

# Impact of Diesel Exhaust Particles on Th2 Response in the Lung in Asthmatic Mice

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**Summary** Although it has been accepted that pulmonary exposure to diesel exhaust particles (DEP), representative constituents in particulate matter of mass median aerodynamic diameter < or 2.5  $\mu\text{m}$  (PM<sub>2.5</sub>), exacerbates murine allergic asthma, the *in vivo* effects of DEP on their cellular events in the context of allergen-specific Th response have never been examined. The aim of this study is to elucidate whether *in vivo* repetitive exposure to DEP combined with allergen (ovalbumin) facilitate allergen-specific Th response in the lung using a simple *ex vivo* assay system. As a result, repetitive pulmonary exposure to DEP *in vivo*, if combined with allergen, amplifies *ex vivo* allergen-specific Th2 response in the lung compared to that to allergen alone, characterized by high levels of interleukin (IL)-4 and IL-5. The result suggests that in asthmatic subjects, DEP promote Th2-prone milieu in the lung, which additively/synergistically augment asthma pathophysiology *in vivo*.

**Key Words:** diesel exhaust particles, asthma, Th2 response

## Introduction

We experimentally demonstrated that repeated pulmonary exposure to diesel exhaust particles (DEP), representative constituents in particulate matter of mass median aerodynamic diameter < or 2.5  $\mu\text{m}$  (PM<sub>2.5</sub>), exacerbates murine asthma [1, 2]. In these studies, we have shown that DEP enhance lung expression of Th2 cytokines (interleukin [IL]-4, IL-5) with corresponding specific Ig production in the presence of allergen [1], suggesting that the exacerbation is likely accompanied by amplified Th2 response in the lung. In contrast, it cannot be excluded that the observation resulted from a larger number of recruited effector leukocytes such as lymphocytes, mast cells/basophils, and eosinophils into the lung in DEP plus allergen-treated mice than in allergen alone-treated mice. Here, we report the effects of

*in vivo* DEP exposure on asthma in the context of allergen-specific Th responses in the lung using a simple *ex vivo* assay system.

## Materials and Methods

### Methods

The studies were carried out in accordance with the Guide for the Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health. ICR mice (Japan Clea Co., Tokyo, Japan), were divided into three experimental groups, and intratracheally exposed to phosphate-buffered saline (PBS: Invitrogen Co., Carlsbad, CA) containing Tween 80 (Nakalai Tesque, Kyoto, Japan), ovalbumin (OVA; grade IV, Sigma Chemical, St. Louis, MO) dissolved in the same vehicle, or OVA plus DEP, suspended in the same vehicle, as described previously [3, 4]. Exposure to these solutions was given every 2 week for 6 weeks, followed by a rest for approximately 8 weeks, and the animals were then re-exposed every 2 weeks for 6 weeks according to the protocol by Fattouh *et al.* [5], with

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Table 1. Effects of *in vivo* exposure to diesel exhaust particles (DEP) on Th2 response in the lung *in vitro*.

Group	IL-4	IL-5 pg/ml	IFN- $\gamma$
vehicle	5.45 $\pm$ 1.67	0.47 $\pm$ 0.27	10.3 $\pm$ 3.03
OVA	6.09 $\pm$ 1.00	1.49 $\pm$ 0.15	11.6 $\pm$ 1.40
OVA plus DEP	9.23 $\pm$ 1.32**	2.97 $\pm$ 0.15***	12.8 $\pm$ 1.81

\* $p < 0.05$  vs vehicle-instilled mice, \*\* $p < 0.01$  vs vehicle-instilled mice; # $p < 0.05$  vs ovalbumin (OVA)-instilled mice. Values are the mean  $\pm$  SEM.

modification. Five h after the last exposure, mice were sacrificed and the lungs were removed. Five h after the last intratracheal administration, mice were sacrificed and the lungs were collected for *ex vivo* experiments.

#### Lung cells culture

Lung cells were isolated and resuspended in R10, which was RPMI 1640 medium (GIBCO BRL, Eggenstein, Germany) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan), 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin (Sigma, St. Louis, MO). Lung cells ( $2.5 \times 10^6$  cells/ml) were cultured in R10 with or without OVA (400  $\mu$ g/ml) for 5 d. Thereafter, supernatants were collected for cytokine measurement as previously conducted [3].

#### Cytokine measurements

Levels of IL-4 (Amersham, Buckinghamshire, UK), IL-5 (Endogen, Cambridge, MA), IL-13 (R&D systems, Minneapolis, MN), and interferon (IFN)- $\gamma$  (R&D systems) were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions. The secondary antibodies were conjugated to horseradish peroxidase. Subtractive reading of 550 nm from the reading at 450 nm was converted to pg/ml using values obtained from standard curves generated with varying concentrations of recombinant IL-4, IL-5, IL-13, and IFN- $\gamma$  with limits of detection of 5 pg/ml, 5 pg/ml, 1.5 pg/ml, and 10 pg/ml, respectively ( $n = 6$  for IL-4,  $n = 14$ –15 in each group, respectively).

#### Statistical analysis

Data were reported as mean  $\pm$  SEM. Differences were determined using analysis of variance (Stat view version 4.0; Abacus Concepts, Inc., Berkeley, CA). If differences between groups were significant ( $p < 0.05$ ), Bonferroni's protected least significant difference test was used to distinguish between pairs of groups.

## Results and Discussion

As a result, both levels of Th2 cytokines produced by lung

cells from the DEP + OVA group were higher than those from the vehicle (Table 1;  $p < 0.05$  in IL-4;  $p < 0.01$  in IL-5) or the OVA ( $p < 0.05$ ) group after *in vitro* stimulation with OVA. OVA-stimulated IFN- $\gamma$  production was not different among cells from the experimental groups.

This cutting-edge study, for the first time, demonstrated that repetitive pulmonary exposure to DEP *in vivo*, if combined with allergen, enhances *ex vivo* allergen-specific Th2 response in the lung compared to that to allergen alone. This implies that in asthmatic subjects, DEP promote Th2-prone milieu as well as infiltration of inflammatory leukocytes in the lung as we previously reported [1, 3]; in other words, both mechanisms may additively/synergistically augment asthma pathophysiology *in vivo* and perhaps in humans.

## References

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