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Ozone primes alveolar macrophage-derived innate immunity in healthy human subjects

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Summary

The present study demonstrates that acute ozone exposure of healthy human subjects enhances lung immune responses to subsequent bacterial stimuli. This highlights common air pollutant exposures as modifiers of the intensity of pulmonary immune activation.

Keywords

innate immunity; LPS; TLR4; CD14

To the Editor

Our previous work in mouse models support that inhalation of ambient ozone can prime the biological response to inhaled bacterial LPS[1]. Mice exposed to inhaled ozone then subsequently challenged to low levels of LPS exhibited increased innate immune response as evidenced by higher levels of pro-inflammatory cytokines. In that study, alveolar macrophages from ozone-exposed animals demonstrated enhanced response to LPS and enhanced surface expression of the LPS receptor TLR4. Consistent with animal studies, evidence from sputum macrophages isolated from humans exposed to ozone identified enhanced surface expression of TLR4 and CD14 by flow cytometry[2–4]. However, the functional relevance of these initial observations in healthy human subjects remained unknown. Therefore, the current study was designed to test the hypothesis that *in vivo* inhalation of relevant levels of ambient ozone would enhance human alveolar macrophage responses *ex vivo* to bacterial stimulation with LPS.

Adult healthy human subjects (N=34) were exposed to either filtered air (FA) or ozone (200 ppb) in an exposure chamber for 135 minutes with intermittent exercise in a cross-over study design with at least a 21 day washout period between exposures as previously reported[5]. The

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full details of the study design, exposure and processing of samples are available in this article's Online Repository at www.jacionline.org. The subjects enrolled were healthy and predominantly male (Table 1). Approximately 20 hours after each exposure, subjects underwent a flexible bronchoscopy with bronchoalveolar lavage. Alveolar macrophage cells were isolated with 95% purity and cultured. To determine innate immune response, cultured cells were stimulated with bacterial lipopolysaccharide (LPS), which is a prototypic toll-like receptor 4 (tlr4)-2 dependent ligand[6]. Cells were also challenged with phorbol-12-myristate-13-acetate (PMA), a specific activator of protein kinase C, which is directly involved in intracellular TLR-signaling[7], but does not require intact surface TLR4. *Ex vivo* macrophages were challenged to saline (control), LPS (100 ng/mL), or phorbol-12-myristate-13-acetate (PMA, 1μM) for 2 hours. Cell lysates were analyzed by RT-PCR for mRNA expression level of TNFα. Inhalation of ozone resulted in increased mRNA expression of TNFα in alveolar macrophages, when compared to filtered air (Figure 1A/B). We also observed an enhanced response to bacterial LPS challenge in alveolar macrophages from subjects previously exposed to inhaled ozone (Figure 1A). Furthermore, we identified that inhalation of ozone enhanced macrophage response to PMA challenge (Figure 1B). These findings demonstrate that in healthy human subjects inhalation of ambient levels of ozone can prime the biological response of alveolar macrophages to both bacterial LPS and PMA.

Previous work supports that ozone can enhance the surface expression of TLR4 and CD14 on sputum macrophages isolated from human subjects[3, 4]. To extend prior observations from human subjects exposed to ozone, we measured the mRNA expression of TLR4 and CD14 in isolated alveolar macrophages from BAL samples. Inhalation of ozone, when compared to filtered air, resulted in significantly increased mRNA expression of both TLR4 and CD14 (Figure 1C/D). These findings demonstrate that human subjects exposed inhalation of ozone can induce the expression of genes that are required for the biological response to LPS.

We should highlight that there is a great degree of inter-individual variability in the biological response to ozone. There were clearly some individuals with extremely high response to ozone as measured by TNFα and by their enhanced priming of innate immunity. This variability is consistent with previous reports and further supports that host factors contribute to the biological response to ozone. Understanding of the specific host factors that regulate susceptibility to ozone will be an area of continued investigation.

Tight regulation of the intensity of pulmonary innate immunity is highly clinically relevant. It is recognized that intact innate immunity is required for effective antibacterial host defense. However, it is also recognized that over-exuberant innate immune responses can contribute to lung injury. Intensity of innate immunity can contribute to both allergic disease[8] and emphysema[9]. Elevated short-term ozone exposures are associated with enhanced mortality and hospitalizations for respiratory disorders[E1, E2]. Mechanisms supporting this epidemiologic evidence are poorly understood; but it is plausible that altered innate pulmonary immune responses are important contributors. The present study supports that ozone exposure augments pulmonary innate immune responses in humans. Additionally, these responses are subject in inter-individual variability suggesting that host factors regulate ozone priming responses and potentially an individual's susceptibility to ozone-derived

health effects. Appreciation of the mechanisms that common environmental exposures can contribute to reprogramming of the intensity of innate immune response, in susceptible individuals, is therefore highly clinically significant. Our findings support that inhalation of environmentally relevant levels of ambient ozone in adult healthy subjects can result in increased mRNA expression of macrophage-derived TNFα, TLR4, and CD14. Furthermore, inhalation of ozone can enhance the functional response of alveolar macrophages to both bacterial LPS and PMA. Similar to our previous observations in rodent models, these new findings support that inhalation of ambient ozone can enhance pulmonary innate immunity in healthy human subjects.

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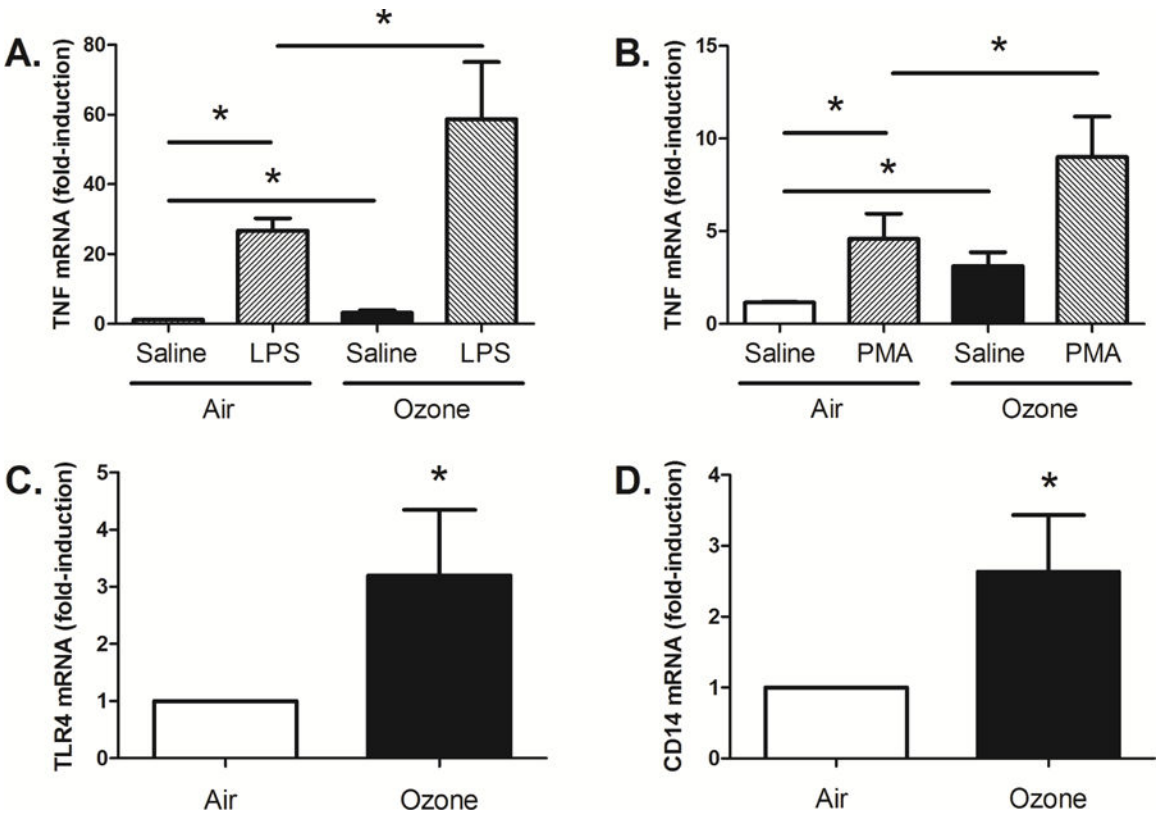

Figure 1.

Ozone enhanced gene expression and functional response to LPS and PMA. Ozone exposure enhanced alveolar macrophage expression of TNFα and the functional response to both bacterial LPS **(A)** and PMA **(B)** (N=34, *p<0.05, two-tailed unpaired Student T test). Ozone exposure resulted in enhanced alveolar macrophage mRNA expression of both TLR4 **(C)** and CD14 **(D)**, when compared to filtered air. (N=34, *p<0.05, one-tailed paired Student T-Test).

Table 1

Baseline characteristics of subjects

	Female	Male
Subject Number	6	28
Age	25.5 ± 2.81	24.71 ± 4.13
Body Mass Index (kg/m^2)	22.27 ± 2.25	24.27 ± 2.67
Ethnicity		
Caucasian	3	17
African American	3	1
Hispanic	0	2
Asian	0	8