

# Effect of six-hour exposure to nitrogen dioxide on early-phase nasal response to allergen challenge in patients with a history of seasonal allergic rhinitis

Jia Hua Wang, MD, Jagdish L. Devalia, PhD, Judith M. Duddle, RGN, Siobham A. Hamilton, RGN, and Robert J. Davies, MD *London, England*

**Background:** Recent studies have suggested that exposure to air pollutants may enhance the airway responsiveness of susceptible individuals to inhaled allergen.

**Methods:** To investigate the effect of exposure to nitrogen dioxide (NO<sub>2</sub>) on nasal airways resistance (NAR) and inflammatory mediators in nasal lavage fluid, eight subjects with a history of seasonal allergic rhinitis, who were tested out of season, were exposed in a randomized single-blind, crossover study to either air or 400 ppb NO<sub>2</sub> for 6 hours. The changes in NAR and eosinophil cationic protein (ECP), mast cell tryptase (MCT), neutrophil myeloperoxidase (MPO), and interleukin-8 (IL-8) in nasal lavage fluid before and after exposure were evaluated. Another group of eight subjects with a history of seasonal allergic rhinitis were also randomized to exposure to air or 400 ppb NO<sub>2</sub> for 6 hours and then challenged with allergen, before evaluation for changes in NAR and changes in ECP, MCT, MPO, and IL-8 in nasal lavage fluid.

**Results:** Exposure to air or NO<sub>2</sub> did not alter either NAR or the levels of ECP, MCT, MPO, or IL-8 in nasal lavage fluid. Allergen challenge after exposure to both air and NO<sub>2</sub> significantly ( $p < 0.05$ ) increased levels of MCT, but not MPO and IL-8, in the nasal lavage fluid. In addition, allergen challenge after exposure to NO<sub>2</sub>, but not air, significantly increased levels of only ECP in nasal lavage fluid ( $p < 0.05$ ).

**Conclusions:** These results suggest that acute exposure to NO<sub>2</sub>, at concentrations found at the curbside in heavy traffic during episodes of pollution, may "prime" eosinophils for subsequent activation by allergen in individuals with a history of seasonal allergic rhinitis. (*J ALLERGY CLIN IMMUNOL* 1995;96:669-76.)

**Key words:** Nitrogen dioxide (NO<sub>2</sub>), allergic rhinitis, eosinophil cationic protein, mast cell tryptase, myeloperoxidase, interleukin-8

Epidemiologic studies have demonstrated that the incidence of allergic rhinitis has increased over the last 2 to 3 decades and have suggested that this may in part be a consequence of increased air pollution.<sup>1,2</sup> Studies from Japan have suggested that an increased incidence of allergic rhinocon-

## Abbreviations used

|                   |                                                  |
|-------------------|--------------------------------------------------|
| ECP:              | Eosinophil cationic protein                      |
| GM-CSF:           | Granulocyte-macrophage colony-stimulating factor |
| IL:               | Interleukin                                      |
| MCT:              | Mast cell tryptase                               |
| MPO:              | Myeloperoxidase                                  |
| NAR:              | Nasal airways resistance                         |
| NO <sub>2</sub> : | Nitrogen dioxide                                 |
| SO <sub>2</sub> : | Sulfur dioxide                                   |

From the Department of Respiratory Medicine and Allergy, St. Bartholomew's Hospital, London.

Supported by the National Asthma Campaign (UK), Joint Research Board of St. Bartholomew's Hospital and Glaxo Group Research Limited.

Received for publication Sept. 13, 1994; revised Feb. 8, 1995, accepted for publication Feb. 10, 1995.

Reprint requests: Robert J. Davies, MD, Department of Respiratory Medicine and Allergy, St. Bartholomew's Hospital, London EC1A 7BE, England, UK.

Copyright © 1995 by Mosby-Year Book, Inc.  
0091-6749/95 \$5.00 + 0 1/1/64141

junctivitis in individuals living near motorways lined with old cedar trees and having heavy car traffic all day long, compared with the incidence in individuals living near cedar forests with less in-

tense traffic, is likely to be due to increased exposure of these individuals to vehicle exhaust pollutants.<sup>3</sup> Similarly, studies from Germany have suggested that a higher incidence of hay fever in cities previously in West Germany, compared with the incidence in cities previously in East Germany, may be due to increased levels of nitrogen dioxide (NO<sub>2</sub>), resulting from greater use of liquid petroleum fuel by industry and drivers of motor vehicles in West Germany.<sup>4,5</sup>

More recent studies from the United Kingdom have indicated that direct exposure of patients with mild asthma to NO<sub>2</sub>, either alone or in combination with sulfur dioxide (SO<sub>2</sub>), increases the responsiveness of the lower airways to inhaled house dust mite allergen. Tunnicliffe et al.<sup>6</sup> have investigated the effect of 1-hour exposure to concentrations of 100 ppb or 400 ppb NO<sub>2</sub>, through a mouthpiece, and demonstrated that compared with exposure to air, prior exposure to 400 ppb NO<sub>2</sub> led to a significant increase in the maximal percentage change from baseline in forced expiratory volume in 1 second after inhalation of a fixed dose of house dust mite allergen. Devalia et al.<sup>7</sup> have investigated the effect of 6-hour exposure to 400 ppb NO<sub>2</sub>, 200 ppb SO<sub>2</sub> or 400 ppb NO<sub>2</sub> + 200 ppb SO<sub>2</sub>, in a modified exposure chamber, and demonstrated that only the combination of the two pollutants significantly decreased the dose of house dust mite allergen required to reduce forced expiratory volume in 1 second by 20%, when compared with prior exposure to air.

Although a number of studies have investigated the mechanisms underlying the effect of exposure to NO<sub>2</sub> in the lower airways,<sup>8-10</sup> to date there are no such studies of the effect of exposure to this pollutant in the upper airways in either atopic or nonatopic individuals. More importantly, there are no studies on the effects of acute exposure to NO<sub>2</sub> on allergen-induced changes in the upper airways of susceptible individuals, such as those with seasonal allergic rhinitis. In view of these findings, the aims of this study were: (1) to investigate the effect of 6-hour exposure to 400 ppb NO<sub>2</sub> on nasal symptoms and release of mediators in the nasal mucosa of patients with a history of seasonal allergic rhinitis, tested outside the pollen season, and (2) to investigate the effect of prior 6-hour exposure to 400 ppb NO<sub>2</sub> on allergen-induced changes in nasal symptoms and mediator release in these patients. Only a single concentration of 400 ppb NO<sub>2</sub> was investigated because it was not possible to implement any dose-response studies as a consequence of the patient volunteers' inability

and/or lack of desire to be exposed to NO<sub>2</sub> on numerous occasions, which such studies would necessitate.

## METHODS

### Subjects

Because no data were available for comparison from similar studies, it was not possible to perform power statistics at the outset of this study, which was conducted in randomized, single-blind, and crossover manner outside the pollen season in the United Kingdom. Consequently, we performed our study in a total of 16 patients with a history of seasonal allergic rhinitis who were free of symptoms, on the basis of the study by Bascom et al.,<sup>11</sup> which investigated the effect of 4-hour exposure to 500 ppb ozone in 12 patients with rhinitis. Six men and 10 women, aged 18 to 55 years (mean age, 26.4 years) participated in the study, and all had documented allergy to grass pollen on the basis of history of provocation of allergic rhinitis on contact with grass pollen and a positive skin test response to an extract of mixed grass pollen allergen (Allerayde Ltd., Nottingham, U.K.). None had smoked cigarettes or tobacco in the previous 2 years. None were receiving inhaled corticosteroids or sodium cromoglycate, and all subjects were asked to abstain from use of nasal ipratropium bromide for 24 hours and antihistamines for 48 hours before testing. Ethical approval for this study was obtained from the Ethics Committee of St. Bartholomew's Hospital, and all patients gave written consent at the time of entry.

### Study design

On the first visit of the study, each volunteer was skin prick tested with mixed grass pollen allergen, and nasal airways resistance (NAR) was measured. After measurement of resting NAR, the subject was challenged with normal saline solution (0.9%), and NAR was measured again. Nasal reactivity of the individual was then assessed by measurement of NAR after nasal provocation with increasing concentrations of grass pollen allergen, as described below. Only those subjects who showed a threefold increase in NAR after nasal provocation with one of the concentrations of allergen, compared with the value of NAR measured after normal saline challenge, were included in the study.

The 16 subjects who met the entry criteria were divided into two groups of eight: one group (group 1) was investigated after exposure to air or 400 ppb NO<sub>2</sub>, and the other group (group 2) was investigated after nasal allergen challenge after exposure to air or 400 ppb NO<sub>2</sub>.

The individuals in group 1 were randomized to exposure to air or 400 ppb NO<sub>2</sub>. Before exposure, resting NAR was measured and a nasal lavage was carried out in each subject. The subjects were then exposed to an atmosphere of either air or air containing 400 ppb NO<sub>2</sub> for 6 hours in an exposure chamber, during which the number of times the subject sneezed was recorded. At

the end of exposure, NAR measurement and nasal lavage were repeated. All lavage samples were centrifuged at 1000 g for 10 minutes, and supernatants were collected and stored at  $-70^{\circ}\text{C}$  until they were analyzed for various mediators. After a 2-week washout period, the subjects were exposed to the alternate atmosphere, and all the tests were repeated as before. The subjects in group 2 followed a similar experimental protocol, except that they were challenged with mixed grass pollen allergen immediately after measurement of NAR after exposure to air or 400 ppb  $\text{NO}_2$  for 6 hours. Nasal allergen provocation was performed with different concentrations of allergen until the NAR increased threefold from the NAR after normal saline challenge. All nasal discharge produced after allergen provocation was collected into polypropylene funnels, and the nose was lavaged after 30 minutes. The nasal discharge and lavage were pooled and processed as described previously.

### Exposure chamber

Exposures of all volunteers to a concentration of 400 ppb  $\text{NO}_2$  in air were conducted in an environmental chamber. A medical quality "stock" 200 ppm  $\text{NO}_2$ /air gas mix (BOC Special Gases, London, U.K.), specifically approved and licensed (license number ML/0735/01 Specials) for use in these studies, was diluted with room air by using a Quantitec Model 853 V5 gas blender (Quantitec Ltd., Milton Keynes, U.K.). The diluted mix of 400 ppb  $\text{NO}_2$  in air was transferred to the environmental chamber through Teflon tubing, and the chamber was equilibrated quickly and efficiently by dissipating the incoming gas mixtures by means of a fan. The atmospheric concentration of  $\text{NO}_2$  in the chamber was maintained at 400 ppb by continually purging the chamber with the diluted gas mixture and was monitored continuously with a CEA Instruments  $\text{NO}_2$  monitor (model TGM 555, Quantitec Ltd.), which has a detection limit of 5 ppb for  $\text{NO}_2$  and measures with an accuracy of greater than 98%.

### Measurement of NAR

NAR was measured during both inspiration and expiration by means of active posterior rhinomanometry with a Mercury NR8 rhinomanometer (Mercury Instruments Ltd., Glasgow, U.K.). A pressure tube was placed in the mouth, and patients were instructed to breathe normally through both nostrils, with their lips sealed tightly around the pressure tube. Airflow was measured with a pneumotachograph, so that under stable conditions the pressure developed in the mouth was equal to that behind the nasal passages. NAR was calculated by dividing pressure by flow rate.

### Nasal allergen provocation

Freeze-dried extracts of mixed grass pollen (Pharmacia, Milton Keynes, U.K.) were made up in normal saline solution to produce final concentrations of 250, 2500, 25,000, and 125,000 BU/ml. One hundred microliters of

normal saline control was nebulized at an airflow rate of 7 L/min, from a cuvette with a modified airbrush (Humbrol, Hull, U.K.), and NAR was measured. This was followed by nebulization of allergen solutions of increasing concentrations, until NAR was increased threefold above the saline control value. During nebulization, each subject was instructed to hold his or her breath at the end of inspiration for approximately 3 seconds, and the opposite nostril was gently compressed by external finger pressure: these maneuvers were undertaken to prevent the passage of solutions to the lower respiratory tract and the opposite nostril.

### Nasal lavage

A modification of the technique of Hilding<sup>12</sup> was used. A size 12F Foley catheter (Rusch UK Ltd., High Wycombe, U.K.), modified by cutting off the tip distal to the balloon to avoid trauma to the nasal mucosa, was placed in the nasal vestibule. The balloon was inflated to the maximum comfortably tolerated by the volunteer (approximately 5 to 8 ml of air) to create a seal. Each subject was asked to flex his or her head slightly forward to prevent escape of fluid through the posterior choanae, and lavage was performed by connecting the catheter to a bladder syringe containing 7 ml of normal saline solution warmed to  $37^{\circ}\text{C}$ . The cycle of lavage and aspiration was performed three times, and after the final aspiration, the balloon was deflated and the catheter removed. Both nostrils were lavaged, the lavage fluids were pooled, and 3.0 ml aliquots of the pooled lavage fluid was centrifuged at 1000 g for 10 minutes at  $4^{\circ}\text{C}$ . The supernatants from each aliquot were aspirated and stored at  $-70^{\circ}\text{C}$  until analysis was performed.

We have determined that this technique of nasal lavage was well tolerated by the volunteers because the lavage fluid could not easily be swallowed and consequently did not cause much discomfort. More importantly, compared with other techniques, this technique allowed a greater "dwell time" for the lavage fluid in the nasal passages, and the cycles of lavage and aspiration resulted in more thorough nasal washing and recovery of nasal secretions.

### Measurement of mediators in nasal lavage fluid

Before analysis, all supernatant samples were concentrated by freeze-drying overnight in a model SB6C freeze dryer (Bewhay Vacuum & Technical Ltd., Boreham Wood, U.K.). The concentrated samples were resuspended in 0.3 to 0.5 ml Tris-buffered saline and analyzed, by an investigator blinded to the experimental protocol, for eosinophil cationic protein (ECP), mast cell tryptase (MCT), neutrophil myeloperoxidase (MPO), and interleukin (IL)-8 with commercially available immunoassay kits. The concentrations, in nanograms per milliliter, of ECP, MCT, and MPO in lavage supernatant were measured with CAP fluoroimmunoas-

**TABLE I.** Effect of 6-hour exposure to air or NO<sub>2</sub> on NAR during inspiration and expiration and number of sneezes

|                 | Air exposure |            | NO <sub>2</sub> exposure |            |
|-----------------|--------------|------------|--------------------------|------------|
|                 | Inspiration  | Expiration | Inspiration              | Expiration |
| NAR (Pa/ml/sec) |              |            |                          |            |
| Before exposure | 0.21         | 0.29       | 0.22                     | 0.33       |
| Range           | 0.13-0.35    | 0.20-0.50  | 0.14-0.53                | 0.18-0.51  |
| After exposure  | 0.22         | 0.28       | 0.20                     | 0.32       |
| Range           | 0.18-0.38    | 0.22-0.44  | 0.16-0.30                | 0.19-0.56  |
| No. of sneezes  | 0.63 ± 0.50  |            | 1.75 ± 0.94              |            |

Results for NAR are expressed as median values, and results for number of sneezes are expressed as means ± SEM ( $n = 8$ ).

say system, RIACT immunoradiometric assay system, and radioimmunoassay, respectively (Pharmacia, Milton Keynes, U.K.). The concentration of IL-8 was measured in picograms per milliliter by ELISA (R&D Systems, Abingdon, U.K.).

### Statistical analysis

The normality of results was tested with a normal probability plot and the Shapiro-Wilk test. The results for allergen concentration used and number of sneezes counted were normally distributed and were expressed as means ± SEM. Paired Student's *t* tests were applied to assess the significance of changes for these results. Results for measurements of NAR and mediators in the lavage were not normally distributed and were expressed as median values. The significance of the changes for these results was assessed by Wilcoxon's test. All statistical tests were performed with the Minitab data analysis software (Minitab Inc., State College, Pa.), and *p* values of less than 0.05 were regarded as significant.

### RESULTS

No significant changes were found in NAR, during either inspiration or expiration after 6-hour exposure to air or 400 ppb NO<sub>2</sub>, in the subjects in group 1. Exposure for 6 hours to air or 400 ppb NO<sub>2</sub> did not induce rhinorrhea nor did it significantly alter the mean number of sneezes in this group of volunteers (Table I). Analysis of ECP, MCT, MPO, and IL-8 levels in nasal lavage fluid demonstrated that these values were also not altered significantly after 6-hour exposure to air or 400 ppb NO<sub>2</sub> (Fig. 1).

Assessment of NAR, immediately after exposure, and the mean number of sneezes during 6-hour exposure to air or 400 ppb NO<sub>2</sub> in the subjects in group 2 demonstrated that this was not significantly altered by NO<sub>2</sub>, confirming the findings for these parameters in the first group of subjects (Table II). As demonstrated for the previous group, 6-hour exposure to air or 400 ppb NO<sub>2</sub> did not induce rhinorrhea in this group of

patients with rhinitis either. Comparison of the concentration of allergen required to produce a threefold increase in NAR after exposure to air or NO<sub>2</sub> with that determined at the first visit (screening visit) showed that the concentration required was not changed after exposure to either of the gases. Similarly, comparison between the concentrations of allergen required to produce threefold increases in the NAR after 6-hour exposure to air or 6-hour exposure to NO<sub>2</sub> showed that these were not significantly different (Table II).

Analysis of ECP in nasal lavage fluid collected 0.5 hour after allergen challenge demonstrated that prior exposure to NO<sub>2</sub> significantly increased the concentration of this mediator from a pre-exposure value of 2.21 ng/ml to a postexposure allergen challenge value of 6.72 ng/ml ( $p < 0.05$ ) (Fig. 2). In contrast, prior 6-hour exposure to air did not significantly alter the concentration of ECP in nasal lavage fluid after allergen challenge (1.89 ng/ml pre-exposure, compared with 2.50 ng/ml postexposure allergen challenge; Fig. 2). Analysis of changes in the concentration of MCT in the nasal lavage fluid showed that unlike ECP, postexposure allergen challenge-induced increase in MCT was not dependent on prior exposure of the individuals to NO<sub>2</sub>. Prior exposure to both air and NO<sub>2</sub> led to a significant increase in the concentration of MCT in the nasal lavage fluid, from pre-exposure values of 0.24 ng/ml and 0.19 ng/ml to postexposure allergen challenge values of 0.62 ng/ml ( $p < 0.05$ ) and 0.55 ng/ml ( $p < 0.05$ ), respectively (Fig. 2). Analysis of the results of measurements of MPO and IL-8 showed that these were not significantly altered by allergen challenge after 6-hour exposure to either air or NO<sub>2</sub> (Fig. 2).

### DISCUSSION

Our studies have demonstrated that exposure of subjects with a history of seasonal allergic rhinitis

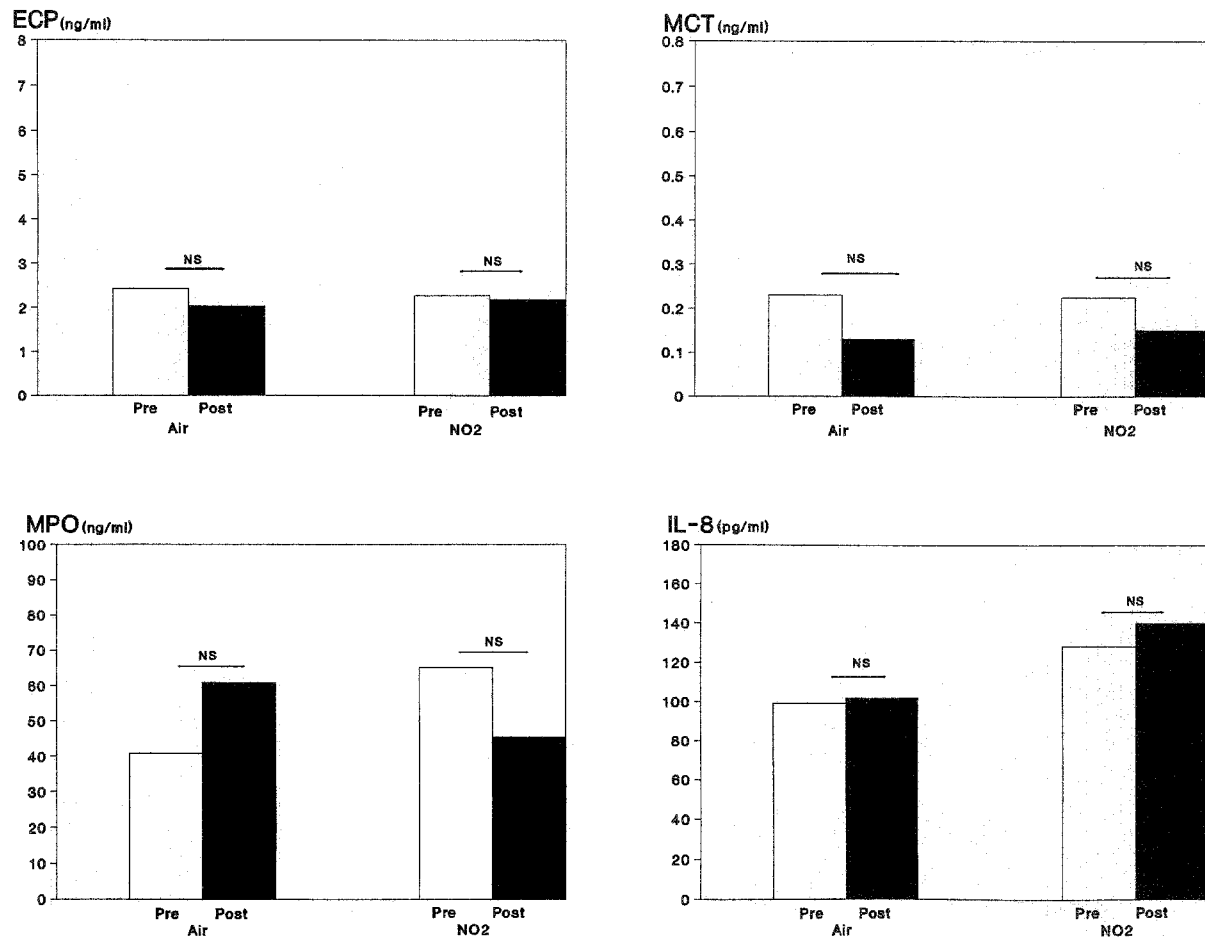


**FIG. 1.** Effect of 6-hour exposure to air or 400 ppb NO<sub>2</sub> on ECP, MCT, MPO, and IL-8 concentrations in nasal lavage fluid. Results are expressed as median values ( $n = 8$ ). Wilcoxon signed-rank test was applied to determine significance.

**TABLE II.** Effect of 6-hour exposure to air or NO<sub>2</sub> on NAR during inspiration and expiration, number of sneezes, and concentration of allergen needed to produce a threefold increase in NAR

|                                | Air exposure    |            | NO <sub>2</sub> exposure |            |
|--------------------------------|-----------------|------------|--------------------------|------------|
|                                | Inspiration     | Expiration | Inspiration              | Expiration |
| NAR (Pa/ml/sec)                |                 |            |                          |            |
| Before exposure                | 0.22            | 0.30       | 0.18                     | 0.26       |
| Range                          | 0.13-0.25       | 0.15-0.40  | 0.14-0.26                | 0.17-0.37  |
| After exposure                 | 0.21            | 0.30       | 0.21                     | 0.27       |
| Range                          | 0.14-0.29       | 0.18-0.61  | 0.12-0.30                | 0.20-0.50  |
| No. of sneezes                 | 0.5 ± 0.33      |            | 0.5 ± 0.33               |            |
| Allergen concentration (BU/ml) |                 |            |                          |            |
| Screening visit                | 87,500 ± 18,298 |            | 87,500 ± 18,298          |            |
| After exposure                 | 75,000 ± 18,898 |            | 112,500 ± 12,500         |            |

Results for NAR are expressed as median values, and results for number of sneezes and allergen concentration are expressed as means ± SEM ( $n = 8$ ).

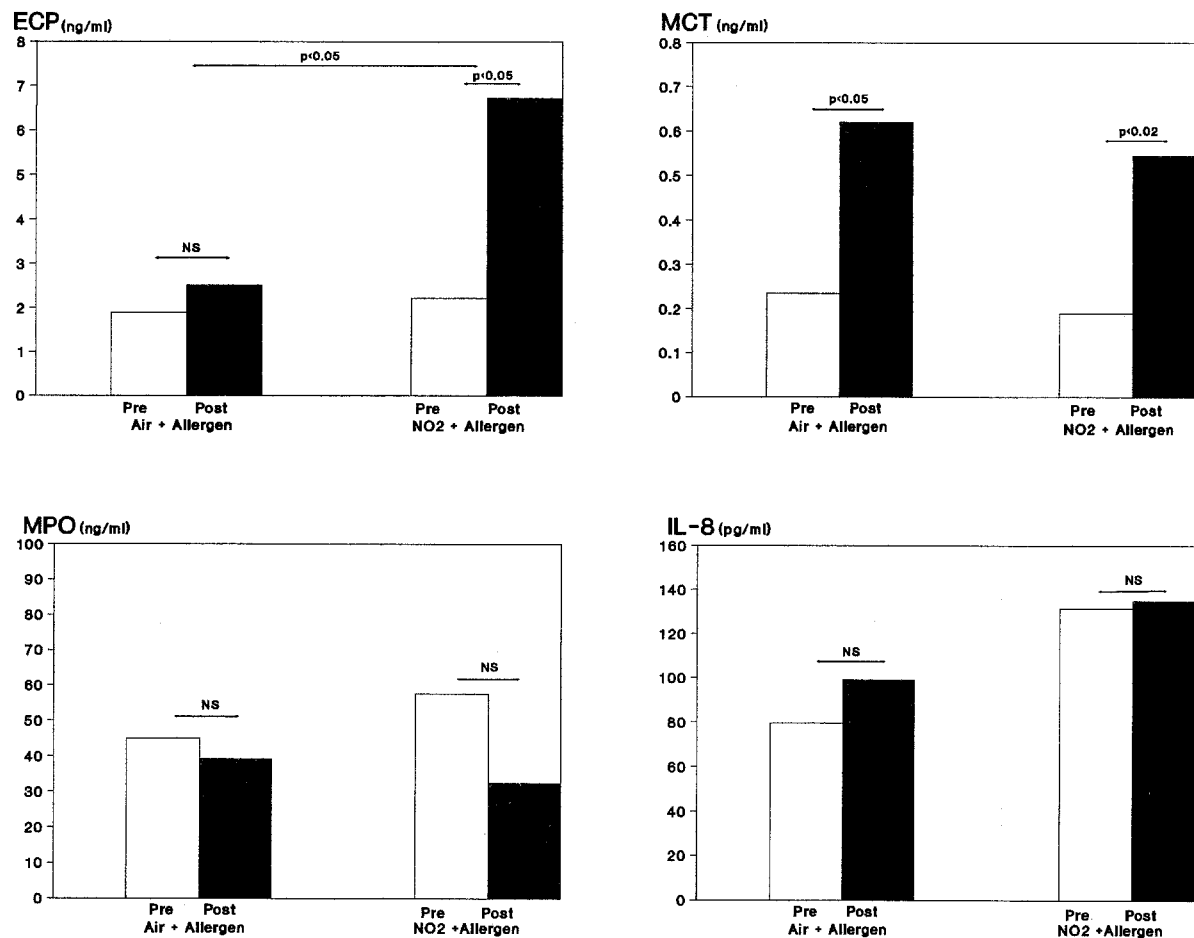


**FIG. 2.** Effect of 6-hour prior exposure to air or 400 ppb NO<sub>2</sub> on ECP, MCT, MPO, and IL-8 concentrations in nasal lavage fluid after allergen challenge. Results are expressed as median values ( $n = 8$ ) (statistics as in Fig. 1).

who are free of symptoms to 400 ppb NO<sub>2</sub> for 6 hours neither altered the nasal patency (NAR) nor increased nasal symptoms (sneezing) or the levels of inflammatory mediators (ECP, MPO, MCT, and IL-8) measured in nasal lavage fluid immediately after exposure. Although prior exposure to NO<sub>2</sub> and air for 6 hours followed by allergen challenge led to a significant increase in the concentration of tryptase in nasal lavage fluid, only prior exposure to NO<sub>2</sub> followed by allergen challenge caused a significant increase in ECP. Because ECP, MPO, and MCT, respectively, were measured as markers of activation for eosinophils,<sup>13,14</sup> neutrophils,<sup>14</sup> and mast cells<sup>15,16</sup> in this study, these results suggest that 6-hour exposure to NO<sub>2</sub> primarily influences the activity of eosinophils.

To our knowledge, this is the first report of the effect of NO<sub>2</sub> inhalation on both the direct nasal response and the allergen-induced nasal response

in subjects with a history of seasonal allergic rhinitis who are free of symptoms. The lack of a direct effect of NO<sub>2</sub> on the nose in this study is consistent with the findings of several studies of the response of the lower airways to inhalation of NO<sub>2</sub>, which failed to demonstrate any effect on lung function in patients with asthma, even after acute exposure to NO<sub>2</sub> at concentrations as high as 4000 ppb.<sup>17-19</sup> Sandström et al.<sup>10</sup> demonstrated that exposure to 2250 to 5500 ppb NO<sub>2</sub> for 20 minutes led to a significant increase in the numbers of lymphocytes, lysozyme-positive alveolar macrophages, and mast cells in bronchoalveolar lavage fluid of healthy nonsmoking subjects doing light exercise. However, these results are probably the consequence of the unusually high concentrations of NO<sub>2</sub> to which the subjects were exposed, which may be encountered occupationally but would not normally be attained even in unventilated areas in

which gas is burned for cooking.<sup>17</sup> Bascom et al.<sup>11</sup> investigated the effect of 4-hour exposure to 500 ppb ozone on the nasal response of patients with seasonal allergic rhinitis who were free of symptoms and demonstrated that ozone itself caused significant increases in both upper and lower respiratory symptoms and a mixed inflammatory cell influx, as evidenced by seven- to 10-fold increase in neutrophils, eosinophils, and mononuclear cells in nasal lavage fluid collected after exposure. Although it is possible that the discrepancy between our findings for NO<sub>2</sub> and those of Bascom et al.<sup>11</sup> for ozone may be a result of differences in experimental protocols used, it is more likely a result of the difference in the chemical nature of the gaseous agents investigated in the two studies. Ozone is a more potent oxidant than NO<sub>2</sub> and consequently may lead to more profound biochemical, functional, and symptomatic changes.

Our observation that prior exposure to NO<sub>2</sub>, but not to air, significantly increased the concentration of ECP in the nasal lavage fluid after allergen challenge indicates that NO<sub>2</sub> may be acting either by attracting more eosinophils into the nasal mucosa or "priming" existing eosinophils to the effects of allergen exposure. Peden et al.<sup>20</sup> have recently demonstrated that exposure of atopic subjects for 2 hours to 400 ppb ozone increased the number of eosinophils in nasal lavage fluid and that prior exposure to ozone augmented the eosinophil response to allergen 4 hours after allergen challenge. Although our finding of an increased concentration of ECP in nasal lavage fluid in this study does not necessarily indicate an increase in the total number of eosinophils migrating into the nasal mucosa after exposure to NO<sub>2</sub>, a recent study of nasal biopsy specimens in our laboratory demonstrated that treatment of patients with asymptomatic allergic rhinitis for 2 weeks with topical fluticasone propionate (200 µg once daily), significantly attenuated the allergen-induced increase in the concentration of ECP in nasal lavage fluid and the numbers of EG2-staining cells (activated eosinophils). EG1-staining cells (total number of eosinophils) in the nasal mucosa of these subjects were not altered, however, suggesting that eosinophil activation and eosinophil numbers are important in allergen-induced nasal disease.<sup>21</sup> An indication of the mechanisms by which air pollutants may influence the number and activation state of eosinophils has come from recent *in vivo* and *in vitro* studies. Aris et al.<sup>22</sup> demonstrated that exposure of healthy, athletic subjects for 4 hours to 200 ppb ozone significantly increased the proximal

airway and bronchoalveolar lavage fluid concentrations of IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF), cytokines known to influence eosinophil activity.<sup>23,24</sup> More recently, we have demonstrated that exposure of primary cultures of human bronchial epithelial cells to NO<sub>2</sub> significantly increased release of IL-8, GM-CSF, and tumor necrosis factor-α from these cells,<sup>9</sup> suggesting that pollutant-induced release of airway epithelial cytokines may modulate airway inflammation by influencing the recruitment and activation of inflammatory cells. Although we have not demonstrated any effects of 6-hour exposure to NO<sub>2</sub> on the concentration of IL-8 in nasal lavage fluid in our investigation, this does not preclude the possibility that other cytokines, such as GM-CSF, RANTES, and IL-5, which are potent activators and chemoattractants of eosinophils,<sup>25</sup> may have been increased.

Although symptomatic and functional effects were not observed in the nose after 6-hour exposure to NO<sub>2</sub> in this study, time-delayed effects of this pollutant gas cannot be ruled out. Epidemiologic studies of exposure to other pollutants, including SO<sub>2</sub> and respirable particulate matter (PM<sub>10</sub>), have indicated that in patients with asthma at least, impairment in lung function may be delayed by 24 to 48 hours.<sup>26,27</sup> Further, Rasmussen et al.<sup>28</sup> investigated the effect on the lower airways of 5-hour inhalation of 2300 ppb NO<sub>2</sub> in healthy nonsmoking individuals in a time-dependent manner. They demonstrated that such exposure leads to a decrease in alveolar permeability and serum glutathione peroxidase activity 11 and 24 hours later.

On the basis of these findings, it would be of interest to investigate further the effect of exposure to NO<sub>2</sub> with and without allergen on both the number and activation state of inflammatory cells and the expression of cytokines influencing these cells in nasal tissue. This could be studied in nasal biopsy specimens obtained either immediately or 24 to 48 hours after exposure and would give an indication of the mechanistic and time-lagged effects of both direct effect of exposure to NO<sub>2</sub> and the effect of NO<sub>2</sub> on allergen-induced inflammation in the nose. Additionally, it would also be of interest to investigate the effect of exposure to a concentration higher than 400 ppb NO<sub>2</sub> for a shorter period and exposure to a concentration lower than 400 ppb NO<sub>2</sub> for a longer period on allergen-induced effects in the nose, in both patients with atopic rhinitis and nonatopic subjects without rhinitis.

In conclusion, our study suggests that exposure

of subjects with a history of allergic rhinitis who are free of symptoms to NO<sub>2</sub>, at concentrations, which can be encountered at the curbside in heavy traffic,<sup>29-31</sup> increases the number and/or activation state of eosinophils in the nasal mucosa, possibly enhancing the upper airway response to inhaled allergen and exacerbating their disease.

We thank Ms. Janice Thomas, in the Department of Statistical Analysis, St. Bartholomew's Hospital, for assistance in statistical analysis.

## REFERENCES

- Ninan TK, Russell G. Respiratory symptoms and atopy in Aberdeen schoolchildren: evidence from two surveys 25 years apart. *Br Med J* 1992;304:873-5.
- Aberg N. Asthma and allergic rhinitis in Swedish conscripts. *Clin Exp Allergy* 1989;19:59-63.
- Ishizaki T, Koizumi K, Ikemori R, Ishiyama Y, Kushibiki E. Studies of prevalence of Japanese cedar pollinosis among residents in a densely cultivated area. *Ann Allergy* 1987;58:265-70.
- von Mutius E, Fritzsche C, Weiland SK, Roll G, Magnussen H. Prevalence of asthma and allergic disorders among children in united Germany. *Br Med J* 1992;305:1395-9.
- Kramer U, Altus C, Behrendt H, et al. Epidemiologische Untersuchungen zur Auswirkung der Luftverschmutzung auf die Gesundheit von Schulanfängern. *Forum Stadte Hygiene* 1992;43:82-7.
- Tunnicliffe WS, Burge PS, Ayres JG. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet* 1994;344:1733-36.
- Devalia JL, Rusznak C, Herdman MJ, Trigg CJ, Tarraf H, Davies RJ. Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. *Lancet* 1994;344:1668-71.
- Devalia JL, Sapsford RJ, Cundell DR, et al. Human bronchial epithelial cell dysfunction following in vitro exposure to nitrogen dioxide. *Eur Respir J* 1993;6:1308-16.
- 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.
- 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.
- Bascom R, Naclerio RM, Fitzgerald TK, Kagey-Sobotka A, Proud D. Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects. *Am Rev Respir Dis* 1990;140:594-601.
- Hilding AC. Simple method for collecting near-normal human nasal secretion. *Ann Otol Rhinol Laryngol* 1972;81:422-4.
- Venge P, Hakansson L, Peterson CG. Eosinophil activation in allergic disease. *Int Arch Allergy Appl Immun* 1987;82:333-7.
- Lacoste JY, Bousquet J, Chanez P, et al. Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis, and chronic obstructive pulmonary disease. *J ALLERGY CLIN IMMUNOL* 1993;92:537-48.
- Schwartz LB, Metcalfe MD, Miller JS, Earl HE, Sullivan T. Tryptase levels as an indicator of mast cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1987;316:1622-6.
- Castells M, Schwartz LB. Tryptase levels in nasal-lavage fluid as indicator of the immediate allergic response. *J ALLERGY CLIN IMMUNOL* 1988;82:348-55.
- Department of Health Advisory Group on the Medical Aspects of Air Pollution Episodes: Third Report—Oxides of Nitrogen. London: HMSO, 1993.
- Linn WS, Solomon JC, Trim SC, et al. Effect of exposure to 4 ppm nitrogen dioxide in healthy and asthmatic volunteers. *Arch Environ Health* 1985;40:234-9.
- Linn WS, Shamoo DA, Avol EL, et al. Dose-response study of asthmatic volunteers exposed to nitrogen dioxide during intermittent exercise. *Arch Environ Health* 1986;41:292-6.
- Peden DB, Carter J, Dailey LA, Devlin R. The effect of ozone on house dust mite allergen-induced nasal inflammation in asthmatics. [Abstract]. *Am J Respir Crit Care Med* 1994;149:A154.
- Lozewicz S, Wang JH, Duddle J, et al. Topical glucocorticoids inhibit activation by allergen in the upper respiratory tract. *J ALLERGY CLIN IMMUNOL* 1992;89:951-7.
- 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.
- Warringa RA, Mengelers HJ, Raaijmakers JA, Bruilnzeel PL, Koenderman L. Upregulation of formyl-peptide and interleukin-8-induced eosinophil chemotaxis in patients with allergic asthma. *J ALLERGY CLIN IMMUNOL* 1993;91:1198-205.
- Soloperto M, Mattoso VL, Fasoli A, Mattoli S. A bronchial epithelial-derived factor in asthma that promotes eosinophil activation and survival as GM-CSF. *Am J Physiol* 1991;260:L530-8.
- Robinson DS, Durham SR, Kay AB. Cytokines in asthma. *Thorax* 1993;48:845-53.
- Walter S, Griffiths RK, Ayres JG. Temporal association between hospital admissions for asthma in Birmingham and ambient levels of sulphur dioxide and smoke. *Thorax* 1994;49:133-40.
- Schwartz J, Slater D, Larson TV, Pierson WE, Koenig JQ. Particulate air pollution and hospital emergency room visits for asthma in Seattle. *Am Rev Respir Dis* 1993;147:826-31.
- Rasmussen TR, Kjaergaard SK, Tarp U, Pedersen OF. Delayed effects of NO<sub>2</sub> exposure on alveolar permeability and glutathione peroxidase in healthy humans. *Am Rev Respir Dis* 1992;146:654-9.
- Air Monitoring Group: Warren Spring Laboratory. Air pollution in the UK: 1992/93. Abingdon: 1994.
- Williams ML, Broughton GJ, Bower JS, Drury VJ, Lilley K. Ambient NO<sub>x</sub> concentrations in the UK 1976-1984: a summary. *Atmospheric Environ* 1988;22:2819-40.
- London Scientific Services. London air pollution monitoring network report for 1989. London: London Scientific Services, 1989.