



STATEMENT

Synopsis of Research Report 204

HEALTH
EFFECTS
INSTITUTE

Effects of Ozone on Acute Lung Inflammation and Injury in Mice

BACKGROUND

Ozone is one of the six criteria pollutants regulated by the U.S. Environmental Protection Agency under the Clean Air Act. Given that importance, investigation into what biological mechanisms underlie ozone's effects continues to be of scientific and policy interest. Currently, the precise mechanisms by which acute exposure to ambient ozone triggers inflammation in the airways are not well understood.

In this study, Dr. Kymberly Gowdy, a recipient of HEI's Walter A. Rosenblith New Investigator Award, and her colleagues evaluated how acute exposure to ozone affected markers of inflammation and injury in the lung, both during initiation and resolution of the response. Gowdy and colleagues were particularly interested in evaluating two features of the resolution phase: the role of specialized pro-resolving mediators, lipid mediators that act as a key signal to switch to the resolution phase; and efferocytosis, the process by which cells that have been activated during the inflammatory response and are facing cell death (apoptosis) are removed by macrophages. The latter process helps the lung return to baseline (homeostasis), preventing detrimental effects if inflammation were to continue (see Statement Figure).

APPROACH

The investigators exposed mice to 1 ppm ozone for 3 hours and evaluated effects in the lungs mostly at 24 hours after the end of exposure. They used standard techniques to measure markers of inflammation — including levels of leukocytes, macrophages, and cytokines and chemokines — as well as injury (protein leak) in lung fluid and tissue. They also developed a sensitive high-performance liquid chromatography tandem mass spectrometry

technique to measure low levels of specialized lipid mediators in homogenized lung tissue. Because the rapid reaction of ozone with components of the respiratory tract produces oxidized phospholipids, which in turn can induce an inflammatory response, the investigators also measured levels of oxidized phospholipids in lung homogenates.

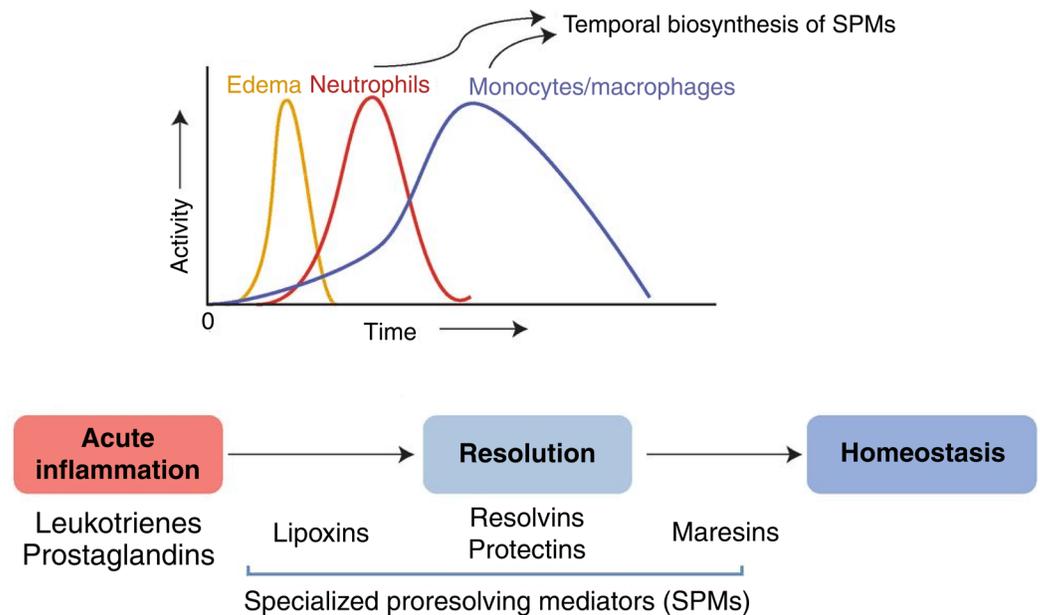
To assess efferocytosis, Gowdy and colleagues gave mice an easily visualized T cell line undergoing cell death by apoptosis by dispensing the cells into the back of the throat 24 hours after ozone exposure

What This Study Adds

- The study evaluated how acute exposure of mice to ozone affects initiation and resolution of the inflammatory response in the lung.
- It is the first to examine the role of specialized pro-resolving mediators, key lipids of the resolution phase of inflammation, in how lung cells are activated and later removed by macrophages.
- Acute ozone exposure resulted in changes in levels of specialized pro-resolving mediators and other markers of lung inflammation and injury in mice. Supplementation of mice with specialized pro-resolving mediators before ozone exposure decreased some of those markers.
- This provides a rationale for future research to evaluate whether supplementation with specialized pro-resolving mediators may mitigate human conditions that involve chronic inflammation, such as chronic respiratory and cardiovascular diseases.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Kymberly M. Gowdy at East Carolina University (currently at the Ohio State University College of Medicine, Columbus, Ohio), and colleagues. The complete report, *Novel Mechanisms of Ozone-Induced Pulmonary Inflammation and Resolution, and the Potential Protective Role of Scavenger Receptor BI* (© 2021 Health Effects Institute), can be obtained from HEI or our website (see last page).

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Statement Figure. Key events in the induction and resolution of the inflammatory response. (Adapted with permission from Serhan CN, Chiang N, Dalli J, Levy BD. 2014. Lipid mediators in the resolution of inflammation. *Cold Spring Harb Perspect Biol* 7:a016311; © Cold Spring Harbor Laboratory Press.)

so that they would move directly into the airspace. The investigators then isolated alveolar macrophages from these mice and microscopically evaluated what percentage of macrophages had taken up the T cell line. Because oxidized phospholipids bind to scavenger receptor (SR)-BI that is expressed on macrophages and many other cells, the investigators also evaluated mice lacking the SR-BI receptor (SR-BI knockout mice). They also administered specialized pro-resolving mediators prior to ozone exposure and measured their levels as well as markers of lung inflammation and injury.

KEY RESULTS AND INTERPRETATION

Exposure to 1 ppm ozone for 3 hours resulted in increases in some of the expected markers of a standard inflammatory response at 24 hours. For example, Gowdy and colleagues reported increases in numbers of macrophages and neutrophils in lung fluid, as well as in levels of pro-inflammatory cytokines and chemokines in lung tissue. Ozone exposure did not consistently affect lung injury; protein leak increased 2-fold, but only in male mice.

Levels of specialized lipid mediators increased at 6 hours, decreased at 24 hours, and were back to baseline levels 72 hours after mice were exposed to ozone, compared with mice exposed to filtered air. Levels of

oxidized phospholipids increased approximately 2- to 3-fold at 6 hours after ozone exposure. Effects of ozone on efferocytosis were difficult to interpret: one experiment in female mice showed a decrease, but effects in male mice were variable.

Pretreatment of mice with specialized pro-resolving mediators prior to ozone exposure decreased levels of some markers of the inflammatory response, such as neutrophils and macrophages in lung fluid, and of some pro-inflammatory cytokines and chemokines in lung tissue compared with levels of those markers in mice that had not been pretreated. Thus, pretreatment appeared to mitigate some of the inflammatory effects of ozone exposure.

The effects of ozone on the inflammatory response and efferocytosis were similar in mice that either expressed or did not express the receptor SR-BI, apart from an increase in neutrophils in mice lacking SR-BI.

HEI REVIEW COMMITTEE EVALUATION

In its independent review, the HEI Review Committee considered the work by Gowdy and colleagues in ozone-exposed mice to be an interesting new approach for evaluating the events involved in both the early and later phases of the inflammatory response (reflecting initiation and resolution) after acute exposure to ozone.

In particular, the study was a valuable initial attempt to understand the role of different types of lipid mediators produced during those early and later phases of inflammation.

The investigators successfully used a sensitive technique to identify and quantify low levels of oxidized phospholipids that were generated early in the response to ozone, as well as specialized pro-resolving lipid mediators that play a key role in the resolution of the inflammatory response. In addition, the investigators showed that pre-treating mice with lipid mediators prior to ozone exposure partially mitigated the resulting inflammatory response. These results offer the possibility that pretreatment with lipid mediators can be used in a clinical or dietary setting to offset inflammation induced by air pollutants or perhaps even pathogens.

The investigators concluded that the SR-BI receptor plays a protective role in either ozone-induced inflammation or resolution of the response. However, the Committee disagreed, based on findings that most markers of the inflammatory and injury response were similar in mice that did or did not express SR-BI, and that the effect of ozone on efferocytosis was almost identical in these two sets of mice.

The Committee noted several important limitations in the study design that reduced confidence in the generalizability of the results. One major limitation was

that the investigators used only one concentration of ozone, 1 ppm. Based on uptake of radioactive ozone by lung cells of rodents and humans, this exposure concentration of 1 ppm in mice was estimated to correspond to a fairly high human exposure concentration of 200 ppb (or 42.8 $\mu\text{g}/\text{m}^3$) of ozone. A further limitation of the study design was that the investigators measured only a limited set of markers of inflammation without performing histopathology, which would have shown both the extent of inflammatory damage and injury to multiple lung cell types, and how the damage and injury might have resolved. The Committee also considered the results and interpretations of the efferocytosis assay to be of uncertain significance, because the system used to evaluate efferocytosis did not clearly model the process in the body by which activated cells are removed during the inflammatory response.

In summary, the Committee thought this study provided a good foundation for further research to assess the role of specialized lipid mediators in mitigating inflammatory responses. Given that exposure to ozone exacerbates chronic inflammatory conditions such as asthma and cardiovascular disease, it will be worth exploring whether ozone affects the resolution of inflammation in these conditions, and whether enhancement of lipid mediator levels through diet or other interventions may be clinically useful in mitigating such conditions.

Novel Mechanisms of Ozone-Induced Pulmonary Inflammation and Resolution, and the Potential Protective Role of Scavenger Receptor BI

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INVESTIGATORS' REPORT *by Gowdy et al.*

Abstract

Background

Aim 1: Role of Scavenger Receptor BI in O₃-Induced Pulmonary Inflammation and the Resolution of Injury

Aim 2: Role of O₃ in the Resolution of Lung Inflammation

Overall Conclusions

Limitations

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Materials Available on the HEI Website

About the Authors

Other Publications Resulting from this Research

CRITIQUE *by the Review Committee*

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Summary of the Study

HEI Review Committee Evaluation