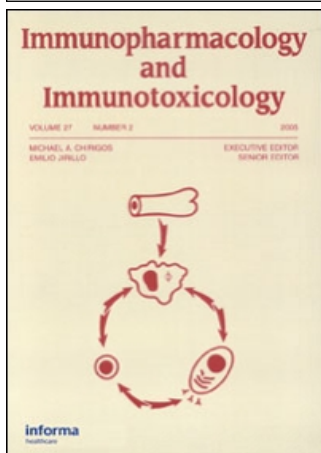


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Effects of Components Derived from Diesel Exhaust Particles on Lung Physiology Related to Antigen

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Our previous study has shown that diesel exhaust particles (DEP), main constituents in ambient particulate matters (PM), enhance airway hyperresponsiveness in a murine model of allergic asthma (Takano et al., 1998). However, it remains unknown which components in DEP are responsible for the enhancement. The present study investigated the effects of repeated pulmonary exposure to DEP components (extracted organic chemicals in DEP; DEP-OC, carbonaceous nuclei of DEP after extraction; washed DEP) on lung physiology in the presence or absence of antigen. ICR mice were divided into six experimental groups. Vehicle, DEP components, ovalbumin (OVA), or DEP components plus OVA was administered intratracheally for 6 weeks. Twenty-four hr after the last instillation, cholinergic lung reactivity was examined. DEP components alone did not induce any facilitation of lung function as compared to vehicle alone. The values of total respiratory system resistance (R), elastance (E), Newtonian resistance (R_n), tissue damping (G), and tissue elastance (H) were higher and the value of compliance (C) was lower in the OVA or the DEP component+OVA groups than in the vehicle group. In particular, the hyperreactivity was most prominent in the washed DEP+OVA group. The values in the DEP-OC+OVA group were not significantly different from those in the OVA group. These data suggest that carbonaceous component in DEP, rather than organic chemical one, can be attributable to the enhancement of lung hyperresponsiveness in allergic asthma.

Keywords Diesel Exhaust Particles, Components, Antigen, Airway Hyperresponsiveness.

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INTRODUCTION

Previous epidemiological studies have indicated that exposure to ambient particulate matter (PM) is linked to increases in mortality and morbidity related to respiratory diseases.^(1,2) The concentration of PM with a mass median aerodynamic diameter (a density-dependent unit of measure used to describe the diameter of the particle) $\leq 10\mu\text{m}$ (PM_{10}) is related to daily hospital admissions for several respiratory diseases.⁽³⁾ Additionally, recent data have shown that PM with a mass median aerodynamic diameter $\leq 2.5\mu\text{m}$ ($\text{PM}_{2.5}$) are more closely associated with both acute and chronic respiratory effects and subsequent mortality than PM_{10} .⁽⁴⁾ In particular, diesel exhaust particles (DEP), main constituents of $\text{PM}_{2.5}$ in urban areas, reportedly affect a variety of respiratory diseases such as asthma,⁽⁵⁾ pulmonary edema,⁽⁶⁾ and lung cancer.⁽⁷⁾

Bronchial asthma is characterized by airway inflammation and reversible airflow obstruction with hyperresponsiveness. We have previously shown that DEP exacerbate airway inflammation related to antigen.⁽⁸⁾ DEP consist of carbonaceous nuclei, a vast number of organic chemicals,⁽⁹⁾ and metals.⁽¹⁰⁾ We have also demonstrated that DEP components augment the asthma model *in vivo*.⁽¹¹⁾ In brief, pulmonary exposure to DEP components has aggravated antigen-related airway inflammation characterized by infiltration of eosinophils and lymphocytes around the airway and an increase in goblet cells in the bronchial epithelium and the adverse effects have been more prominent with organic chemical components of DEP (DEP-OC) than with residual carbonaceous ones (washed DEP) with overall trend.⁽¹¹⁾

Otherwise, lung hyperresponsiveness, including the capacity of airways to constrict excessively in response to a variety of nonspecific stimuli, is another cardinal hallmark of asthma.⁽¹²⁾ Asthmatic subjects are frequently hyperresponsive to contractile agonists such as methacholine (MCh). We have also demonstrated that DEP worsen airway responsiveness related to antigen in the previous study.⁽¹³⁾ However, it remains unidentified which components in DEP are critical for the hyperresponsiveness.

The present study, therefore, was designed to determine whether organic chemical components of DEP and/or residual carbonaceous ones aggravate lung physiology in the presence or absence of antigen.

MATERIALS AND METHODS

Animals

Male ICR mice 6 to 7 weeks of age and weighing 29 to 34 g (Japan Clea Co., Tokyo, Japan) were used in all experiments. They were fed a commercial diet (Japan Clea Co.) and given water *ad libitum*. Mice were housed in an

animal facility that was maintained at 24 to 26 °C with 55 to 75% humidity and a 12-hr light/dark cycle.

Preparation of DEP

A 4JB1-type, light-duty, 4-cylinder, 2.74 liter, Isuzu diesel engine (Isuzu Automobile Company, Tokyo, Japan) under computer control was connected to a dynamometer (Meiden-sha, Tokyo, Japan). The engine was operated on standard diesel fuel at 1500 rpm under a load of 10 torques (kg/m). DEP were collected as previously described.⁽¹⁴⁾ The mass median aerodynamic diameter of DEP was 0.4 µm by analysis of an Anderson Air Sampler (Sibata Science Technology, Tokyo, Japan). Most of the particles were globular in shape.

Preparation of DEP-OC and Washed DEPs

DEP were extracted with benzene-ethanol as previously described.⁽¹¹⁾ Briefly, 10 g of DEP were suspended in 800 ml of benzene-ethanol (3:1, v/v) and were ultrasonicated for 30 min. The suspension was centrifuged at 600 g for 20 min. The supernatants were transferred to another tube, and then were filtered with membrane filter (pore size; 0.45 µm). This procedure was repeated twice. The residual particles of DEP were prepared as washed DEP. The extracts were evaporated and dissolved in 100% DMSO (Nacalai Tesque, Kyoto, Japan), and prepared as DEP-OC. They were stored at -80 °C until use. The suspension was sonicated for 3 min using an Ultrasonic disrupter (UD-201; Tomy Seiko, Tokyo, Japan).

Study Protocol

Mice were divided into 6 experimental groups (n=7–8 in each group) as exhibited in our recent study (shown as Figure 1 in the paper by Yanagisawa et al.⁽¹¹⁾). The vehicle group received PBS at pH 7.4 (GIBCO BRL, Life Technology, Grand Island, NY) containing 0.025% Tween 80 (Nacalai Tesque, Kyoto, Japan) and 0.25% DMSO. The washed DEP group or the DEP-OC group received 50 µg of washed DEP or DEP-OC in the same vehicle, respectively. The OVA group received 1 µg of OVA (Sigma Chemical Co, St Louis, MO) dissolved in the vehicle. The washed DEP+OVA group or the DEP-OC+OVA group received combined administration of washed DEP or DEP-OC together with OVA in the same vehicle, respectively. Then all groups received OVA or vehicle every 2 wk for 6 wk, and DEP components or vehicle once a week for 6 weeks. In each group, vehicle, DEP-OC, washed DEP, OVA, DEP-OC+OVA, or washed DEP+OVA was dissolved in 0.1-ml aliquots and inoculated by the intratracheal route through a polyethylene tube under anesthesia with 4% halothane (Takeda Chemical Industries, Ltd., Osaka, Japan).

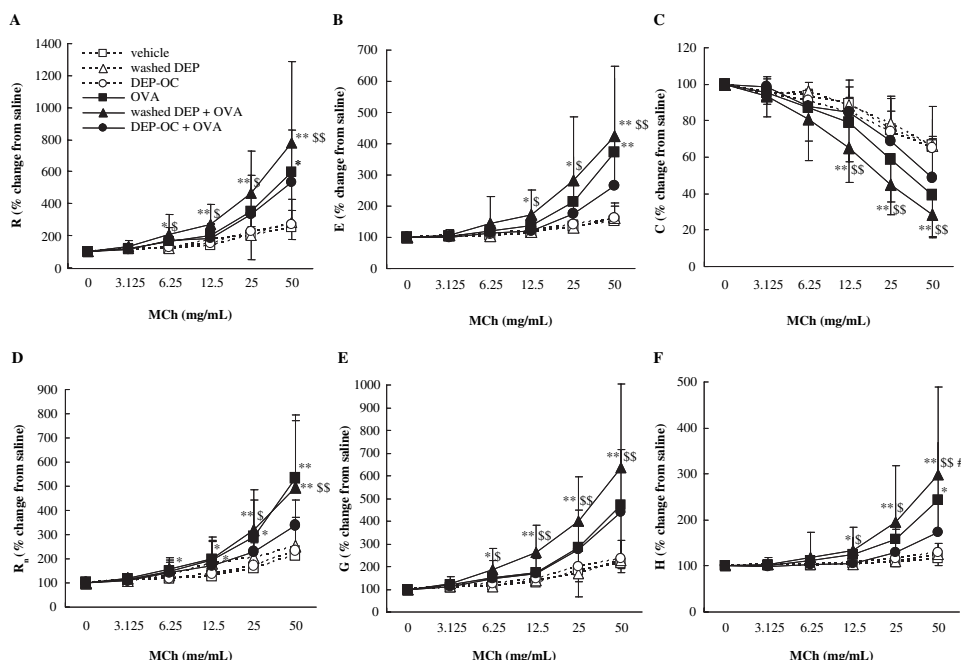


Figure 1: Dose-response curve to inhaled methacholine (MCh). The animals were randomized into 4 experimental groups that received repeated intratracheal instillation with vehicle, diesel exhaust particles (DEP) components (extracted organic chemicals in DEP; DOC, carbonaceous nuclei of DEP after extraction; WD), ovalbumin (OVA), or the combination of DEP components+OVA for 6 wk. Total respiratory system resistance (R: A), elastance (E: B), compliance (C: C), Newtonian resistance (R_n : D), tissue damping (G: E), or tissue elastance (H: F) was measured 24 h after the last instillation. Data are shown as mean $\pm$ SD ($n=7-8$ in each group). * $P < 0.05$ vs. vehicle, ** $P < 0.01$ vs. vehicle, \$ $P < 0.05$ vs. DEP component, \$\$ $P < 0.01$ vs. DEP component, # $P < 0.05$ vs. OVA.

Analysis of Lung Function

Assessment of cholinergic lung constrictor responsiveness was done with a computer-controlled small-animal ventilator (FlexiVent; Scireq, Montreal, Canada) as previously described.⁽¹⁵⁻¹⁸⁾ In brief, the mice were anesthetized with 0.1 ml per 10 g body weight of a 40 mg/ml ketamine hydrochloride given intraperitoneally. Anesthesia was maintained by supplemental administration of 30% of the initial dose at ~ 25 -min intervals, as required. Mice were tracheostomized with a 5-mm section of metallic tubing and ventilated (FlexiVent) at $180 \text{ b} \cdot \text{min}^{-1}$ with a tidal volume of 10 mL/kg and a positive end expiratory pressure of $3 \text{ cm H}_2\text{O}$.

Both the single-compartment model (using snap shot method) and the constant-phase model (using forced oscillation technique [FOT] method) of respiratory mechanics were applied to assess lung function and airway responses to methacholine (MCh). For the single-compartment model, total

respiratory system resistance (R), elastance (E), compliance (C) were determined essentially as described previously.^(15,16) For the constant-phase model, R_n (Newtonian resistance), tissue damping (G), and tissue elastance (H) were determined as described previously.^(15,18) All data points were determined by the FlexiVent software (version 5.0) by using multiple linear regressions to fit each data point to the single-compartment or the constant-phase model, as appropriate.

The lung volume history of the mice was standardized prior to measurement of lung mechanics using two deep inflations. P and V data were generated by applying a 2 s sine wave volume perturbation (SW) with an amplitude of 0.2 ml and a frequency of 2.5 Hz. After 5 min of regular mechanical ventilation, the SW perturbation was applied three times and the average was taken to generate a baseline measurement. The respiratory system input impedance (Z_{rs}) was measured during periods of apnea using a 3 s signal containing 19 mutually prime sinusoidal frequencies ranging from 0.25 to 19.625 Hz; these maneuvers generated data that were fit to the single-compartment or the constant-phase compartment models, respectively. The averages of these measurements for each mouse served as its baseline values.

Following acquisition of baseline data, airway responsiveness to aerosolized MCh (3.125 to 50 mg/mL saline; delivered by ultrasonic nebulizer) was assessed using both snap shot and FOT methods. Aerosols were delivered for 10 s with a ventilation at 180 b.min⁻¹ with a tidal volume of 4 ml/kg, after which the snap shot and the FOT methods were applied consecutively every 6 s for 5 min. Peak responses during each 5-min period were determined, and only values with a coefficient of determination of 0.95 or greater were used.

Statistical Analysis

Data were reported as mean $\pm$ standard deviation (SD). Differences were analyzed by Kruskal–Wallis test followed by the Mann–Whitney *U*-test using the SPSS 8.0 statistical package for Windows (Chicago, IL). Values of $P < 0.05$ or $P < 0.01$ were considered significant.

RESULTS

Effects of DEP Components on Lung Function in the Presence or Absence of Antigen

To evaluate the effects of DEP components on MCh responsiveness, we investigated R (Figure 1A), E (Figure 1B), C (Figure 1C), R_n (Figure 1D), G (Figure 1E), and H (Figure 1F) in 6 groups of mice 24 h after the last intratracheal instillation. R (Figure 1A) and E (Figure 1B) were higher in the OVA or the DEP-OC+OVA group than in the vehicle group ($P < 0.05$ vs. the OVA

group). The value was further higher in the washed DEP+OVA group than in the vehicle ($P < 0.05$), the washed DEP group ($P < 0.05$), or the OVA group (N. S.). C (Figure 1C) was lower in the OVA or the DEP-OC+OVA group than in the vehicle group.

The value was further lower in the washed DEP+OVA group than in the vehicle ($P < 0.01$), the washed DEP group ($P < 0.05$), or the OVA group (N. S.). R_n was significantly higher in the OVA group than in the vehicle group ($P < 0.01$; Figure 1D). The value was also higher in DEP component+OVA groups than in the vehicle ($P < 0.01$ vs. the washed DEP+OVA group) or the DEP component group ($P < 0.01$ vs. the washed DEP+OVA group). G (Figure 1E) was higher in the OVA or the DEP-OC+OVA group than in the vehicle group (N. S.). The value was further higher in the washed DEP+OVA group than in the vehicle ($P < 0.01$), the washed DEP group ($P < 0.01$), or the OVA group (N. S.). H (Figure 1F) was higher in the OVA or the DEP-OC+OVA group than in the vehicle group ($P < 0.05$ vs. the OVA group). The value was significantly higher in the washed DEP+OVA group than in the vehicle ($P < 0.01$), the washed DEP group ($P < 0.01$), or the OVA group ($P < 0.05$).

DISCUSSION

Our previous study has demonstrated that DEP repeatedly administered by intratracheal route deteriorates antigen-related airway inflammation⁽⁸⁾ and airway hyperresponsiveness⁽¹³⁾ in mice. Furthermore, our ongoing study has demonstrated that DEP components facilitate antigen-related airway inflammation, which is more prominent with DEP-OC than with washed DEP in overall trend.⁽¹¹⁾ The present study expands the previous one to show that DEP components, in particular washed DEP, enhance lung hyperresponsiveness to MCh related to antigen.

It has been demonstrated that DEP generated from several types of diesel engines exaggerate airway hyperresponsiveness related to antigen^(13,19,20) *in vivo*. However, all these studies have used enhanced pause (Penh), which is derived from unrestricted barometric plethysmograph, to measure changes in lung physiology. As Penh cannot differentiate between constriction in the airways and changes in the tissue compartment of the lungs, it should be difficult to tell when the responses to MCh are localized, if they are true physiological responses.^(21–24) Further, there is no study about relative contribution of the compounds containing in DEP to lung physiology related to antigen. As far as we know, therefore, the present study first elucidated the effects of each main compound in DEP on airway, tissue, or mixed compartment response to MCh using FOT in detail.

Our previous study on the same protocol as the present one has demonstrated that antigen-related airway inflammation including lung eosinophilia and goblet cell hyperplasia is more deteriorated by DEP-OC than by washed

DEP.⁽¹¹⁾ In contrast, the cholinergic airway hyperreactivity (R and R_n) in the presence of antigen was enhanced by washed DEP rather than DEP-OC in the present study. The current findings, thus, seem to be somewhat different from our previous one. However, some experimental studies have shown that airway exposure to ambient-derived particles worsens the airway hyperresponsiveness in allergic or nonallergic animals.^(25–27) Furthermore, a recent report has demonstrated that inhalation of elemental carbon nanoparticles (median diameter of particle < 100 nm) enhances airway hyperresponsiveness related to antigen using Penh.⁽²⁸⁾ As well, we are being evidenced that pulmonary exposure to two sizes of carbon nanoparticles facilitates antigen-related lung hyperreactivity on the same protocol (manuscript under submission). Given the previous reports and current investigation, the present observation is not inconsistent with these ones.

Mechanisms for the enhancing effects of washed DEP on the airway physiology remains unidentified. The enhancing effects of washed DEP may be due to amplification of Th1-mediated airway hyperresponsiveness,⁽³⁰⁾ since our previous work has confirmed that washed DEP amplified lung expression of Th1 cytokines such as interferon- γ and IL-2⁽¹¹⁾. Otherwise, our previous study has demonstrated that intratracheal administration of washed DEP, rather than DEP-OC, can induce endothelial damage.⁽²⁹⁾ Thus, it can be speculated that washed DEP-induced endothelial damage devastate nitric oxide (NO) release and corresponding soluble geranyl cyclase /cyclic GMP production, resulting in impairment of relax for airway smooth muscles.

For other considerable speculation, it is possible that washed DEP influence autonomic nerve system to lead to vagotonia-sympathicotonia imbalance. Otherwise, this adverse reaction might be mediated through the activation of (EGF)-EGF receptor (EGFR) pathway, since the signaling reportedly plays a role in airway remodeling with hyperresponsiveness.⁽³¹⁾ Further elucidations are needed to clarify the possibilities.

On the other hand, DEP-OC did not enhance antigen-related lung hyperresponsiveness in the present study. The mechanisms have not been clarified yet; however, DEP-OC may not be critical for the augmentation of the pathways regarding antigen-related airway hyperresponsiveness such as vagotonia-sympathicotonia systems, epithelial growth factor EGF-EGFR signaling, NO/cGMP pathway, and/or neuropeptides and their receptors signaling.⁽³²⁾ In addition, some previous reports have implicated the independency of airway hyperresponsiveness from airway inflammation.^(33,34) Thus, our present results combined with the previous one could suggest that DEP components differently affect asthma manifestations, leading to the profound enhancement induced by “whole” DEP (containing both washed DEP and DEP-OC).^(8,11,13)

Our detailed analyses for lung functions revealed that antigen exposure worsened the values of E, C, G, and H in the present study. As well, the values

were further worsened in the washed DEP+OVA group. E and C positively reflect functional consequence of airway remodeling with narrowing.⁽³⁵⁾ On the other hand, G and H are reportedly consistent with the stiffing of the respiratory tissues, particularly that of the lungs.^(36,37) Thus, our findings suggest that washed DEP might be an important contributor in the context of antigen-related airway remodeling.

In conclusion, the present study has shown that washed DEP can enhance antigen-related lung hyperresponsiveness, whereas DEP-OC cannot. On the other hand, our previous study has demonstrated that DEP-OC can facilitate antigen-related airway inflammation, whereas washed DEP cannot.⁽¹¹⁾ Given the present findings and previous ones, it is suggested that DEP, complicated particles with several components, aggravate asthma manifestations in combination with differential effects of each component.

REFERENCES

1. Abbey, D.E.; Nishino, N.; McDonnell, W.F.; Burchette, R.J.; Knutsen, S.F.; Lawrence Beeson, W.; Yang, J.X. Long-term inhalable particles and other air pollutants related to mortality in nonsmokers. *Am. J. Respir. Crit. Care Med.* **1999**, *159*, 373–382.
2. Cohen, A.J.; Pope, C.A., 3rd. Lung cancer and air pollution. *Environ. Health Perspect.* **1995**, *103*, 219–224.
3. Dockery, D.W.; Pope, C.A., 3rd.; Xu, X.; Spengler, J.D.; Ware, J.H.; Fay, M.E.; Ferris, B.G., Jr.; Speizer, F.E. An association between air pollution and mortality in six U.S. cities. *N. Engl. J. Med.* **1993**, *329*, 1753–1759.
4. Peters, A.; Wichmann, H.E.; Tuch, T.; Heinrich, J.; Heyder, J. Respiratory effects are associated with the number of ultrafine particles. *Am. J. Respir. Crit. Care Med.* **1997**, *155*, 1376–1383.
5. Saito, Y.; Azuma, A.; Kudo, S.; Takizawa, H.; Sugawara, I. Long-term inhalation of diesel exhaust affects cytokine expression in murine lung tissues: Comparison between low- and high-dose diesel exhaust exposure. *Exp. Lung Res.* **2002**, *28*, 493–506.
6. Ichinose, T.; Furuyama, A.; Sagai, M. Biological effects of diesel exhaust particles (DEP). II. Acute toxicity of DEP introduced into lung by intratracheal instillation. *Toxicology.* **1995**, *99*, 153–167.
7. Ichinose, T.; Yajima, Y.; Nagashima, M.; Takenoshita, S.; Nagamachi, Y.; Sagai, M. Lung carcinogenesis and formation of 8-hydroxy-deoxyguanosine in mice by diesel exhaust particles. *Carcinogenesis.* **1997**, *18*, 185–192.
8. Takano, H.; Yoshikawa, T.; Ichinose, T.; Miyabara, Y.; Imaoka, K.; Sagai, M. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. *Am. J. Respir. Crit. Care Med.* **1997**, *156*, 36–42.
9. Research of Cancer: Evaluation of carcinogenic risks to humans: diesel and gasoline exhaust and some nitroarenes. Lyon, France: International Agency for Research on Cancer (IARC), 1989: 46.
10. Vouk, V.B.; Piver, W.T. Metallic elements in fossil fuel combustion products: amounts and form of emissions and evaluation of carcinogenicity and mutagenicity. *Environ. Health Perspect.* **1983**, *47*, 201–225.

11. Yanagisawa, R.; Takano, H.; Inoue, K.I.; Ichinose, T.; Sadakane, K.; Yoshino, S.; Yamaki, K.; Yoshikawa, T.; Hayakawa, K. Components of diesel exhaust particles differentially affect Th1/Th2 response in a murine model of allergic airway inflammation. *Clin. Exp. Allergy*. **2006**, *36*, 386–395.
12. Duguet, A.; Biyah, K.; Minshall, E.; Gomes, R.; Wang, C.G.; Taoudi-Benchekroun, M.; Bates, J.H.; Eidelman, D.H. Bronchial responsiveness among inbred mouse strains. Role of airway smooth-muscle shortening velocity. *Am. J. Respir. Crit. Care Med*. **2000**, *161*, 839–848.
13. Takano, H.; Ichinose, T.; Miyabara, Y.; Yoshikawa, T.; Sagai, M. Diesel exhaust particles enhance airway responsiveness following allergen exposure in mice. *Immunopharmacol. Immunotoxicol*. **1998**, *20*, 329–336.
14. Sagai, M.; Furuyama, A.; Ichinose, T. Biological effects of diesel exhaust particles (DEP). III. Pathogenesis of asthma like symptoms in mice. *Free Radic. Biol. Med*. **1996**, *21*, 199–209.
15. Card, J.W.; Carey, M.A.; Bradbury, J.A.; Graves, J.P.; Lih, F.B.; Moorman, M.P.; Morgan, D.L.; DeGraff, L.M.; Zhao, Y.; Foley, J.F.; et al.: Cyclooxygenase-1 over-expression decreases Basal airway responsiveness but not allergic inflammation. *J. Immunol*. **2006**, *177*, 4785–4793.
16. Gavett, S.H.; Madison, S.L.; Chulada, P.C.; Scarborough, P.E.; Qu, W.; Boyle, J.E.; Tiano, H.F.; Lee, C.A.; Langenbach, R.; Roggli, V.L.; et al.: Allergic lung responses are increased in prostaglandin H synthase-deficient mice. *J. Clin. Invest*. **1999**, *104*, 721–732.
17. Kang, H.S.; Blink, S.E.; Chin, R.K.; Lee, Y.; Kim, O.; Weinstock, J.; Waldschmidt, T.; Conrad, D.; Chen, B.; Solway, J.; et al.: Lymphotoxin is required for maintaining physiological levels of serum IgE that minimizes Th1-mediated airway inflammation. *J. Exp. Med*. **2003**, *198*, 1643–1652.
18. Lee, Y.C.; Lee, K.S.; Park, S.J.; Park, H.S.; Lim, J.S.; Park, K.H.; Im, M.J.; Choi, I.W.; Lee, H.K.; Kim, U.H. Blockade of airway hyperresponsiveness and inflammation in a murine model of asthma by a prodrug of cysteine, L-2-oxothiazolidine-4-carboxylic acid. *FASEB J*. **2004**, *18*, 1917–1919.
19. Dong, C.C.; Yin, X.J.; Ma, J.Y.; Millecchia, L.; Wu, Z.X.; Barger, M.W.; Roberts, J.R.; Antonini, J.M.; Dey, R.D.; Ma, J.K. Effect of diesel exhaust particles on allergic reactions and airway responsiveness in ovalbumin-sensitized brown Norway rats. *Toxicol. Sci*. **2005**, *88*, 202–212.
20. Nordenhall, C.; Pourazar, J.; Ledin, M.C.; Levin, J.O.; Sandstrom, T.; Adelroth, E. Diesel exhaust enhances airway responsiveness in asthmatic subjects. *Eur. Respir. J*. **2001**, *17*, 909–915.
21. Mitzner, W.; Tankersley, C. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med*. **1998**, *158*, 340–341.
22. Hantos, Z.; Brusasco, V. Assessment of respiratory mechanics in small animals: The simpler the better? *J. Appl. Physiol*. **2002**, *93*, 1196–1197.
23. Adler, A.; Cieslewicz, G.; Irvin, C.G. Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *J. Appl. Physiol*. **2004**, *97*, 286–292.
24. Bates, J.; Irvin, C.; Brusasco, V.; Drazen, J.; Fredberg, J.; Loring, S.; Eidelman, D.; Ludwig, M.; Macklem, P.; Martin, J.; et al. The use and misuse of Penh in animal models of lung disease. *Am. J. Respir. Cell Mol. Biol*. **2004**, *31*, 373–374.

25. Lei, Y.C.; Chen, M.C.; Chan, C.C.; Wang, P.Y.; Lee, C.T.; Cheng, T.J. Effects of concentrated ambient particles on airway responsiveness and pulmonary inflammation in pulmonary hypertensive rats. *Inhal. Toxicol.* **2004**, *16*, 785–792.
26. Walters, D.M.; Breyse, P.N.; Wills-Karp, M. Ambient urban Baltimore particulate-induced airway hyperresponsiveness and inflammation in mice. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 1438–1443.
27. Walters, D.M.; Breyse, P.N.; Schofield, B.; Wills-Karp, M. Complement factor 3 mediates particulate matter-induced airway hyperresponsiveness. *Am. J. Respir. Cell Mol. Biol.* **2002**, *27*, 413–418.
28. Alessandrini, F.; Schulz, H.; Takenaka, S.; Lentner, B.; Karg, E.; Behrendt, H.; Jakob, T. Effects of ultrafine carbon particle inhalation on allergic inflammation of the lung. *J. Allergy Clin. Immunol.* **2006**, *117*, 824–830.
29. Inoue, K.; Takano, H.; Sakurai, M.; Oda, T.; Tamura, H.; Yanagisawa, R.; Shimada, A.; Yoshikawa, T. Pulmonary exposure to diesel exhaust particles enhances coagulatory disturbance with endothelial damage and systemic inflammation related to lung inflammation. *Exp. Biol. Med.* **2006**, *231*, 1626–1632.
30. Cui, J.; Pazdziorko, S.; Miyashiro, J.S.; Thakker, P.; Pelker, J.W.; Declercq, C.; Jiao, A.; Gunn, J.; Mason, L.; Leonard, J.P.; et al. TH1-mediated airway hyperresponsiveness independent of neutrophilic inflammation. *J. Allergy Clin. Immunol.* **2005**, *115*, 309–315.
31. Morcillo, E.J.; Cortijo, J. Mucus and MUC in asthma. *Curr. Opin. Pulm. Med.* **2006**, *12*, 1–6.
32. De Swert, K.O.; Joos, G.F. Extending the understanding of sensory neuropeptides. *Eur. J. Pharmacol.* **2006**, *533*, 171–181.
33. Cockcroft, D.W.; Davis, B.E. Mechanisms of airway hyperresponsiveness. *J. Allergy Clin. Immunol.* **2006**, *118*, 551–559.
34. Leckie, M.J.; ten Brinke, A.; Khan, J.; Diamant, Z.; O'Connor, B.J.; Walls, C.M.; Mathur, A.K.; Cowley, H.C.; Chung, K.F.; Djukanovic, R.; et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet.* **2000**, *356*, 2144–2148.
35. Kaminsky, D.A.; Irvin, C.G.; Lundblad, L.; Moriya, H.T.; Lang, S.; Allen, J.; Viola, T.; Lynn, M.; Bates, J.H. Oscillation mechanics of the human lung periphery in asthma. *J. Appl. Physiol.* **2004**, *97*, 1849–1858.
36. Barnas, G.M.; Sprung, J. Effects of mean airway pressure and tidal volume on lung and chest wall mechanics in the dog. *J. Appl. Physiol.* **1993**, *74*, 2286–2293.
37. Hirai, T.; McKeown, K.A.; Gomes, R.F.; Bates, J.H. Effects of lung volume on lung and chest wall mechanics in rats. *J. Appl. Physiol.* **1999**, *86*, 16–21.