STATEMENT

Synopsis of Research Report 191

HEALTH EFFECTS INSTITUTE

Protective Role of Eosinophils and TNFα after Ozone Inhalation

INTRODUCTION

Exposure to ozone induces deleterious responses in the airways that include shortness of breath, inflammation, and bronchoconstriction. People with asthma have increased airway sensitivity to ozone and other irritants. Dr. Allison Fryer and colleagues addressed how exposure to ozone affects the immune and physiological responses in guinea pigs. Guinea pigs are considered a useful animal model for studies of respiratory and physiological responses in humans; their response to airborne allergens is similar to that in humans and shares some features of allergic asthma.

Fryer and colleagues had previously observed that within 24 hours of exposure, ozone not only induced bronchoconstriction but also stimulated the production of new cells in the bone marrow, where all white blood cells develop. As a result of ozone exposure, increased numbers of newly synthesized white blood cells, particularly eosinophils, moved into the blood and lungs.

The central hypothesis of the current study was that newly synthesized eosinophils recruited to the lungs 3 days after ozone exposure were beneficial to the animals because they reduced ozone-induced bronchoconstriction. The investigators also hypothesized that the beneficial effect seen in normal (*nonsensitized*) animals was lost in animals that had been injected with an allergen, ovalbumin (*sensitized*). They also planned to explore the effects of inhibitors of certain cytokines (cell-signaling molecules).

Immune responses in sensitized animals are dominated by a *Th2 pattern*, which is characterized by the synthesis of cytokines (interleukin [IL]-4, IL-5, and IL-13) and the Th2 subset of $CD4^+$ T lymphocytes and the cells they activate (predominantly eosinophils, and B lymphocytes that switch to making immunoglobulin E [IgE]). Thus, sensitized animals were used as a model of allergic humans, whose immune responses tend to be dominated by IgE.

APPROACH

Fryer and colleagues exposed normal and sensitized (allergic) guinea pigs to 2 ppm ozone or filtered air for 4 hours and measured changes in cell numbers and airway responses 1 or 3 days later. They counted the numbers of eosinophils and other white blood cells (macrophages, neutrophils, and lymphocytes) in bone marrow, blood, and bronchoalveolar lung lavage fluid. The investigators

What This Study Adds

- Eosinophils are white blood cells that play an important role in inflammation, allergies, and allergic asthma, and can modify the airway response to ozone inhalation. This study tested a novel hypothesis: that allergic guinea pigs react differently to ozone than normal animals because of newly synthesized eosinophils that travel from bone marrow to the lungs.
- The study confirmed that newly formed eosinophils found in the lungs 3 days after ozone exposure had a beneficial effect in normal, but not allergic, animals. Tumor necrosis factor-alpha may be involved in the regulation of eosinophil synthesis and movement.
- These findings suggest how the responses to ozone may differ in people who have allergies or asthma from those in people who do not.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Allison D. Fryer at the Division of Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, Oregon, and colleagues. The complete report, *Protective Role of Eosinophils and Tumor Necrosis Factor-α after Ozone Inhalation* (© 2017 Health Effects Institute), can be obtained from HEI or our Web site (see next page).

also measured important physiological responses, including bronchoconstriction. Some animals were pretreated with etanercept and monoclonal anti-IL-5, which block tumor necrosis factor-α (TNFα) and IL-5, respectively. TNFα and IL-5 blockers have been used to treat patients with asthma.

A key feature of the study was a technique to distinguish which white blood cells were synthesized after exposure from those that already existed, by injecting animals with bromodeoxyuridine (BrdU). BrdU is a thymidine analogue that is incorporated into the DNA of dividing cells, serving as a marker of newly produced cells. Therefore, a snapshot can be obtained of the proportion of newly synthesized (BrdU-positive) versus pre-existing (BrdU-negative) cell types.

KEY RESULTS

1. *Allergic and normal animals differed in the time course of bronchoconstriction and changes in cell types after ozone exposure.* In normal animals, bronchoconstriction increased substantially at day 1 but decreased by day 3 after ozone exposure. In contrast, in allergic animals bronchoconstriction remained high at day 3. Ozone also increased the percentage of newly formed, BrdU−positive eosinophils in the bone marrow and lungs of normal but not allergic animals.
2. *Pretreatment with the TNFα blocker etanercept had complex effects, which differed between normal and allergic animals.* In normal animals, etanercept decreased ozone-induced new synthesis of eosinophils in the bone marrow and blocked eosinophil migration to the lung; it also increased bronchoconstriction at day 3 (relative to day 1 without etanercept). In allergic animals, etanercept had no effect on any cell type in the bone marrow or lung after exposure to ozone and did not change bronchoconstriction compared with allergic animals not treated with etanercept.

   Etanercept tended to increase the numbers of blood monocytes and lymphocytes in air- and ozone-exposed normal and allergic animals at day 3, but had no effect on eosinophils in blood at this time point. This was one of the few statistically significant findings in the blood of exposed animals in the study.
3. *Anti-IL-5 reduced bronchoconstriction at day 3 after exposure of allergic animals to ozone.* In contrast, bronchoconstriction was greatly increased in normal animals treated with anti-IL-5.

CONCLUSIONS

Fryer and colleagues explored the airway and cellular responses in guinea pigs exposed to ozone. The HEI Review Committee, which conducted an independent review of the study, agreed that the findings supported the authors' hypothesis (1) that exposure to ozone stimulates production of eosinophils in bone marrow, (2) that these newly formed eosinophils migrate to the lungs, and (3) that those eosinophils play a delayed but potentially beneficial role in reducing ozone-induced inflammation in the airways of healthy normal animals, but not in allergen-sensitized animals. The Committee also agreed that guinea pigs were a good model for studying responses to an allergen, because a major subtype of asthma (the high Th2 or allergic type) is associated with high levels of eosinophils in the blood.

A novel finding was that the TNFα blocker etanercept decreased ozone-induced formation of eosinophils in the bone marrow and blocked eosinophil migration to the lung in normal animals. However, because injecting etanercept had little effect on eosinophils and did not decrease bronchoconstriction in allergic guinea pigs, the potential for treating patients with allergic asthma with TNFα blockers is uncertain. This is consistent with the poor performance of TNFα blockers in clinical studies of asthma treatment.

Blocking the cytokine IL-5 with an anti-IL-5 antibody substantially decreased bronchoconstriction in sensitized animals. This suggests that therapies targeting IL-5 and eosinophils would be promising in at least some types of asthma. The Committee expressed caution toward experiments with cytokine blockers, both in animal models and humans, because such blockers are often not specific to a particular cell type and may differ at different sites in the body. Without further detailed confirmation of the effects of the blockers, interpreting these experiments can be challenging.

The Committee concluded that the study by Fryer and colleagues raises several intriguing directions for future research, including exploring ways in which newly formed eosinophils differ from pre-existing ones, and how such findings apply to humans with allergy or asthma.

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