

Letter to the Editor

The glutathione-S-transferase mu 1 (*GSTM1*) null genotype and increased neutrophil response to low-level ozone (0.06 ppm)

To the Editor:

We have previously reported that the glutathione-S-transferase mu 1 (*GSTM1*) null genotype is associated with an increased inflammatory response to inhaled ozone (O_3)¹ and that O_3 modifies markers of innate immunity and inflammation in healthy subjects.²⁻⁴ Although it is unclear what inflammatory risk factors account for increased susceptibility to O_3 , the neutrophil (PMN) response to O_3 is a hallmark inflammatory marker that varies in magnitude across subjects; hence it has been used to identify O_3 -responsive and nonresponsive subjects in terms of inflammatory responsiveness.^{3,5,6} We hypothesized that PMN-responsive subjects would have modified cell responses and airway cytokine levels and that genetic factors would modulate the risk of a PMN response to O_3 .

In a recently published study by our group,⁷ 24 healthy adults (age, 20-33 years) were examined 18 hours after a 6.6-hour controlled exposure to 0.06 ppm O_3 or clean air (CA). That study revealed a significant O_3 -induced decrease in FEV₁ and an increase in neutrophilic inflammation in the airways. In this report we provide new measurements from the Kim et al⁷ study, including genotyping for genes reported to affect the risk for response to O_3 (*GSTM1*, *NQO1*, and *TNF*) and sputum cell assessment of markers of innate immune activation and function and inflammatory cells and cytokines. Moreover, in this analysis we stratified the 24 subjects according to their PMN response to O_3 defined as the percentage PMN O_3 -percentage PMN CA value, such that PMN-responsive subjects (responders) were those who had percentage PMN O_3 -percentage PMN CA values of 10 or greater ($n = 13$) versus those who had percentage PMN O_3 -percentage PMN CA values of less than 10 (nonresponders, $n = 11$). Specifically, by means of flow cytometry (BD LSR-II flow cytometer with BD FACSDiva 6.1 software; BD Biosciences Immunocytometry Systems, San Jose, Calif), we examined phenotype responses known to be modified by O_3 , such as cell-surface marker expression (mean fluorescence intensity) of innate immune receptors (CD11b, CD14, CD16, CD86, and HLA-DR, CD54), phagocytosis (percentage of cells undergoing phagocytosis of IgG opsonized zymosan A bioparticles), and inflammatory cytokines (IL-8, IL-1 β , IL-6, hyaluronic acid, and TNF- α), as measured by Meso Scale Discovery/MSD (Gaithersburg, Md).^{2,4} All flow cytometric end points and markers of inflammation were analyzed by using nonparametric paired t tests comparing post- O_3 with post-CA exposure time points. Regression analysis was used to determine the association between genotype status and PMN responsiveness. The following regression model was used:

$$\text{Logit}(P(\text{Responder})) = \alpha_0 + \alpha_1 \text{Age} + \alpha_2 I(\text{Female}) + \alpha_3 \text{BSA} \\ + \alpha_4 \text{BMI} + \alpha_5 I(GSTM1-),$$

where BSA is defined as body surface area and BMI is defined as body mass index. Statistical significance was determined at a P value of less than .05 for all analyses.

TABLE I. Mean (SEM) cytokine levels after CA and O_3 exposure

Cytokine (pg/mL)	Responders		Nonresponders	
	CA	O_3	CA	O_3
IL-8	6,465 (1,618)*†	9,794 (2,241)‡	2,772 (572)*	4,441 (831)
IL-6	137 (53)†	134 (32)‡	39 (10)	57 (11)
IL-1 β	190 (68)	171 (37)	67 (13)	101 (28)
TNF- α	15 (5)†	16 (4)	4 (1)	9 (3)
HA	11,737 (1,428)	18,019 (5,615)‡	9,064 (1,474)	6,976 (1,522)

HA, Hyaluronic acid.

* $P < .05$, CA versus O_3 .

† $P < .05$, CA versus CA.

‡ $P < .05$, O_3 versus O_3 .

We observed that the mean $\pm$ SEM PMN response to O_3 was significantly increased in responders versus nonresponders (27 ± 3 vs 3 ± 2 , $P < .05$) and that the mean $\pm$ SEM FEV₁ response (percentage change from CA) was not significantly different between subject cohorts ($-1.23\% \pm 0.8\%$ vs $-2.41\% \pm 2.1\%$). Logistic regression analysis showed that responder status was strongly associated with *GSTM1* status. The *GSTM1* null genotype significantly ($P < .05$) increased the probability of being a responder such that the calculated odds ratio of being a responder for the *GSTM1* null genotype was estimated to be 13 (95% CI, 1.071-157.8). *NQO1* and *TNFA* genotypes did not have a significant association with PMN response in this cohort. After CA, responders had significantly ($P < .05$) increased levels of the proinflammatory cytokines IL-8, IL-6, and TNF- α compared with those seen in nonresponders, and along with hyaluronic acid, which has been implicated in the inflammatory response to O_3 ,^{8,9} levels of these cytokines remained significantly increased after O_3 when compared with post- O_3 levels in nonresponders (Table I). Only IL-8 levels were significantly increased after O_3 versus after CA in both responders and nonresponders (Table I). Sputum macrophage phagocytosis was also significantly increased after CA in responders versus nonresponders ($51\% \pm 2\%$ vs $45\% \pm 3\%$, $P < .05$), which, together with the inflammatory cytokine data, suggests that PMN responders might have primed immunoinflammatory features under non- O_3 -exposed conditions. We found that O_3 significantly enhanced the expression of immune cell-surface receptors on macrophages (CD11b, CD14, and CD16) and monocytes (HLA-DR, CD86, and CD54) in responders but not in nonresponders (Fig 1).

In summary, we report that subjects with an increased PMN response to low-level O_3 are 13 times more likely to have the *GSTM1* null genotype than nonresponders. Furthermore, responders have increased immunoinflammatory responses to O_3 compared with nonresponders and have increased markers of inflammation after CA, suggesting the presence of a primed inflammatory airway in non- O_3 -exposed conditions. PMN responsiveness was also confirmed to be independent of the spirometric (FEV₁) response to low-level O_3 in healthy subjects. Because *GSTM1* is a risk factor for asthma exacerbation and O_3 , these data support the hypothesis that genetic modifiers of oxidative stress modulate the health effects of O_3 in patients with allergic airways disease.

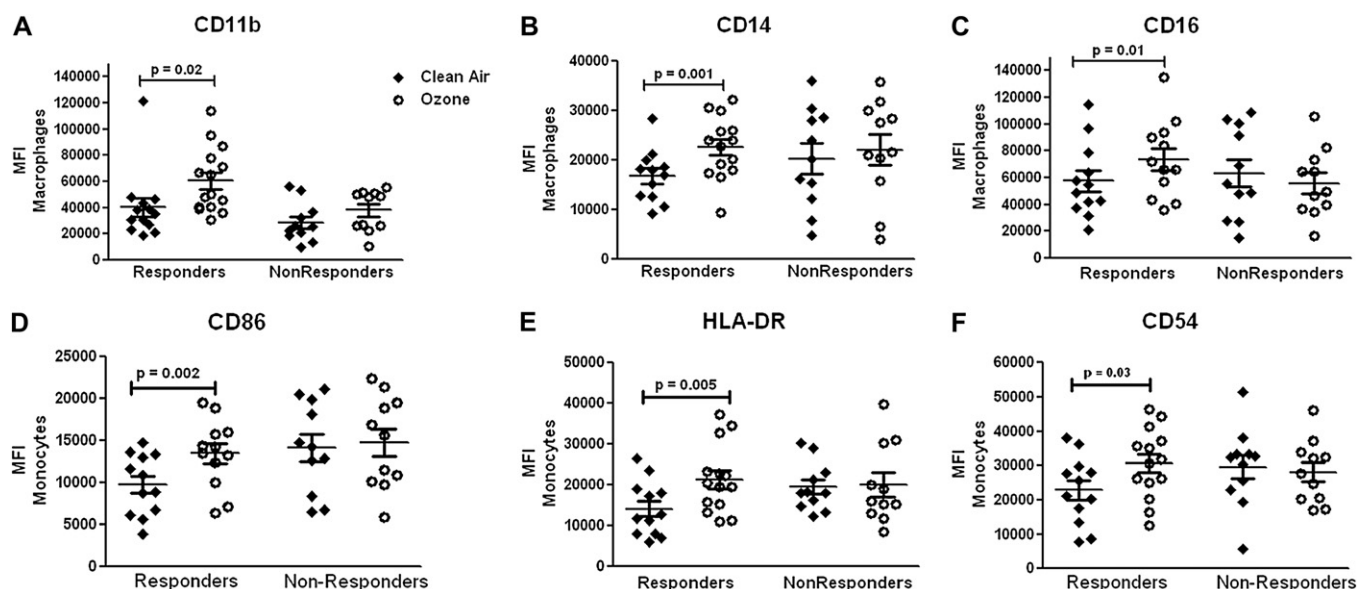


FIG 1. Mean $\pm$ SEM cell-surface phenotype expression (mean fluorescence intensity [MFI]) in responders and nonresponders after CA (solid diamonds) and O₃ (open circles) exposure on sputum macrophages (A-C) and monocytes (D-F). Responders, but not nonresponders, had significantly ($P < .05$) increased expression for all markers after O₃ versus CA exposure.

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NCTracs. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

- Alexis NE, Zhou H, Lay JC, Harris B, Hernandez ML, Lu TS, et al. The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. *J Allergy Clin Immunol* 2009;124:1222-8.e1225.
- Alexis NE, Lay JC, Hazucha M, Harris B, Hernandez ML, Bromberg PA, et al. Low-level ozone exposure induces airways inflammation and modifies cell surface phenotypes in healthy humans. *Inhal Toxicol* 2010;22:593-600.
- Alexis NE, Lay JC, Haczk A, Gong H, Linn W, Hazucha MJ, et al. Fluticasone propionate protects against ozone-induced airway inflammation and modified immune cell activation markers in healthy volunteers. *Environ Health Perspect* 2008;116:799-805.
- Lay JC, Alexis NE, Kleeberger SR, Roubey RA, Harris BD, Bromberg PA, et al. Ozone enhances markers of innate immunity and antigen presentation on airway monocytes in healthy individuals. *J Allergy Clin Immunol* 2007;120:719-22.
- Lazaar AL, Sweeney LE, MacDonald AJ, Alexis NE, Chen C, Tal-Singer R. SB-656933, a novel CXCR2 selective antagonist, inhibits ex vivo neutrophil activation and ozone-induced airway inflammation in humans. *Br J Clin Pharmacol* 2011;72:282-93.
- Holz O, Khalilieh S, Ludwig-Sengpiel A, Watz H, Stryczak P, Soni P, et al. SCH527123, a novel CXCR2 antagonist, inhibits ozone-induced neutrophilia in healthy subjects. *Eur Respir J* 2010;35:564-70.
- Kim CS, Alexis NE, Rappold AG, Kehrl H, Hazucha MJ, Lay JC, et al. Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. *Am J Respir Crit Care Med* 2011;183:1215-21.
- Hernandez ML, Lay JC, Harris B, Esther CR Jr, Brickley WJ, Bromberg PA, et al. Atopic asthmatic subjects but not atopic subjects without asthma have enhanced inflammatory response to ozone. *J Allergy Clin Immunol* 2010;126:537-44.
- Garantziotis S, Li Z, Potts EN, Kimata K, Zhuo L, Morgan DL, et al. Hyaluronan mediates ozone-induced airway hyperresponsiveness in mice. *J Biol Chem* 2009;284:11309-17.

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Supported by the US Environmental Protection Agency Internal Fund, US Environmental Protection Agency cooperative agreement CR83346301, and National Institutes of Health grants RC1ES018417, R01ES012706, and U19AI077437 (D.B.P.). Although the research described herein has been funded in part by the US Environmental Protection Agency (EPA), it has not been subjected to the EPA's required peer and policy review. The findings contained in this report do not necessarily reflect the views of the EPA or the National Institutes of Health, and no official endorsement should be inferred.

Disclosure of potential conflict of interest: N. E. Alexis, J. C. Lay, C. S. Kim, and D. B. Peden have been supported by one or more grants from the National Institutes of Health (NIH) and the US Environmental Protection Agency (EPA). M.L. Hernandez has been supported by one or more grants from the National Institute of Environmental Health Sciences and the National Institute of Allergy and Infectious Diseases. M. J. Hazucha has been supported by one or more grants from the NIH and the US EPA, has consultancy arrangements with HEI, is employed by UNC, and has received one or more grants from or has one or more grants pending with