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The IL-1 axis is associated with airway inflammation after O₃ exposure in allergic asthmatics

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To the Editor

Ozone (O₃) can injure or activate airway epithelial cells and macrophages and is the most common non-infectious environmental cause of asthma exacerbations. The typical airway response to O₃ exposure includes neutrophilia, increased pro-inflammatory cytokine levels, and transient decrements in lung function. Although it was previously hypothesized that exacerbation of allergic inflammation mediated an allergic asthmatic's (AA) increased susceptibility to O₃, we have shown that sputum from AA displays evidence of an augmented innate immune response to O₃ exposure compared to healthy volunteers (HV) (1, 2).

The IL-1 protein family members, including IL-1 α and IL-1 β , are important regulators of inflammatory responses. Both IL-1 α and IL-1 β signal through IL-1 receptor type 1 (IL-1R1) with downstream signaling leading to NF κ B and AP-1-dependent transcription of pro-inflammatory genes, including genes for IL-8 and IL-6 (3). In the lung, IL-1 β is produced by epithelial cells, macrophages, and mast cells and is notably upregulated in asthmatics (4, 5). O₃ exposure of rat alveolar macrophages increases expression of IL-1 α and IL-1 β and promotes secretion of pro-inflammatory cytokines from epithelial cells (6). Similarly, mice lacking IL-1R1 have reduced expression of IL-6 and MIP-2 (a murine IL-8 homolog) after

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subacute O₃ exposure (7), suggesting that signaling by either or both IL-1 α and IL-1 β are important regulators of the airway response to O₃. IL-1 receptor antagonist (IL-1ra) is an anti-inflammatory protein released by monocytes and macrophages in response to IL-1 β that blocks IL-1 signaling and mitigates a hyper-inflammatory state (8).

We previously demonstrated that, compared to HV, AA have elevated baseline and post-O₃ sputum levels of IL-1 β and elevated post-O₃ sputum neutrophilia (1). Recently, we have shown that airway IL-1 responses to inhaled lipopolysaccharide (characterized by sputum neutrophilia and pro-inflammatory cytokine production) can be safely and effectively reduced by systemic administration of recombinant IL-1ra in HV (9). Due to similarities between airway inflammatory responses between O₃ and LPS exposure, we hypothesized that the IL-1 axis is a key regulator of O₃-induced airway inflammation. To explore this, we performed a retrospective correlation analysis of a subset of subjects from a previously published cohort of HV and AA before and 4 hours after the end of a 2 hour 0.4 ppm O₃ exposure (1, 2). The subset of subjects was restricted to those with available sputum samples for further cytokine level analyses (HV, n=21; AA, n=18). Demographics and induced sputum cellularity for this subset are available in Table E1 of the online repository. We compared previously measured sputum neutrophilia, IL-1 β , and IL-8 levels with newly measured sputum IL-1ra and IL-1 α levels. IL-1 α and IL-1ra levels were determined by ELISA (Mesoscale Discovery, Gaithersburg, MD, and R&D Systems, Minneapolis, MN, respectively). Spearman correlation analyses were performed to assess the association of O₃-induced changes in IL-1 α and IL-1 β levels on the percentage of neutrophils of total sputum leukocytes (%Neutrophils), Neutrophils/mg of sputum, and IL-8 and IL-1ra levels in each cohort in response to O₃ exposure. Criterion for significance was taken to be $p < 0.05$.

We first assessed the relationship of IL-1 α and IL-1 β with %Neutrophils and Neutrophils/mg in response to O₃ exposure. We found that changes in IL-1 β had a significant and positive correlation with changes in %Neutrophils in AA (Figure 1B), but not in HV ($r=-0.06$, $p=0.8$). There was no association between IL-1 α and %Neutrophils or Neutrophils/mg in either HV or AA (Table 1), perhaps because there was no significant change in IL-1 α levels after O₃ exposure within each cohort, nor in comparison to each other (9.7 (-1356 to 2108) versus 75.37 (-1879 to 1218) pg/mL [IL-1 α , median (range)], respectively). Because IL-8 is such a strong chemokine for neutrophils, and because IL-1 is known to upregulate IL-8 expression, we next assessed the relationship of IL-1 α and IL-1 β with IL-8 in response to O₃ exposure. Whereas changes in IL-1 α had no correlation with changes in IL-8 in either HV or AA, changes in IL-1 β in response to O₃ was significantly and positively correlated with changes in IL-8 in both HV and AA (Table 1). These data suggest that IL-1 β is more strongly associated with pro-inflammatory signals in response to O₃ than IL-1 α . Furthermore, these data suggest that O₃-induced IL-1 β is a stronger pro-inflammatory signal in AA than in HV. Interestingly, O₃-induced changes in IL-8 are significantly correlated with changes in %Neutrophils (Figure 1D) and Neutrophils/mg in AA ($r=0.6$, $p=0.02$) but not HV ($r=0.1$, $p=0.68$ for %Neutrophils; $r=0.2$, $p=0.45$ for Neutrophils/mg).

Given that IL-1ra is a natural antagonist to IL-1 signaling, we measured the levels of IL-1ra in the sputum after O₃ exposure. There was no significant change in sputum IL-1ra levels

after O₃ exposure within either HV or AA cohorts, nor in comparison to each other (–10 (–70 to 219) versus –24 (–181 to 114) ng/mL [IL-1ra, median (range)], respectively). Because IL-1 β has been shown to increase IL-1ra secretion (8), we next investigated if there were any correlations between O₃-induced changes in IL-1 β and IL-1ra. There was no significant correlation between O₃-induced changes in IL-1 β or IL-1 α with changes in IL-1ra in either HV or AA (Table 1). We found that O₃-induced changes in IL-1 α were positively correlated with IL-1ra in AA (p=0.05), but not in HV. IL-1 α -induced expression of IL-1ra in airway cells is not well described, therefore the clinical relevance of this suggestive finding is unclear. In conclusion, our data support the notion that IL-1 β is associated with airway neutrophil recruitment in AA after O₃ exposure, which may be mediated through regulation of IL-8. O₃-induced neutrophilia was uncoupled from both IL-1 β and IL-8 responses in HV, but not in AA. This is consistent with our previous report, where we found that despite similarities in neutrophil and macrophage proportions in induced sputum samples after O₃ exposure, gene array profiles from AA showed increased innate immune signaling involving the NF κ B pathway (1, 2), while HV attempted to mitigate the airway response to O₃.

A limitation to the interpretation of these data for asthmatics is the early 4 hour timepoint analyzed after O₃ exposure; the kinetics of airway inflammation have been well studied in HV (E1) but not in asthmatics. Although airway neutrophilia peaks 6 hours after O₃ exposure in HV, most asthma exacerbations occur the day after exposure to O₃. Others have found that asthmatics requiring inhaled corticosteroids had increased airway neutrophilia 18 hours after O₃ and evidence of mast cell inflammation (E2), though the degree of airway inflammatory responses was not compared to earlier or later timepoints. It is noteworthy that even at the early 4 hour timepoint after O₃ exposure, we saw augmentation of the innate immune response in AA. We suspect that the primed inflammatory state in AA airways with elevated baseline IL-1 β levels was further exacerbated after O₃ through the IL-1 pathway. Further investigation of IL-1 β regulation and kinetics of airway inflammatory responses in AA airways will provide key insights into mechanisms of environmentally-induced asthma exacerbations that can guide the development of focused therapeutic targets for susceptible populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Changes in IL-1 β and IL-8 correlate with changes in %Neutrophils in sputum from AA. Linear regression with Spearman correlation analysis of O₃-induced changes in sputum IL-1 β and %Neutrophils in HV (A) and AA (B); correlations of O₃-induced changes in sputum IL-8 and %Neutrophils in HV (C) and AA (D)

Table 1

Correlation of sputum IL-1 α and IL-1 β levels with PMNs/mg, IL-1ra, and IL-8

Cohort	Independent analyte	Neutrophils/mg		IL-8		IL-1ra	
		R	P	R	P	R	P
HV	IL-1 α	0.3	0.2	-0.01	0.97	0.39	0.08
	IL-1 β	-0.02	0.9	0.84	<0.0001*	0.01	0.96
AA	IL-1 α	0.3	0.19	0.04	0.89	0.48	0.05
	IL-1 β	0.4	0.09	0.71	0.001*	0.42	0.08

Spearman correlation analyses were performed to assess the relationship of sputum IL-1 β & IL-1 α levels with Neutrophils/mg of sputum, IL-1ra, and IL-8, and IL-6 levels in HV and AA subjects.