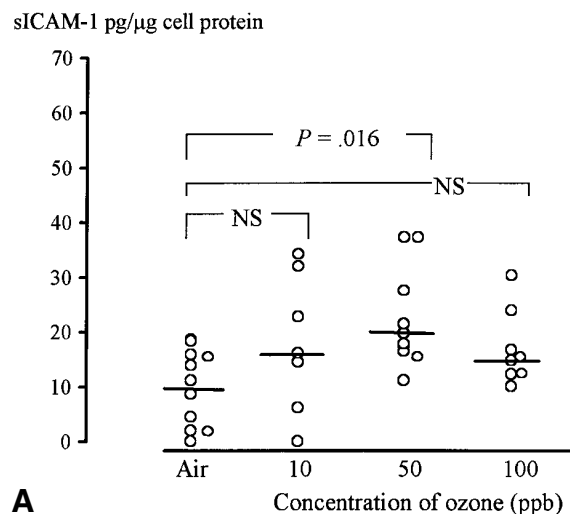
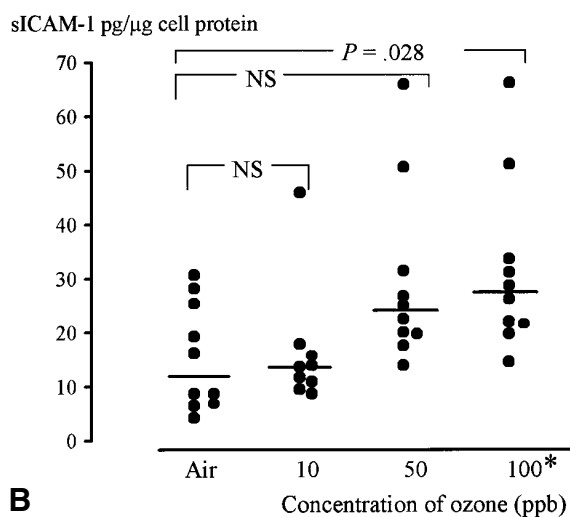
**A****B**

FIG 7. Effect of exposure for 6 hours to 10 to 100 ppb O_3 on the release of sICAM-1 from bronchial epithelial cell cultures of nonatopic nonasthmatic subjects (**A**) and atopic patients with mild asthma (**B**). Results are expressed as median and individual values (* $P < .05$ vs nonatopic nonasthmatic subjects).

nonatopic nonasthmatic subjects.¹⁶ To our knowledge, these studies are the first of their kind and demonstrate that HBECs of both nonasthmatic and asthmatic subjects constitutively release proinflammatory mediators and that exposure to gaseous pollutants, such as O_3 and NO_2 , lead to a differential release of these mediators from cells derived from the different study groups.

The present studies have demonstrated that there was no significant difference between the constitutive release of IL-8, GM-CSF, and sICAM-1 from HBECs cultured from asthmatic and nonasthmatic subjects under these experimental conditions. This is in contrast to our previous findings from HBEC cultures established on cell culture dishes, which showed that the cells of asthmatic subjects release significantly greater quantities of IL-8, GM-CSF, and sICAM-1 compared with the cells of

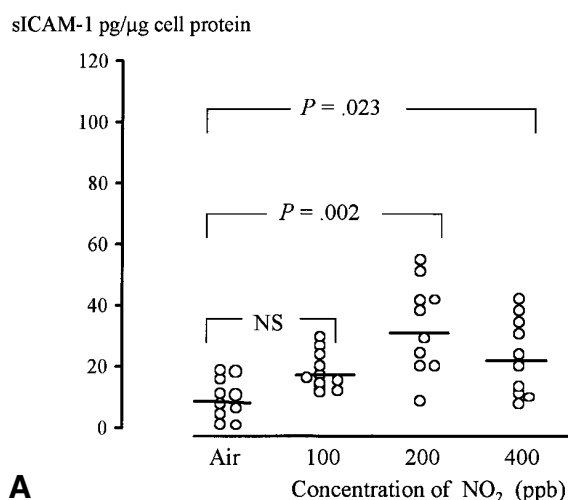
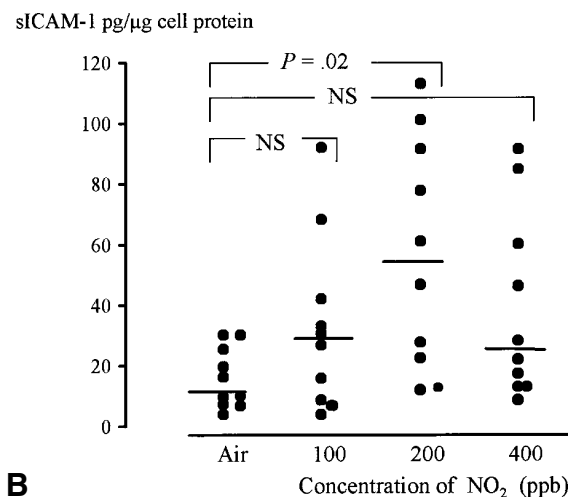
**A****B**

FIG 8. Effect of exposure for 6 hours to 100 to 400 ppb NO_2 on the release of sICAM-1 from bronchial epithelial cell cultures of nonatopic nonasthmatic subjects (**A**) and atopic patients with mild asthma (**B**). Results are expressed as median and individual values.

nonasthmatic subjects.¹⁶ Because the present studies were conducted with cultures that were established from bronchial biopsy specimens used for the second time and were therefore effectively 4- to 6-weeks old at confluence, this raises the possibility that the phenotypic characteristics of the epithelial cells on the explants are either altered or downregulated with the age of the explant in vitro. Consequently, it is not unreasonable to postulate that the differences observed between HBECs of asthmatic and nonasthmatic subjects cultured at the outset from relatively fresh biopsy specimens merely reflect the already stimulated status of the bronchial epithelial cells in situ. The heightened stimulation of epithelial cells would result from their interaction with environmental factors and the effect of cytokines released by resident inflammatory cells, such as T lymphocytes, eosinophils, mast cells, and macrophages, into the milieu. Indeed, it

has been demonstrated that the bronchial epithelium of asthmatic subjects is infiltrated by these inflammatory cell types,²⁰ which can produce cytokines, including IL-1 and TNF- α , that induce the release of IL-8, GM-CSF, RANTES, and sICAM-1 from HBECs.^{21,22}

Our findings, however, that the ability of HBECs of asthmatic subjects, but not the cells of nonasthmatic subjects, grown in cell culture inserts to release RANTES, a potent chemokine affecting the activity of eosinophils,¹² is maintained over a prolonged culture period suggest that there may also be some difference between HBECs of asthmatic and nonasthmatic subjects. Additionally, on the basis of our observation that exposure to 100 ppb O₃ can induce significantly greater release of GM-CSF and sICAM-1 from HBECs of asthmatic subjects compared with the cells of nonasthmatic subjects, it is tempting to speculate that a prolonged culture period may lead to some form of a metabolic quiescence, which is reversible by exposure of these cells to a stimulus. Indeed, this hypothesis is in accordance with the observation that asymptomatic asthmatic subjects may be made transiently symptomatic by allergen inhalation or by exposure to increased concentrations of air pollutants. Also, the demonstration by Calderon and colleagues²³ that the nasal epithelial cells of subjects with atopic rhinitis release significantly greater quantities of IL-1 β , GM-CSF, RANTES, and TNF- α during the pollen season compared with outside of the pollen season lends further support to this hypothesis.

Our studies demonstrated that exposure to O₃ and NO₂ induced release of IL-8 and sICAM-1 from HBECs of both asthmatic and nonasthmatic subjects and, additionally, release of GM-CSF and RANTES from HBECs of asthmatic subjects. Investigating the effects of O₃, Rusznak et al⁹ also demonstrated that exposure to 50 to 100 ppb O₃ increases the release of IL-8, TNF- α , GM-CSF, and sICAM-1 from HBECs cultured from surgical explants. Similarly, Devalia et al¹⁰ showed that exposure of HBECs derived from surgical explants to 400 ppb NO₂ for 6 hours leads to an increase in the release of IL-8, GM-CSF, and TNF- α from these cells. More recently, we have demonstrated that 6 hours' exposure to 400 ppb NO₂ significantly enhanced release of IL-8, RANTES, and sICAM-1 from the cells obtained from surgical explants.¹¹ Studies of others using BEAS-2B S6 human airway cell lines also reported that exposure to 0.1 to 1.0 ppm O₃ induced the release of IL-6, IL-8, and fibronectin from these cells.²⁴

Our findings that both O₃ and NO₂ enhanced the release of GM-CSF and RANTES from only the cells of asthmatic subjects and that 100 ppb O₃-induced release of GM-CSF and sICAM-1 was greater in the cells of asthmatic subjects compared with cells of nonasthmatic subjects suggest that asthmatic cells may be more susceptible to the deleterious effects of these pollutants. Indeed, our recent studies of DEPs demonstrated that although incubation with this pollutant led to increased levels of IL-8, GM-CSF, and sICAM-1 from HBECs cultured from both asthmatic and nonasthmatic subjects, the

DEP-induced release from asthmatic cells was significantly greater than the release from the cells of nonasthmatic subjects.¹⁶

Our results of asthmatic subjects complement findings of *in vivo* exposure studies reporting greater response of asthmatic subjects to the effects of O₃ and NO₂. Indeed, studies demonstrated that exposure to 200 ppb O₃ results in increased levels of PMNs, lactate dehydrogenase, total protein, myeloperoxidase, fibronectin, and IL-8 in BAL and proximal airway lavage fluid obtained from asthmatic subjects compared with nonasthmatic subjects.⁵ More recently, Peden et al⁶ have reported that exposure of allergic asthmatic subjects to 160 ppb O₃ on separate occasions for 4 weeks led to increased levels of eosinophils in bronchial lavage fluid and BAL fluid from asthmatic subjects compared with exposure to air. Although studies have suggested that unlike O₃, NO₂ exposure at near-ambient levels (eg, <2.0 ppm) does not have much effect on markers of inflammation in BAL, Jörres et al⁸ have reported that exposure of healthy and asthmatic subjects to 1 ppm NO₂ for 3 hours increases the levels of thromboxane B₂ in BAL fluid from both asthmatic and nonasthmatic subjects and PGD₂ in BAL fluid of only asthmatic subjects.

Together, these studies suggest that O₃ and NO₂ may increase the production of inflammatory mediators and inflammatory cell influx in asthmatic subjects, possibly leading to an acute exacerbation at a later stage. Indeed, studies by Molino et al²⁵ have demonstrated that exposure of mild asthmatic subjects for 1 hour to 120 ppb O₃ leads to a significant decrease in the provocation dose of allergen required to decrease the FEV₁ by 15% (PC₁₅ FEV₁) compared with prior exposure to air. Similarly, Tunnicliffe et al²⁶ have demonstrated that exposure for 1 hour to 400 ppb NO₂ significantly increased the airway response of asthmatic subjects to inhaled allergen during both the immediate and late phase when compared with exposure to air. Devalia et al²⁷ have also shown that exposure for 6 hours to a combination of 400 ppb NO₂ and 200 ppb SO₂ led to a significant increase in the airway response to inhaled allergen in subjects with mild asthma compared with exposure to air.

One of the interesting findings of these studies is, however, that both pollutants (O₃ and NO₂) significantly induced release of sICAM-1, which plays an important role in migration of eosinophils and neutrophils into the airway,^{13,14} from HBECs of both nonasthmatic and asthmatic subjects. These results are in accordance with those of studies of O₃ reported by Rusznak et al⁹ and ourselves,¹¹ with NO₂ showing increased release of sICAM-1 from HBECs by these pollutants. We think this is of particular significance, not only because of the role of this adhesion molecule in inflammatory cell trafficking but also the fact that ICAM-1 presents as a receptor for rhinoviruses, which cause common colds.¹⁴ Consequently, O₃ and NO₂ may play a role in the development of viral respiratory system infections by inducing generation of sICAM-1 by airway epithelial cells, which may lead to adhesion of these viruses to airway epithelium.

Furthermore, rhinoviruses are reported to be the most common viral respiratory pathogens associated with acute asthma attacks.^{14,28} Thus our finding that higher concentrations of 100 ppb O₃ led to significantly greater quantities of sICAM-1 in cells of asthmatic subjects may further support the role of this pollutant in the exacerbation of asthma.

In conclusion, these studies suggest that air pollutants, such as O₃ and NO₂, may modulate airway disease, such as asthma, by increasing the release of proinflammatory mediators from airway epithelial cells, which form the first line of cellular defense against inhaled irritants.

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