

Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies

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Background: Increased ambient ozone levels have been associated with increased asthma morbidity in epidemiologic studies. Given that asthma is characterized by airway inflammation and increased sensitivity to airway irritants, it has been suggested that asthmatic subjects may be particularly sensitive to the effect of ozone.

Objective: The objective of this study was to determine whether exposure to 0.16 ppm ozone induces eosinophilic inflammation in the lower airways of asthmatic subjects.

Methods: Eight asthmatic subjects sensitive to mites were exposed to 0.16 ppm ozone and clean air on separate occasions no less than 4 weeks apart in a double-blind, randomized fashion followed by bronchoscopy 18 hours later. Bronchoalveolar lavage fluid and bronchial lavage fluid were examined for eosinophils.

Results: Ozone induced significant increases in airway eosinophils, especially in bronchial lavage fluid.

Conclusions: Ozone exposure results in increased eosinophilic inflammation in the lower airways of asthmatic subjects with allergies. (*J Allergy Clin Immunol* 1997;100:802-8.)

Key words: Ozone, asthma, eosinophils, air pollution

In normal subjects, ozone causes a functional restrictive defect in lung function that is associated with chest discomfort and characterized by decreases in the forced vital capacity (FVC), with little effect on the FEV₁/FVC ratio.^{1,2} This pollutant also evokes inflammatory responses in the respiratory tract, including increases in the levels of neutrophils and mediators such as IL-6, IL-8, and fibronectin in bronchoalveolar lavage fluid,

Abbreviations used

BALF:	Bronchoalveolar lavage fluid
ECP:	Eosinophil cationic protein
FVC:	Forced vital capacity
ILF:	Initial lavage fluid
PMN:	Polymorphonuclear neutrophil

even at ozone levels as low as 0.08 ppm with prolonged exposures.^{3,4} However, there has been no correlation noted between the changes in pulmonary function mechanics and respiratory tract inflammation induced by ozone in normal subjects.²

Increased ambient ozone levels have been associated with increased asthma morbidity in epidemiologic studies.⁵⁻⁷ Given that asthma is characterized by airway inflammation and increased sensitivity to airway irritants, it has been suggested that asthmatic subjects may be particularly sensitive to the effect of ozone. However, although several investigators have examined the effect of ozone on the lung function of asthmatic subjects, few studies have demonstrated a bronchoconstrictive effect of this pollutant in these subjects.^{1,2,5,8,9} Consequently, an alternate explanation for the health effects of ozone in asthma has been proposed that suggests that ozone may enhance airway sensitivity to inhaled allergen in extrinsic asthmatic subjects.⁹

This idea was initially supported by studies by Molino et al., who examined seven asthmatic subjects with seasonal allergies after exposure to 0.12 ppm ozone at rest for 1 hour and subsequent challenge with inhaled allergen. Six of the seven subjects required less inhaled allergen to induce a 15% decrease in FEV₁ after an ozone exposure than after a clean air exposure.¹⁰ Although others have been unable to confirm this finding,¹¹ Jorres et al. described increased sensitivity of 18 of 23 mildly asthmatic subjects to inhaled allergens after exposure to 0.25 ppm ozone for 3 hours with intermittent exercise.¹² Given that both the degree of reactivity to inhaled allergens has been linked to the degree of inflammation present in the airway, it seems reasonable that ozone may exert its effect by enhancing airway inflammation in asthmatic subjects.

Although many studies have demonstrated ozone-induced neutrophilic inflammation in nonasthmatic subjects, there are few studies that examine the effect of

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TABLE I. Effect of ozone on cell differentials observed in BALF recovered from asthmatic subjects

Subject no.	Total cells ($\times 10^6$)		Macs (%)		PMNs (%)		Eosinophils (%)		Lymphocytes (%)		Monocytes (%)		Epi (%)	
	Air	O ₃	Air	O ₃	Air	O ₃	Air	O ₃	Air	O ₃	Air	O ₃	Air	O ₃
1	11	15	80.5	92.5	1.5	0.0	0.5	0.0	15.0	7.5	2.0	0.0	0.5	0.0
2	13	16	89.0	90.5	1.5	3.0	0.5	0.5	7.0	6.0	2.0	0.0	0.0	0.0
3	24	13	67.5	58.5	3.0	14.0	4.0	6.5	22.0	17.0	3.5	2.5	0.0	1.0
4	28	44	82.0	74.0	9.5	8.5	1.5	7.5	3.5	7.5	3.5	2.0	0.0	0.0
5	9	14	75.0	77.5	1.5	7.0	4.5	8.0	15.5	5.0	2.0	1.5	1.5	1.0
6	30	40	96.0	78.5	0.5	5.5	0.5	1.0	3.0	13.0	0.0	2.0	0.0	0.0
7	31	37	81.0	58.0	0.5	26.5	1.0	2.0	14.0	10.5	2.5	2.5	1.0	0.5
8	12	28	84.3	77.5	1.7	4.0	1.7	4.5	10.3	13.5	0.7	0.0	1.3	0.5
Mean	19.8	25.9	81.9	75.9	2.5	8.6	1.8	3.8	11.3	10.0	2.0	1.3	0.5	0.4
SEM	3.3	4.6	3.0	4.5	1.0	3.0	0.6	1.2	2.3	1.5	0.4	0.4	0.2	0.2
<i>p</i> value	NS		NS		<i>p</i> < 0.05		<i>p</i> < 0.05		NS		NS		NS	

Macs, Macrophages; Epi, epithelial cells.

such exposure on bronchial inflammation in subjects with allergies or asthmatic subjects. Nasal studies of subjects with allergic rhinitis or extrinsic asthma suggest that ozone may induce eosinophilic influx onto airway mucosal surfaces. Bascom et al. have reported that subjects with allergic rhinitis have increased nasal eosinophils after exposure to 0.5 ppm ozone for 4 hours without exercise.¹³ In extrinsic asthmatic subjects, ozone (0.4 ppm for 2 hours without exercise) was found to directly cause eosinophil influx as well as to enhance Eosinophil influx and eosinophil cationic protein levels after allergen challenge to the nasal airway.¹⁴

To date, two groups have compared the effect of ozone exposure on lower airway inflammation in asthmatic and nonasthmatic subjects.^{15, 16} Both studies reveal increased neutrophil influx into the lower airways of asthmatic subjects when compared with that observed in bronchoalveolar lavage fluid (BALF) from normal volunteers collected 18 hours after relatively brief (≤ 4 hours) ozone exposure. Asthmatic subjects also had increased IL-8 levels in BALF after ozone exposure. Taken together, these data suggest that asthmatic subjects may be more sensitive to the inflammatory effect of ozone than are nonasthmatic subjects. It is interesting that neither group reported ozone-induced increases in airway eosinophils in asthmatic subjects.

One common criticism of controlled exposure studies is that many regimens use vigorous exercise (to increase ventilation) and high levels of ozone (often as high as 0.4 ppm) to achieve an effective dosing of ozone in a minimal amount of time. This concern led to a series of studies by our group comparing the effect of a prolonged exposure (7.6 hours) with a level of ozone often reported in the ambient atmosphere in many cities (0.16 ppm) in normal subjects and asthmatic subjects. The first of these studies compared the effect of this exposure regimen on lung function of normal subjects and asthmatic subjects. Although both groups experienced decreases in FVC consistent with the previously reported effect of ozone, asthmatic subjects also had true bronchospasm, as indi-

cated by decreases in both the FEV₁ and the FEV₁/FVC ratio.¹

This study examines the effect of this exposure regimen on airway inflammation in asthmatic subjects with allergies. Eighteen hours after exposure, bronchoscopy was performed and both BALF and the initial fraction of lavage fluid (ILF, defined as the first 20 ml of fluid recovered during bronchoalveolar lavage) were recovered and analyzed for Eosinophil and neutrophil influx. As outlined below, we report that prolonged exposure to 0.16 ppm ozone induces eosinophilic inflammation in the bronchial airways of asthmatic subjects with allergies.

METHODS

Study design

Eight asthmatic subjects were recruited and underwent exposures from January 3, 1995, through August 7, 1996. All subjects had mild asthma and had positive skin test results for the house dust mite *Dermatophagoides farinae*. The principal end points in the study were the percentage of eosinophils recovered in BALF and ILF after exposure to air and ozone. Changes in FEV₁ and FVC (preexposure vs postexposure values) associated with air and ozone exposure were also examined. Changes in other end points (polymorphonuclear neutrophils [PMNs] recovered in lavage fluid, soluble components of lavage fluid, and correlation between PMNs or eosinophils and spirometric end points) were considered as exploratory hypotheses. Subject selection criteria, exposure regimens, and bronchoscopy procedures are outlined below.

Subject selection

Only subjects with physician-diagnosed asthma by appropriate history of respiratory symptoms (wheezing, chest tightness, or reversible airway obstruction) or those found to have a baseline unmedicated FEV₁/FVC of 75% or less were recruited. Only those with mild asthma and sensitized to *D. farinae* as determined by epicutaneous skin tests were subsequently enrolled into the study—criteria similar to those used in previous studies of ozone exposure on nasal inflammation in asthmatic subjects.¹⁴

Asthmatic subjects with air flow obstruction (FEV₁/FVC <

TABLE II. Effect of ozone on cell differentials observed in ILF recovered from asthmatic subjects

Subject no.	Total cells ($\times 10^5$)		Macs (%)		PMNs (%)		Eosinophils (%)		Lymphocytes (%)		Epi (%)	
	Air	O ₃	Air	O ₃	Air	O ₃	Air	O ₃	Air	O ₃	Air	O ₃
1	2.6	2.4	77.0	32.0	14.0	6.0	4.0	33.0	2.0	7.0	3.0	22.0
2	9.2	9.1	97.0	71.0	0.0	12.0	1.0	4.0	0.5	6.0	1.5	5.0
3	1.6	4.5	45.0	56.0	0.0	6.0	7.0	15.0	4.0	3.0	44.0	20.0
4	0.9	19.8	76.0	35.0	4.0	18.0	1.0	38.0	10.0	7.0	9.0	2.0
5	3.6	1.9	61.0	40.0	0.0	7.0	4.0	37.0	4.0	12.0	31.0	4.0
6	3.0	1.6	64.0	57.5	3.5	24.0	5.5	4.5	9.0	4.0	18.0	0.0
7	4.0	3.5	74.0	56.0	0.0	5.0	1.0	8.5	7.0	6.5	18.0	24.0
8	0.7	2.9	69.5	50.5	0.5	14.0	0.0	2.0	4.5	8.0	25.5	25.5
Mean	3.2	5.7	70.4	49.8	2.8	11.5	2.9	17.8	5.1	6.7	18.8	12.8
SEM	1.0	2.2	5.3	4.7	1.7	2.4	0.9	5.5	1.2	1.0	5.1	3.9
p value: air vs O ₃	NS		p < 0.05		p < 0.05		p < 0.05		NS		NS	

Macs, Macrophages; Epi, epithelial cells.

70%) were tested for reversibility of this air flow obstruction. Reversibility was defined as a greater than 15% improvement in two of the following three measures of spirometric performance: FVC, FEV₁, and FEF₂₅₋₇₅. All individuals whose post-bronchodilator FEV₁ was not greater than 80% of predicted value were disqualified from participation. Subjects were disqualified if they had needed any oral steroids within the previous year. None currently used theophylline or inhaled anti-inflammatory agents. These conservative criteria were imposed to ensure subject safety; this was our initial bronchoscopic investigation of ozone-induced inflammation in asthmatic subjects.

The exposure and bronchoscopy protocols outlined below were reviewed and approved by the UNC Committee for the Protection of the Rights of Human Subjects, the UNC General Clinical Research Center Advisory Committee, and the US Environmental Protection Agency. All subjects gave informed consent before participation in the protocol.

Exposure protocol

The exposure protocol used was similar to that outlined by Horstman et al.¹ Briefly, each subject was exposed to clean filtered air and 0.16 ppm ozone over 7.6 hours with exercise sufficient to achieve ventilation of approximately 25 L/min (13 to 15 L/m²). In a given hour of exposure, subjects underwent exercise on a treadmill for 25 minutes, followed by exercise on an exercise ergometer for 25 minutes, followed by a 10-minute rest period. Exercise intensity was adjusted such that subjects had a minute ventilation (V_E) of 13 L/m² per minute.

The clean air and ozone exposures were separated by no less than 4 weeks, and order of exposures was double-blinded and randomized. Experimental exposures were deferred for 4 weeks if subjects had clinical evidence of a viral upper respiratory infection or allergic rhinitis. Temperature was maintained at 65° F. During each exposure, each subject was constantly monitored by closed-circuit television, direct observation, and ECG telemetry. After each exposure, asthmatic subjects were observed overnight at the General Clinical Research Center of the University of North Carolina. All subjects underwent bronchoscopy 18 hours after ending the air and ozone exposures.

Bronchoscopy and bronchoalveolar lavage

Bronchoscopy was performed in the UNC Bronchoscopy Laboratory. Before bronchoscopy, two actuations of inhaled

β-agonist (metaproterenol or albuterol) in a metered dose inhaler was administered to each subject. To qualify for bronchoscopy, each subject's FEV₁ had to be greater than 80% of predicted value and greater than 75% of their preexposure value after administration of the inhaled β-agonist.

Under direct visualization, the bronchoscope was wedged in a 4th to 6th order bronchus (segmental or subsegmental) of the lingula. Twenty milliliters of sterile normal saline was slowly injected from a syringe through the bronchoscope channel, and suction was then applied to recover as much fluid as possible. This procedure was repeated five more times with 50 ml aliquots of saline injected each time, with a total of 270 ml of normal saline being instilled. Recovery of the injected fluid was approximately 60% to 80%. The initial 20 ml lavage was stored separately and labeled as ILF. The remainder of the lavage fluid was labeled as BALF. During a single bronchoscopy procedure, 270 ml lavage of the right middle lobe was also performed if the initial lavage of the lingula was well tolerated. All lavage fluid from the right middle lobe was treated in a fashion similar to that from the lingular lavage. All subjects underwent lavage after both air and ozone exposures, although right middle lobe lavage was performed in only 11 of the 16 total procedures. All collected lavage fluids were stored in sterile plastic centrifuge tubes immediately after collection. Cells and fluid were processed for analysis as described below.

After the procedure, subjects were monitored in the UNC General Clinical Research Center nursing unit for at least 4 hours. Subjects with clinically significant respiratory symptoms or whose postbronchodilator FEV₁ was less than 75% of the preexposure value were to be observed overnight, although no subject required overnight observation.

Analysis of cells and soluble mediators in bronchial and BALF

Cell viability was assessed by trypan blue exclusion; the number of live cells, dead cells, and total cells was determined from unprocessed ILF or BALF with the use of a hemocytometer. Differential counts were determined as previously described.^{3,4,17} Enumeration of total cells recovered in ILF and BALF were expressed as total recovered live cells from the entire lavage fraction. Eosinophil cationic protein (ECP) was used as a marker of eosinophil activation and assayed with an ELISA kit purchased from Pharmacia. The cytokines IL-5,

IL-6, IL-8, IL-10, and RANTES were assayed with ELISA kits purchased from R&D Systems. Fibronectin, lactate dehydrogenase, and total protein were assayed as previously described.¹⁷

Statistics

The principal end points for analysis were the percentages of eosinophils recovered in BALF and ILF. Also examined were the percentages of change between preexposure and postexposure FVC (% Δ FVC) and FEV₁ (% Δ FEV₁) after air and ozone exposures. The percent change in preexposure to postexposure values associated with a given exposure were calculated with the following formulas:

$$\% \Delta \text{FVC} = \left(\frac{\text{preexposure FVC} - \text{postexposure FVC}}{\text{preexposure FVC}} \times 100 \right)$$

$$\% \Delta \text{FEV}_1 = \left(\frac{\text{preexposure FEV}_1 - \text{postexposure FEV}_1}{\text{preexposure FEV}_1} \times 100 \right)$$

Mediator levels in ILF and BALF were considered as exploratory end points.

Statistical analysis for a given end point was made with the use of two-tailed nonparametric tests (Wilcoxon signed rank tests), pairing measurements from each individual observed with clean air and ozone exposures, with significance set at a *p* value greater than 0.05. Statistical analysis was performed with GraphPad Prism software (Version 2.0, GraphPad, San Diego, Calif.).

RESULTS

The total cell count with differentials for each subject recovered from the BALF and ILF are shown in Tables I and II, respectively. For both BALF and ILF samples, there was no significant difference in recovery of total cells or lavage fluid of samples obtained after clean air and ozone exposure. However, there was a significant increase in eosinophils recovered in BALF and ILF after ozone exposure. There was also a significant increase in PMNs recovered in ILF and BALF when comparing air exposure results with that after ozone exposure. Fig. 1 depicts the individual percentage of PMNs and eosinophils of cells recovered in ILF after air and ozone exposure. Ozone-induced changes in percent of PMNs and eosinophils is more pronounced in ILF when compared with BALF. It is interesting to note that despite finding ozone-induced increases in both percentage of PMNs and eosinophils, there was no correlation between these two cell types in ILF when considering data from either all bronchoscopies (air and ozone exposure days, $r_s = 0.23$, $p = 0.58$) or only those after the ozone exposure ($r_s = 0.25$, $p = 0.36$). No significant differences in levels of soluble mediators in either ILF or BALF were found when comparing samples collected after air exposure with those collected after ozone exposure.

Spirometry was performed before and immediately after air and ozone exposures, and preexposure and postexposure FVC, FEV₁, and FEV₁/FVC ratio (FEV₁%) values were recorded. All lung function results are summarized in Table III. There were no significant differences in FVC, FEV₁, or FEV₁/FVC ratio when pre-air exposure values were compared with pre-ozone exposure values. Ozone induced a significant decrease

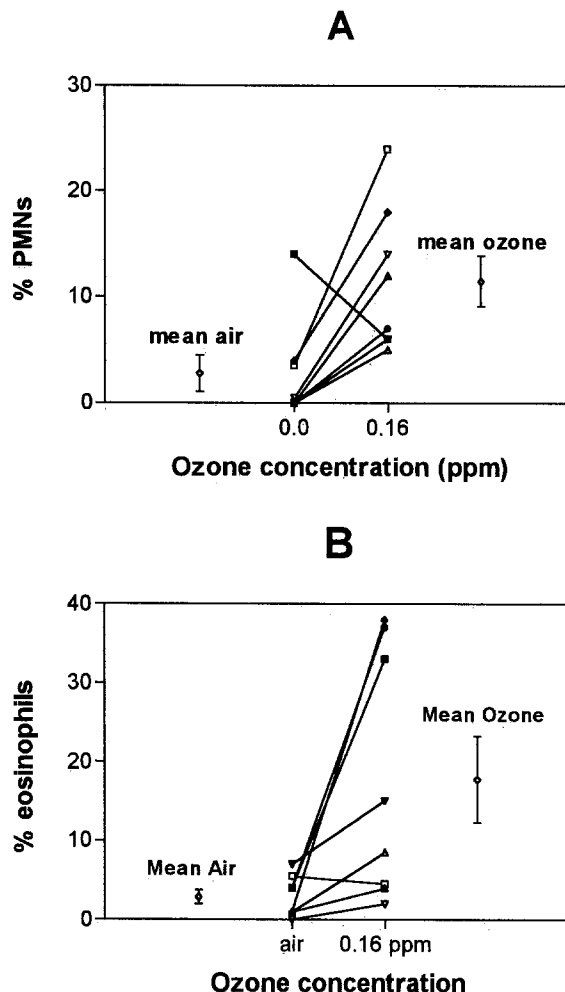


FIG. 1. Recovery of neutrophils and eosinophils in bronchial lavage fluid 18 hours after exposure to clean air and 0.16 ppm ozone in asthmatic subjects with allergies. Percentage of total cells in ILF samples composed of PMNs (%) or eosinophils (%) are expressed on the y-axis of **A** and **B**, respectively. Values from each subject after the air and ozone exposures are shown and are flanked by mean data from each exposure. Mean differences between air- and ozone-induced PMN and eosinophil influx are both significant ($p < 0.05$, two-tailed *t* test).

($p < 0.05$) in postexposure FVC and FEV₁ when compared with preexposure values. There were no differences in preexposure versus postexposure spirometric measures associated with the air exposure.

The percent change in preexposure to postexposure measurements of FVC and FEV₁ after air and ozone are shown in Table IV, with significant differences observed between the % Δ FVC and % Δ FEV₁ values associated with air and ozone exposures. Of note, one subject (subject 4) had a 14.9% decrease of the FEV₁ during the air exposure, which was outside of the 99% CI for measurements of this end point for the entire cohort of subjects. All other spirometric measurements noted for the cohort were within the 95% CI for the given end

TABLE III. Summary of lung function data: preexposure and postexposure to air and ozone

Subject no.	FVC (L)				FEV ₁ (L)				FEV ₁ (%) (FEV ₁ /FVC × 100)			
	Air		O ₃		Air		O ₃		Air		O ₃	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	5.48	5.40	5.57	5.19	4.12	4.16	4.33	3.44	76	77	78	68
2	5.26	5.33	5.44	4.73	4.24	4.37	4.18	3.55	81	84	85	87
3	5.41	5.20	5.51	4.85	4.25	4.26	4.23	4.18	80	84	78	86
4	5.80	5.76	5.26	5.00	4.62	3.93	3.90	3.32	80	69	74	66
5	4.15	4.28	4.12	3.28	2.88	2.96	3.25	2.12	69	70	80	65
6	5.05	5.02	5.20	4.79	3.78	3.82	3.64	3.71	76	76	74	77
7	5.43	5.71	5.05	4.85	4.45	4.62	4.39	3.96	82	81	88	82
8	6.23	6.08	6.35	6.08	4.68	4.63	4.79	4.08	75	76	75	67
Mean	5.35	5.35	5.31	4.84	4.13	4.09	4.09	3.55	77.4	77.1	78.0	74.8
SEM	0.21	0.19	0.22	0.27	0.21	0.19	0.17	0.23	1.5	2.0	1.3	3.3

point. The 14.9% decrease in pre-air exposure to post-air exposure FEV₁ values for subject 4 is virtually identical to that noted after ozone exposure (14.87%) and indicates that the preexposure to postexposure change in FEV₁ values after both the air and ozone exposure in this subject are probably caused by exercise and not by exposure to ozone. When data from this subject are excluded from statistical analysis, the difference between the %ΔFVC and %ΔFEV₁ measurements observed with air and ozone exposures ($n = 7$) remains significant.

DISCUSSION

In this study, exposure to 0.16 ppm ozone for 7.6 hours resulted in a significant increase in eosinophils, as reflected in both ILF and BALF samples recovered 18 hours after completion of the exposure. While ozone is known to induce PMN influx into the airways of both normal subjects and asthmatic subjects, eosinophil influx into the bronchial airways of asthmatic subjects with allergies after exposure to ozone is a novel observation.^{3, 4, 15, 16, 18} The ability of ozone to induce eosinophil influx into the bronchial airways of asthmatic subjects with allergies is consistent with previous reports examining the effect of ozone on nasal inflammation in asthmatic subjects with allergies and allergic rhinitis subjects.^{13, 14} Because eosinophils are key effector cells in asthma, these findings suggest that atopic subjects may be at greater risk for airway dysfunction than are nonatopic subjects in areas with increased ambient ozone.

In this study, BALF was fractionated into ILF (the first 20 ml of lavage fluid recovered during bronchoalveolar lavage) and BALF (the remaining total lavage fluid). The concentration of total cells and the percentage of inflammatory cells (PMNs and eosinophils) after ozone exposure was remarkably greater in ILF samples compared with BALF samples. It has been suggested that initial fractions of BALF preferentially sample the proximal airways.^{16, 19} If this is true, then it might be expected that asthmatic subjects (who have eosinophilic

inflammation of the bronchial airways but not of the alveolar regions) would have increased concentrations of inflammatory cells in ILF, as observed in this study.

We did, however, observe eosinophils in BALF as well, which suggests that the reason for increased recovery of inflammatory cells in the ILF may not be due to preferential sampling of the airway with an initial lavage aliquot. Indeed, increased concentration of inflammatory cells present in ILF may be a function of dilution of recovered cells in 20 ml of lavage fluid rather than in larger volumes of lavage fluid as occurs in subsequent series of five, 50 ml lavages collectively considered as BALF. Additionally, it can be argued that many of the inflammatory cells present in the lavaged portion of the airway may be removed with initial lavage aliquots, with few cells remaining to be recovered with later aliquots. Less ambiguous methods for regional sampling of inflammatory cells in the airway include segmental lavage techniques or bronchial biopsies.^{16, 18, 20} These methods were not used by us in this initial study to minimize manipulation of the airway. Nonetheless, ILF samples had a much higher concentration of inflammatory cells than did BALF samples, indicating that when simple lavage techniques are to be used for subject safety considerations, initial lavage fractions may provide increased sensitivity for the effect of pollutant exposure.

It is interesting to note that other investigators have examined the effect of ozone exposure in asthmatic subjects, and while finding increased numbers of ozone-induced airway PMNs relative to normal subjects, did not report increases in eosinophils.^{15, 16} One possible explanation for the lack of ozone-induced eosinophils could be differences in baseline eosinophilic inflammation in the airways of these subjects. In the studies by Basha et al.¹⁵ and Scannell et al.,¹⁶ no airway eosinophils in lavage fluid were recovered from these subjects after air exposure. In contrast, all the asthmatic subjects in our study had small numbers of eosinophils in either BALF or ILF under baseline conditions (air exposure). Thus asthmatic subjects in our study may have had a greater amount of airway eosinophilic inflammation, which may

in turn be necessary for an eosinophilic response to ozone. If this is true, then decreasing underlying inflammation may serve to diminish subsequent responses to ozone.

Alternatively, it may be that atopy rather than asthma per se is necessary for an eosinophilic response to ozone to occur. This idea is supported by studies of subjects with allergies who demonstrate an eosinophilic response in the nasal airway after ozone exposure.^{13, 14} In the current study, all of our subjects were sensitive to the house dust mite. However, the atopic status of subjects examined by Scannell et al. and Basha et al. was not reported and could be less uniform.^{15, 16} Future ozone exposure studies in which subjects are carefully screened for both asthma (by nonspecific bronchial challenge) and atopy will be required to answer this question.

Another explanation for the differences in the inflammatory responses reported in asthmatic subjects may lie in the duration of exposure. Exposure of asthmatic subjects with allergies to 0.12 and 0.24 ppm ozone for 90 minutes has been noted to cause a dose-dependent increase in leukocyte influx into the nasal airway 10 minutes after exposure.²¹ Six hours after the exposure to all conditions, leukocyte levels dropped. However, with the 0.24 ppm exposure, leukocyte numbers rose again several hours later, which suggests a biphasic leukocyte increase in asthmatic subjects with allergies. There were no changes in either lung function or percentage of eosinophils in nasal lavage in these subjects. Scannell and colleagues exposed asthmatic subjects to 0.2 ppm ozone for 4 hours without finding ozone-induced airway eosinophils. Thus exposure of asthmatic subjects to ozone for 4 hours or less has not been shown to induce airway eosinophils. In contrast, our exposure protocol involves exposure to 0.16 ppm ozone for 7.6 hours with the use of modest exercise only. Additionally, our exposure protocol uses exercise for 50 minutes per hour of exposure, which would maximize exposure of the lower airway to ozone for a great percentage (approximately 75%) of the exposure period. It is possible that eosinophilic airway responses of asthmatic subjects to ozone exposure may not be observed with shorter exposure regimens or regimens that do not allow for persistent, prolonged exposure.

Despite an ozone-induced increase in airway eosinophils, it was somewhat surprising that ozone did not exert any apparent effect on levels of ECP, IL-5, IL-8, IL-10, or RANTES in either BALF or ILF collected 18 hours after exposure. It is possible that mediators that induce influx of eosinophils to the airway are elevated shortly after ozone exposure rather than 18 hours later, the time at which bronchoscopy was performed in this study. This idea is supported by studies of normal subjects, in which recovery of various inflammatory mediators after ozone exposure has been found to be temporally dependent.

Exposure of normal subjects to 0.4 ppm ozone for 2 hours yields increases in PMNs, IL-6, IL-8, and PGE₂ in BALF that are greater 1 hour after exposure compared

TABLE IV. Comparison of the percent change (preexposure vs postexposure) in FVC and FEV₁ during air and ozone exposure

Subject no.	%ΔFVC		%ΔFEV ₁	
	Air (%)	O ₃ (%)	Air (%)	O ₃ (%)
1	-1.46	-6.82	0.90	-20.55
2	1.33	-13.05	3.06	-15.07
3	-4.99	-11.98	0.23	-1.18
4	-0.69	-4.94	-14.90	-14.87
5	3.13	-20.39	4.16	-34.77
6	-0.59	-7.88	1.05	1.92
7	5.16	-3.96	3.28	-9.79
8	2.73	-3.96	-1.06	-14.82
Mean	0.58	-9.16	-0.41	-13.64
SEM	±1.13	±2.01	±2.16	±4.03
Air vs O ₃ :		Air vs O ₃ :		
<i>p</i> < 0.01		<i>p</i> < 0.001		

with 18 hours after exposure.¹⁷ Conversely, increases in BALF fibronectin and plasminogen activator levels are greater at 18 hours rather than immediately after exposure. Likewise, in the nasal airways of asthmatic subjects with allergies, ozone-induced increases of ECP are significant 3 hours but not 18 hours after ozone exposure.¹⁴ In the current study, nearly significant ozone-induced increases in total protein, fibronectin, and IL-6 in BALF and ILF samples were observed. These results are consistent with the time course for these mediators after ozone exposure in normal subjects.¹⁷ Therefore, the lack of ozone-induced changes in mediators associated with eosinophil influx in this study does not rule out that such changes occur before 18 hours after exposure and could have been missed in this study because of the timing of bronchoscopy after exposure.

As with previous studies in normal subjects, no correlation was observed between the PMN influx and decreases in lung function observed in asthmatic subjects after ozone exposure.² However, in these asthmatic subjects with allergies, a nearly significant correlation (*r*_s = -0.69, two-tailed *p* = 0.08, *n* = 7) was noted between increases in the percentage of eosinophils recovered in ILF from asthmatic subjects and decreases in the percent fall in FEV₁ after ozone exposure but not after air exposure (data not shown). It should be cautioned that the correlation between ozone-induced eosinophil influx and changes in the FEV₁ observed in this study are a preliminary finding as the result of the relatively small number of asthmatic subjects in whom both airway lavage fluid and lung function measurements were obtained.

The mechanism by which ozone induces an eosinophil influx in asthmatic subjects is not clear. In vitro studies of epithelial cells reveal that ozone induces increases in PGE₂, IL-6, IL-8, and ICAM-1, all of which may be involved in both neutrophilic as well as eosinophilic airway inflammation.²²⁻²⁴ Ozone-induced neutrophil influx is blunted in mast cell-deficient mice, which sug-

gests that mast cells may be important in mediating the effects of ozone.²⁵ If this is true, then persons with asthma might be expected to more susceptible to the effect of ozone than are nonasthmatic subjects. However, regardless of the molecular mechanisms involved, the results reported herein suggest that prolonged exposure to 0.16 ppm ozone does induce eosinophilic responses in asthmatic subjects with mild allergies. It is known that prolonged exposure of normal subjects to even lesser amounts of ozone (0.08 and 0.10 ppm) causes neutrophilic inflammation.⁴ If these levels also cause eosinophil influx in asthmatic subjects with allergies, then it is possible that asthma morbidity may be increased by levels of ozone currently below the current National Ambient Air Quality Standard. Also, because only mildly asthmatic subjects have been examined for the effect of ozone on airway inflammation, its effect in those with more significant disease remains unknown. Certainly the dose-response characteristics of the proinflammatory effect of ozone in asthmatic subjects remains to be fully elucidated.

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