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Ozone activates pulmonary dendritic cells and promotes allergic sensitization by a TLR4-dependent mechanism

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Abstract

Human exposure to ozone is associated with increased prevalence of allergic asthma. Here, we demonstrate that ozone increases levels of activated dendritic cells in thoracic lymph nodes and promotes allergic sensitization through a TLR4-dependent pathway.

Keywords

ozone; toll-like receptor; asthma; dendritic cells; sensitization; adjuvant

To the Editor

Ozone is a highly reactive component of air pollution, formed by the interaction of sunlight with oxygen, nitrous oxides and volatile organic compounds. Human exposure to air pollution, including ambient ozone, is associated with several health problems, including increased incidence and severity of allergic airways disease. Ozone might exacerbate existing asthma by activating monocytes and macrophages,¹ which could in turn activate allergen-specific memory T cells. However, the ability of ozone to increase the prevalence of *de novo* asthma likely involves additional cell types because monocytes and macrophages are not efficient stimulators of naïve T cells. Effective naïve T cell stimulation is generally thought to require activated dendritic cells (DCs),² which display very high levels of the major histocompatibility complex and also provide strong co-stimulatory signals, such as CD86 (B7.2). Recently, DCs residing in the airway were found to be activated by ozone,³ suggesting that these DCs might promote *de novo* asthma by stimulating naïve T cells. Although naïve T cell stimulation might occur within the lung, a more widely-held view is that antigen-bearing DCs migrate from the lung to draining thoracic lymph nodes (LNs) for antigen presentation to naïve T cells. However, the impact of ozone on LN DCs has not been reported, and it is not known if ozone promotes allergic sensitization through the toll-like receptor (TLR)4, which is required for some, but not all, biological responses to inhaled ozone.^{4, 5}

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Airway instillation of highly purified ovalbumin (OVA) leads to OVA-specific tolerance, not allergic sensitization.⁶ However, instillation of OVA together with 0.1 µg bacterial lipopolysaccharide (LPS-OVA) results in allergic sensitization to OVA.⁷ Thus, LPS can act as an adjuvant in the airway. The increased prevalence of asthma in areas with high ozone levels suggests that ozone might also function as an adjuvant in the airway and thereby promote sensitization to innocuous inhaled antigens. To test this, we performed a series of experiments in a murine model of asthma (Fig 1, A), using OVA that lacks detectable levels of LPS (Hyglos GmbH, Regensburg, Germany). As seen previously⁸, C57BL/6 mice exposed to filtered air prior to oro-pharyngeal aspiration of OVA on days 0 and 6 did not become sensitized because they had negligible accumulation of eosinophils and neutrophils in the airway following subsequent challenge for 1 h with an aerosol of 1% OVA on day 13 (Fig 1, B). However, mice given OVA immediately after exposure to 1 ppm ozone for 2 h⁴ (ozone-OVA) became sensitized, as indicated by their increased levels of airway eosinophils and neutrophils following OVA challenge. These mice also displayed increased levels of serum IgE (Fig 1, C), and had increased levels of the Th2 cytokines, IL-4, IL-5 and IL-9, in lung homogenates (Fig 1, D). As seen previously for LPS-OVA sensitized mice,⁸ ozone-OVA also promoted Th17 responses, as indicated by levels of IL-17 in lungs of challenged mice.

Some, but not all, biological responses to inhaled ozone are dependent on the toll-like receptor, (TLR)4.^{4, 5} We therefore used *tlr4*-deficient mice on a C57BL/6 genetic background to test whether ozone-OVA mediated allergic sensitization through the airway is TLR4-dependent. As expected, these mice were refractory to LPS-OVA sensitization (Fig 1), because TLR4 is required for signaling responses to LPS. Interestingly, *tlr4*-deficient mice were also refractory to ozone-OVA sensitization, as evidenced by their low levels of airway inflammation, serum IgE and cytokines upon subsequent challenge with aerosolized OVA. Therefore, ozone-OVA sensitization occurs through a TLR4-dependent mechanism.

We next studied the impact of ozone on OVA-bearing DCs that migrate from the lung to thoracic lymph nodes (LNs) in WT mice. To track these cells *in vivo*, we used the fluorescent dye, Alexa Fluor (AF) 647, to label OVA (OVA-AF), and delivered it to airways of mice. On the following day, cells were prepared from draining thoracic LNs of OVA-AF treated mice and analyzed by flow cytometry. DCs were identified as live, non-autofluorescent cells bearing the cell surface marker CD11c (Fig. 2, A). Within this DC gate, a subset of OVA-AF⁺ cells was seen (Fig 2, B). The fraction of LN DCs that contained OVA-AF was similar in all OVA-AF treated mice (Fig 2, B), but the total number of AF⁺ DCs, and therefore the percentages of total LN cells that were AF⁺ DCs, were increased in mice that also received LPS (Fig 2, C). These increases were not seen in ozone-treated mice, suggesting that LPS, but not ozone, promotes the migration of DCs to draining LNs.

We next analyzed co-stimulatory molecules on the surface of OVA-AF⁺ DCs. The percentage of total DCs positive for MHCII, CD40, or CD86 were increased in mice receiving LPS-OVA, but not in mice receiving ozone-OVA (Fig. 2, D), in agreement with the ability of LPS, but not ozone, to increase the trafficking of DCs from the lung to draining LNs. However, both ozone and LPS increased levels of CD86 on the cell surface of LN DCs, compared to their counterparts in mice exposed to filtered air. By contrast, LPS, but not ozone, increased levels of CD83 (data not shown). Neither treatment increased levels of MHCII or CD40. Although no single molecule or set of molecules can accurately predict the immunostimulatory capacities of DCs,⁹ CD86 is generally associated with Th2 responses.^{10, 11} Our findings are in agreement with a report that CD86, but not CD83, is increased on monocyte-derived DCs from asthmatics compared to DCs from non-asthmatics.¹² Additional experiments will be required to fully understand the relationship between ozone-induced CD86 on LN DCs and ozone-mediated allergic sensitization through the airway. For example, it will be important to determine if endogenous TLR4 ligands mediate ozone-induced allergic sensitization, as they likely do in

ozone-induced lung injury,¹³ if ozone-activated DCs can promote Th2 differentiation *ex vivo*, and if ozone-OVA sensitized mice develop airway hyperresponsiveness (AHR) following OVA challenge. Nonetheless, our current finding that ozone can activate pulmonary DCs and promote allergic sensitization through the airway provides a plausible explanation for the increased prevalence of asthma in areas with high levels of air pollution.

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Abbreviations

DCs	dendritic cells
LN	lymph nodes
LPS	lipopolysaccharide
OVA	ovalbumin
TLR	toll-like receptor
MFI	mean fluorescent intensity
Th2	T helper 2
AF	Alexa Fluor
BAL	bronchoalveolar lavage

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FIG 1. Ozone functions as a TLR4-dependent adjuvant in the airway

(A). Timeline. Mice were sensitized at the indicated times by airway delivery of OVA together with LPS, or immediately after exposure of the mice to either ozone or filtered air (air). Two days after challenge with aerosolized OVA, mice were euthanized, blood collected for measures of total IgE, and bronchoalveolar lavage (BAL) performed. (B) Leukocyte recruitment to the airway, (C) total serum IgE levels and (D) cytokines in lung homogenates of WT (black rectangles) or *tlr4*-deficient (open rectangles) mice following allergic sensitization and challenge. Data shown are from one of three experiments yielding similar results. Standard errors are shown. * $p < 0.05$ by student's T-test ($n = 5/\text{group}$)

FIG 2. Ozone increases CD86 expression on LN DCs

OVA-AF was delivered to recipient mice together with 0.1 μ g LPS, or following exposure of the mice to ozone or filtered air. DCs in thoracic LNs were identified as live, non-autofluorescent cells displaying the cell surface marker, CD11c (A). OVA-AF⁺ DCs were further identified as a subset of DCs within the CD11c⁺ gate (B). OVA-AF⁺ DCs, presented as total cell number, and as a percentage of total LN cells are shown for each OVA-AF treatment group (C). OVA-AF⁺ DCs that also stained with antibodies against the indicated co-stimulatory molecules are presented as a percentage of total LN cells (D). Display levels (MFI) of individual co-stimulatory molecules are also shown. Data are from one of two experiments yielding similar results. Standard errors are indicated. * $p < 0.05$ by student's T-test ($n = 7/\text{group}$)