

Letter to the Editor

Ozone enhances markers of innate immunity and antigen presentation on airway monocytes in healthy individuals

To the Editor:

Elevated levels of ambient ozone (O_3) are associated with increased respiratory morbidity in the United States, and epidemiologic studies have reported associations between asthma exacerbations and elevated ambient levels of pollutants.¹ Human challenge studies with O_3 , inhaled ambient particles, or inhaled endotoxin have shown that both volunteers with asthma and normal volunteers develop neutrophilic airways inflammation²⁻⁴ and that volunteers with asthma subsequently exhibit increased sensitivity to inhaled allergen,^{5,6} suggesting inflammation may mediate these exacerbations. In addition to neutrophils, antigen-presenting cells (APCs) including monocytes and macrophages are present in the inflammatory milieu. The importance of antigen presentation is increasingly recognized not only in development of allergic responses but also in maintaining T_H2 inflammation in the airways.⁷ We have reported that inhalation challenge with ambient air particles³ or endotoxin² stimulates surface expression of molecules important for innate immunity (CD11b, and membrane-bound [m]CD14) and antigen presentation (CD80, CD86 and HLA-DR) on airway monocytic cells. Similarly, we have reported that exposure to 0.4 ppm O_3 causes enhanced mCD14 expression on airway macrophages and monocytes.⁸ We hypothesized that, like endotoxin, O_3 would also cause increased expression of molecules important in innate immunity and antigen presentation. This hypothesis is supported by experimental evidence in rats that demonstrated enhanced antigen presenting activity and surface expression of MHC class II molecules (Ia) and costimulatory molecules (B7.1 and B7.2) on bronchoalveolar lavage monocytes after O_3 exposure.⁹

To examine the *in vivo* effects of acute ozone exposure on the expression of cell surface receptor molecules on induced sputum-derived and blood-derived leukocytes, 9 healthy nonsmoking volunteers (4 men and 5 women; 21-30 years of age) completed a double blind crossover study in which they were exposed to either filtered air (FA) or 0.4 ppm O_3 for 2 hours (with intermittent exercise; minute ventilation, 30-40 L/min) during 2 study sessions (separated by at least 2 weeks). This study was approved by the Committee on the Protection of the Rights of Human Subjects (Institutional Review Board) at the University of North Carolina at Chapel Hill. The primary outcome measures were changes in the expression of leukocyte cell surface receptors associated with innate immunity (CD11b, mCD14, CD16) and antigen presentation (HLA-DR, CD86). Flow cytometry was used to identify leukocyte populations and measure cell surface receptor expression that was expressed as mean fluorescence intensity. Procedures for sputum induction and processing and flow cytometry analysis were previously described

in detail.³ Secondary endpoints included changes in sputum leukocyte counts, pulmonary function (percent predicted FEV₁ and forced vital capacity, airway resistance), phagocyte function (phagocytosis and oxidative burst), exhaled oral and nasal nitric oxide (NO) excretion, and selected cytokines in sputum supernatants. Nonparametric statistics (Wilcoxon sign-rank test, Friedman test with Dunn *post hoc* analysis, Spearman correlation) were used for comparing means, and the significance α level was set at .05. Values are expressed as the means $\pm$ SEMs.

Ozone induced significant increases in the percentages and number (cells/mg) of sputum neutrophils and monocytes and a significant decrease in the percentages of macrophages (Table I). A positive correlation was found between the relative increases in neutrophils and monocytes per milligram of sputum at 6 hours after O_3 ($R = 0.75$; $P = .05$). We identified 3 populations of mononuclear phagocytes: monocytes, macrophages, and a population of cells intermediate in size and granularity (designated immature macrophages). We previously reported the last to exhibit a dendritic cell phenotype on the basis of upregulation of surface MHC class II (HLA-DR) and costimulatory molecules (CD80 and CD86) after inhalation of endotoxin.² Changes in surface marker expression were observed on sputum leukocytes (none on blood leukocytes) and occurred predominantly in sputum monocytes, which exhibited enhanced expression of all surface receptors at 1 or more time points after O_3 (vs FA; Fig 1). In contrast to our findings with endotoxin,² ozone-induced enhancement of surface molecule expression in the macrophage population as a whole was not a prominent feature. Among the cytokines and chemokines assayed, only IL-6 was significantly elevated at 6 hours after O_3 ($P < .01$). We found no significant differences in phagocyte function or oxidative burst activity after exposure to O_3 (vs FA) for any sputum or blood cell type. Immediately after O_3 exposure, percent predicted FEV₁ and forced vital capacity showed a transient decline of 22% and 14%, respectively. Exhaled oral and nasal NO remained unchanged at all times after O_3 exposure. No significant differences were found between baseline values and post-FA values for sputum surface markers, pulmonary function, or NO measurements.

We report here that acute O_3 exposure causes an increase in the number of airway monocytes and significant upregulation of cell surface molecules associated with innate immunity (mCD14, CD11b, CD16) and antigen presentation (CD86, HLA-DR) on airway monocytic cells. These data suggest that O_3 causes innate immune priming of airway monocytes, increased capacity to present processed antigens to CD4⁺ T cells, and an overall increase in the population of APCs in the airways. We speculate that these events may result in enhanced responsiveness to subsequently inhaled environmental pollutants including allergens. In atopic individuals who have developed

TABLE I. Mean (SEM) total and differential (percent of total) cell counts of nonsquamous cells in induced sputum†

	PMN		Mono		ImMac		Mac	
	Percentage	c/mg	Percentage	c/mg	Percentage	c/mg	Percentage	c/mg
Baseline	33.20 (4.98)	446 (168)	6.52 (0.49)	108 (52)	36.16 (4.02)	498 (156)	21.44 (2.92)	341 (168)
6 h FA	29.63 (6.51)	357 (57)	5.33 (0.55)	59 (13)	31.53 (3.87)	353 (91)	21.77 (4.74)	265 (111)
6 h O ₃	61.72* (5.79)	1122* (417)	12.56* (2.29)	196* (63)	11.56* (2.10)	159 (27)	10.73* (2.99)	132 (28)
24 h FA	37.99 (9.00)	832 (564)	4.68 (0.58)	55 (14)	30.44 (5.11)	289 (34)	20.07 (4.20)	177 (29)
24 h O ₃	61.16* (9.24)	909 (198)	10.46* (1.87)	143 (46)	12.58* (2.82)	145 (38)	12.45* (4.57)	111 (33)

c/mg, Cells per milligram sputum; ImMac, immature macrophage; Mac, macrophage; Mono, monocyte.

*Significantly different from corresponding FA control values ($P < .05$).

†Eosinophils, lymphocytes, and bronchial epithelial cells are not shown.

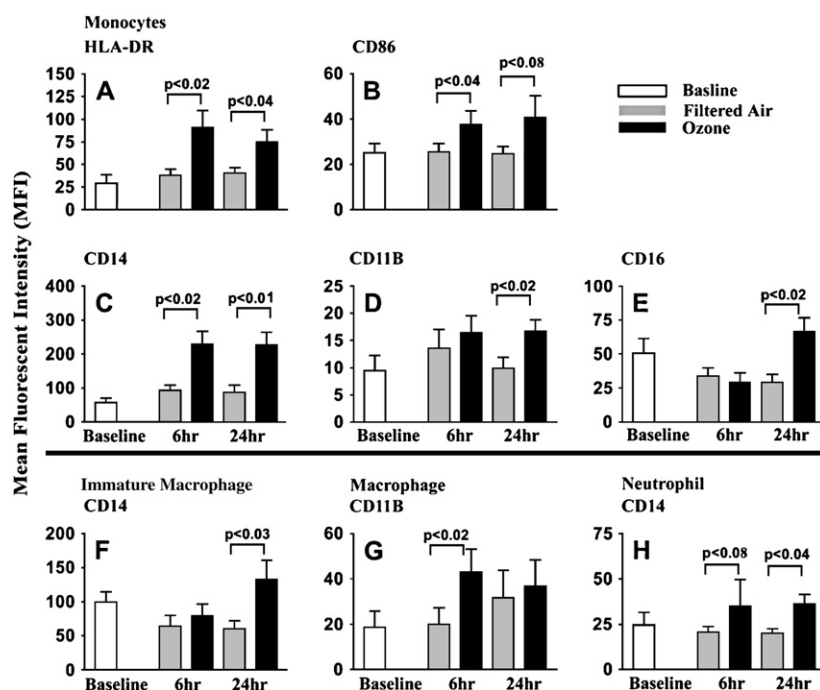


FIG 1. Expression of cell surface molecules on sputum leukocytes after exposure to 0.4 ppm O₃. Significant increases in expression (ie, increased mean fluorescence intensity) of cell surface molecules were observed on sputum monocytes (A-E). Other significant changes in cell surface molecules included mCD14 on immature macrophages (F) and neutrophils (H) and CD11b/CR3 on macrophages (G). Although not statistically significant, CD86 was also slightly elevated on monocytes ($P = .08$) at 24 hours (B), and mCD14 was elevated on neutrophils ($P = .08$) at 6 hours (H). Values are means and SEMs.

IgE-mediated immune responses, increased antigen presentation by airway monocytes is likely to promote exacerbations of asthma that are associated with exposure to ambient O₃. Furthermore, selective T_H2 induction by these primed mononuclear cells would promote an allergic phenotype in the airways and may explain in part the reported adjuvant effect of O₃ on responsiveness to inhaled allergen in individuals with allergy.⁶ It is interesting to speculate that after exposure to an innate immune stimulus like O₃, it may be the combination of innate priming and

immune-modulating factors (IL-6), and not a preprogrammed phenotype, that determines the T-cell lineage commitment by airway mononuclear APCs.

One limitation of this study was that we did not assess the actual ability of sputum monocytes to induce antigen-specific T-cell proliferation. However, similar studies in O₃-exposed rats have shown that airway monocytes with enhanced surface expression of MHC class II and B7 costimulatory molecules did indeed exhibit increased antigen-presenting activity.⁹ In future studies, it will be

necessary to perform additional *in vitro* studies to examine the ability of airway monocytes to stimulate antigen-specific T-cell proliferation.

We thank Fernando Dimeo, Heather Wells, Margaret Herbst, Martha Almond, Carole Robinette, Sally Ivins, Lynn Newlin-Clapp, and Lisa Rothlein (University of North Carolina Center for Environmental Medicine, Asthma and Lung Biology) as well as Maryanne Bassett, Deborah Levin, and Tracy Montilla (US Environmental Protection Agency) for their technical and clinical assistance. Research subjects were recruited through the efforts of Westat Environmental Protection Agency Support Services under contract with the Clinical Research Branch of the US Environmental Protection Agency.

John C. Lay, DVM, PhD^{a,b}
Neil E. Alexis, PhD^{a,b}
Steven R. Kleeberger, PhD^d
Robert A. S. Roubey, MD^c
Bradford D. Harris, MD^{a,b}
Philip A. Bromberg, MD^{a,c}
Milan J. Hazucha, MD, PhD^a
Robert B. Devlin, PhD^e
David B. Peden, MD, MS^{a,b,c}

From ^athe Center for Environmental Medicine Asthma and Lung Biology and the Departments of ^bPediatrics and ^cMedicine, University of North Carolina School of Medicine, University of North Carolina at Chapel Hill; ^dthe Laboratory of Respiratory Biology, National Institute of Environmental Health Sciences, Research Triangle Park, NC; and ^ethe Clinical Research Branch, Human Studies Division, National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC. E-mail: jcl@med.unc.edu.

Supported primarily by grant NIEHS-1RO1ES012706 from the National Institute of Environmental Health Sciences, and supported in part by a grant (RR00046) from the General Clinical Research Centers program of the Division of Research Resources, National Institutes of Health, and a cooperative agreement with the US Environmental Protection Agency (CR829522). Although the research described in this article has been funded in part by the United States Environmental Protection Agency through cooperative agreement CR829522 with the Center for Environmental Medicine, Asthma, and Lung Biology at the University of North

Carolina at Chapel Hill, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Disclosure of potential conflict of interest: D. B. Peden has consulting arrangements with GlaxoSmithKline, Novartis, and Genentech; has received grant support from GlaxoSmithKline; and is on the speakers' bureau for AstraZeneca and Merck. The rest of the authors have declared that they have no conflict of interest.

REFERENCES

1. Peden DB. The epidemiology and genetics of asthma risk associated with air pollution. *J Allergy Clin Immunol* 2005;115:213-9.
2. Alexis NE, Lay JC, Almond M, Bromberg PA, Patel DD, Peden DB. Acute LPS inhalation in healthy volunteers induces dendritic cell maturation in vivo. *J Allergy Clin Immunol* 2005;115:345-50.
3. Alexis NE, Lay JC, Zeman K, Bennett WD, Peden DB, Soukup JM, et al. Biological material on inhaled coarse fraction particulate matter activates airway phagocytes in vivo in healthy volunteers. *J Allergy Clin Immunol* 2006;117:1396-403.
4. Vagaggini B, Carnevali S, Macchioni P, Taccola M, Fornai E, Bacci E, et al. Airway inflammatory response to ozone in subjects with different asthma severity. *Eur Respir J* 1999;13:274-80.
5. Boehlecke B, Hazucha M, Alexis NE, Jacobs R, Reist P, Bromberg PA, et al. Low-dose airborne endotoxin exposure enhances bronchial responsiveness to inhaled allergen in atopic asthmatics. *J Allergy Clin Immunol* 2003;112:1241-3.
6. Kehrl HR, Peden DB, Ball B, Folinsbee L, Horstman D. Increased specific airway reactivity of mild allergic asthmatics following 7.6 hr exposures to 0.16 ppm ozone. *J Allergy Clin Immunol* 1999;104:1198-204.
7. Eisenbarth SC, Piggott DA, Bottomly K. The master regulators of allergic inflammation: dendritic cells in Th2 sensitization. *Curr Opin Immunol* 2003;15:620-6.
8. Alexis NE, Becker S, Bromberg PA, Devlin R, Peden DB. Circulating CD11b expression correlates with the neutrophil response and airway mCD14 expression is enhanced following ozone exposure in humans. *Clin Immunol* 2004;111:126-31.
9. Koike E, Watanabe H, Kobayashi T. Exposure to ozone enhances antigen-presenting activity concentration dependently in rats. *Toxicology* 2004;197:37-46.

doi:10.1016/j.jaci.2007.05.005