

Exposure to Diesel Exhaust Aggravates Nasal Allergic Reaction in Guinea Pigs

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Diesel exhaust particulates (DEP) (1) and exposure to diesel exhaust (DE) induce nasal mucosal hyperresponsiveness to histamine. Therefore, in the present study, we investigated whether or not exposing guinea pigs to DE aggravates the nasal allergic reaction induced by repeated nasal administration of ovalbumin (OVA). Guinea pigs were exposed to filtered air or to DE (DE containing 0.3 or 1.0 mg/m³ of DEP) for 5 wk. During exposure to filtered air or to DE, guinea pigs were administered 1% of OVA in saline into the nasal cavities once a week. Sneezes were counted and nasal secretions were measured as indices of sneezing responses and rhinorrhea for 20 min after OVA administration. Titers of specific anti-OVA-IgG and anti-OVA-IgE and the number of eosinophils infiltrated into both nasal epithelium and subepithelium were measured at the end of the exposure to DE. Exposure to DE enhanced the number of sneezes and the amount of nasal secretions induced by OVA. Titers of specific anti-OVA-IgG and anti-OVA-IgE also significantly increased in DE-exposed animals. Exposure to DE also augmented the number of eosinophils that infiltrated both the nasal epithelium and the subepithelium induced by OVA. These results suggest that exposure to DE enhances the nasal allergic reaction induced by repeated antigen administration in guinea pigs.

The incidence of allergic rhinitis appears to be increasing, particularly in industrialized countries (1, 2). Epidemiologic studies have shown that the prevalence rate of allergic rhinitis in areas of air pollution is higher than that in non-polluted areas (1, 3, 4). In Japan, the number of diesel-powered cars, which emit 20 to 100 times more particulates and 2 to 20 times more nitrogen oxides than gasoline-powered cars, has increased 2- to 3-fold during the past 10 yr. It has been suggested that diesel exhaust (DE) has contributed to the increased prevalence of allergic rhinitis (5). From this viewpoint, it is necessary to elucidate whether or not exposure to DE causes nasal mucosal hyperresponsiveness to chemical mediators released by antigen-antibody reactions, enhances IgE production, and aggravates nasal allergic reactions induced by repeated nasal administration of antigen. In nasal hyperresponsive animals, increases in nasal airway resistance, nasal secretion, and sneezes elicited by major mediators released during an allergic attack are induced by lowering the normal threshold. Therefore, nasal mucosal hyperresponsiveness could be an important factor in the increasing prevalence of allergic rhinitis. The administration of a suspension of diesel exhaust particulates (DEP) induces nasal mucosal hyperresponsiveness to inhaled histamine (His) (6). Short term and relatively long term exposure to DE also induces nasal mucosal hyperresponsiveness to His assessed by number of sneezes and nasal secretions after exposure to DE (7, 8). The administration of DEP or exposure to DE enhances IgE antibody production in mice (5, 9, 10), and in hu-

mans (11). Therefore, we investigated whether or not exposure to DE aggravates the nasal allergic reaction induced by repeated nasal administrations of antigen.

METHODS

Animals

This study was approved by the National Institute for Environmental Studies Ethics Committee for Experimental Animals. Male Hartley guinea pigs (weighing about 250 g and 4 wk of age) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Guinea pigs were used as a histamine-induced rhinitis model, since histamine induces nasal congestion, sneezing, and rhinorrhea well in guinea pigs among small experimental animals. These animals were fed with standard guinea pig chow (RC4 Oriental Yeast Co. Ltd., Tokyo) and given water *ad libitum*. The animals were used for experiments at 8 wk of age when they weighed about 450 to 550 g.

Protocol

Guinea pigs were exposed to filtered air or to various concentrations of DE for 5 wk. During the exposure, guinea pigs were administered 40 μ l/kg of 1% ovalbumin (OVA) in saline into both anterior nares once a week. Sneezes were counted for 20 min after OVA administration. Nasal secretion was also measured 20 min after OVA administration. Titers of specific anti-OVA-IgG and anti-OVA-IgE and the number of eosinophils that infiltrated the nasal epithelium and subepithelium were measured 24 h after the last administration of OVA. The protocol is described in Figure 1.

Exposure to Diesel Exhaust

Groups of eight guinea pigs were exposed to filtered air or to DE (containing 0.3 or 1.0 mg/m³ particulates) for 5 wk in identical stainless steel and glass chambers. Filtered room air or filtered room air mixed with DE to achieve 0.3 or 1.0 mg/m³ of particulates were passed through the chambers. The chambers were maintained at 25 $\pm$ 1 $^{\circ}$ C, 55 $\pm$ 5% humidity, and -5 mm H₂O relative to atmospheric pressure with an air flow of 110 m³/h. The concentrations of nitrogen dioxide (NO₂) and nitric oxide (NO) were feedback-controlled by continuous monitoring with an NOx analyzer (Model 8440; Monitor Labs, USA) operating on a chemiluminescence principle. The DE used in this study was generated by a light-duty (2,740 ml) four-cylinder diesel engine (4jB1 type; Isuzu Automobile Co., Tokyo) using standard diesel fuel. The engine was operated at 2,000 rpm under a load of 6 torque (kg/m) generated by an EDYC dynamometer (Meiden-Sya, Tokyo, Japan). Specifications of the engine and dilution system have been reported elsewhere (12). A summary of the characteristics of the exposure atmospheres is shown in Table 1.

Number of Sneezes

Immediately after DE exposure, an intact, unanesthetized, spontaneously breathing guinea pig was placed in a two-chamber restrainer that kept the neck fixed and isolated from the body behind the neck. Altered airflow at the nose in time with the onset of sneezing was measured using a pneumotachograph (A; Fleish Instruments, Lausanne, Switzerland) connected to a differential pressure transducer (Model MP45-14; Validyne, Northridge, CA). Box pressure changes caused by abdominal movements in time with the onset of sneezing were determined using a differential pressure transducer (Model MP45-14; Validyne) and a carrier demodulator (Model CD72; Validyne). Airflow and box pressure were reported on a recorder (Linearcorder F WR3701; Graphtec, Tokyo, Japan). Airflow and box pressure signals were also displayed simulta-

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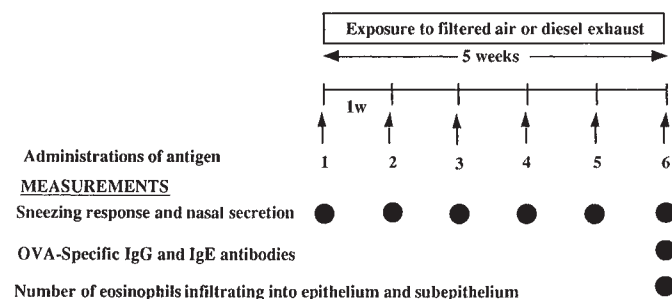


Figure 1. Experimental protocol. Guinea pigs were exposed to filtered air or to DE containing 0.3 mg/m³ or 1.0 mg/m³ DEP for 5 wk. During exposure, guinea pigs were administered 40 μ l/kg of 1% OVA-saline into both anterior nares once each week. After DE exposure, each guinea pig was placed in a two-chamber restrainer. Sneezes were counted for 20 min after administering OVA-saline. Nasal secretion was also measured 20 min after the administration of OVA-saline. Titers of specific anti-OVA-IgG and anti-OVA-IgE and the number of eosinophils that infiltrated the nasal epithelium and subepithelium were measured 24 h after the last administration of OVA-saline.

neously on an oscilloscope (SS-5802; Iwatsu Electric, Tokyo, Japan). The sounds of sneezing (which could readily be distinguished from coughing) were picked up by a microphone (RP-3102E; National, Tokyo, Japan) amplified and reproduced by a loudspeaker (CFD-D77; Sony, Tokyo, Japan). After exposure to DE, the number of sneezes was measured over 10 min. After dripping 100 μ l/kg of 1.5 mM His-saline (0.9% NaCl) into the nostrils, the number of sneezes was counted for 20 min.

Nasal Secretion

Nasal secretion from the nostrils was measured as an index of the exocrine activity of the nasal mucosa. The secretion was wiped off with a piece of weighed paper (100 $\times$ 120 mm: Kimwipe, S-200; Jujo-Kimberly, Tokyo, Japan). The paper was wrapped in a piece of weighed aluminum foil (60 $\times$ 150 mm) and weighed.

Passive Cutaneous Anaphylaxis

At 24 h after the last administration of OVA, guinea pigs were exsanguinated via the axillary artery and vein after deep anesthesia with intraperitoneally administered sodium pentobarbital (50 mg/kg). Serum levels of OVA-specific IgG and IgE antibodies were determined by homologous passive cutaneous anaphylaxis tests with latency periods of 4 h and 7 d, respectively (13, 14). Guinea pigs were passively sensitized by an intradermal injection of 100 μ l/animal of diluted sera. After 4 h or 7 d, these animals were injected intravenously with saline (1 ml) containing OVA (1%) and Evans blue (500 μ g). The titer is defined as a dilution of sera that induces a blue spot with a diameter of 8 mm after 30 min. The titers of OVA-specific IgG and IgE were estimated by extrapolation using a dilution-diameter curve.

Measurement of Eosinophils Infiltrating Nasal Mucosa

After death by exsanguination, the head of each guinea pig was removed. The lower jaw, skin, and musculature were removed, and the head was immersed in 10% neutral phosphate-buffered formalin. After fixation, the heads were decalcified in a decalcifying solution contain-

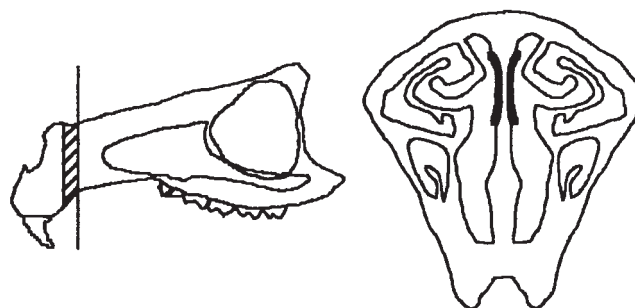


Figure 2. Lateral view of a guinea pig nose and a site selected for counting the infiltrating eosinophils. (Left panel) A tissue block was removed from the anterior nasal cavity by making two transverse cuts perpendicular to the hard palate immediately posterior to the upper incisor teeth. (Right panel) The number of eosinophils that infiltrated the respiratory epithelium and subepithelium were counted. The surface epithelium lining these regions is shown as bold lines.

ing 0.5 M ethylenediaminetetraacetic acid disodium salt (pH, 7.5) for 2 wk and rinsed in tap water. A tissue block was removed from the anterior nasal cavity by making two transverse cuts perpendicular to the hard palate immediately posterior to the upper incisors (Figure 2). The tissue block was imbedded in paraffin, and sections 5 μ m thick were cut from the anterior surface. Sections were stained with hematoxylin-eosin to identify eosinophils. The number of infiltrated eosinophils into respiratory epithelium and subepithelium were counted with a video micrometer (VM-30; Olympus, Tokyo, Japan). The surface epithelium lining these regions was designated as shown in Figure 2. Results were expressed as the number of eosinophils per 10⁴ μ m².

Statistical Analysis

Values are expressed as central values and interquartiles (25th and 75th percentile). Normality was not clear because the sample number was small. Therefore, the Mann-Whitney U test was used to compare groups; p values less than 0.05 were considered significant.

Chemicals

OVA, His dihydrochloride, and Sodium pentobarbital were purchased from Seikagaku Corp. (Tokyo, Japan), Wako Pure Chemicals (Osaka, Japan), and Dainabot (Osaka, Japan), respectively.

RESULTS

DE Augmented the Number of Sneezes Induced by Repeated Nasal Administration of OVA

The effect of DE exposure upon the sneezing response induced by repeated nasal OVA administration is shown in Figure 3. The first nasal OVA administration into guinea pigs prior to exposure induced only a slight sneezing response. This may have been due to physical stimulation of the administration. Among the guinea pigs exposed to filtered air, OVA enhanced the sneezing response at the fourth administration, about 4-fold compared with the first administration. The fifth and the sixth administrations tended to enhance the sneezing response slightly, but not significantly. Exposure to 0.3 and 1.0 mg/m³ DE augmented the number of sneezes induced by OVA from the fourth administration by about 7- to 9- and 12- to 15-fold, respectively, compared with the first administration. In comparison with the filtered air group and the 1.0 mg/m³ DE groups, DE significantly augmented the sneezing response from the third administration of OVA by about 6- to 30-fold compared with the filtered air group. A concentration of 0.3 mg/m³ DE also significantly augmented the sneezing response at the fourth administration by 3-fold compared with the filtered air group.

TABLE 1

A SUMMARY OF THE CHARACTERISTICS OF THE EXPOSURE ATMOSPHERES OF DIESEL EXHAUST

	Air	Diesel Exhaust (0.3 mg/m ³)	Diesel Exhaust (1.0 mg/m ³)
NOx, ppm	0.02 $\pm$ 0.00	3.60 $\pm$ 0.15	10.46 $\pm$ 0.14
NO, ppm	0.02 $\pm$ 0.00	3.35 $\pm$ 0.14	9.64 $\pm$ 0.13
NO ₂ , ppm	0.00 $\pm$ 0.00	0.21 $\pm$ 0.01	0.74 $\pm$ 0.01
SO ₂ , ppm	0.15 $\pm$ 0.00	0.28 $\pm$ 0.01	0.40 $\pm$ 0.02
CO ₂ , ppm	636.07 $\pm$ 38.34	821.52 $\pm$ 17.64	1,553.46 $\pm$ 20.45
SPM, mg/m ³	0.03 $\pm$ 0.00	0.33 $\pm$ 0.00	1.28 $\pm$ 0.24

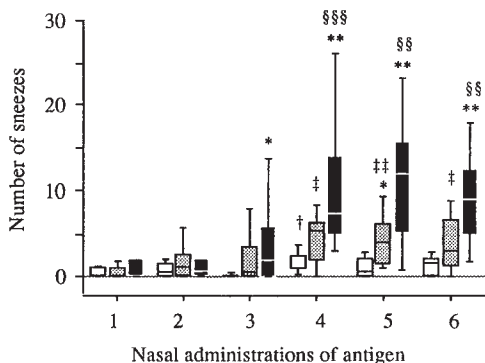


Figure 3. Effect of exposure to DE on sneezing response induced by repeated nasal OVA administration. Guinea pigs were exposed to filtered air or to DE containing 0.3 mg/m³ or 1.0 mg/m³ of DEP (open, shaded, and filled columns, respectively) for 5 wk. During the exposure, guinea pigs were administered 40 µl/kg of 1% OVA-saline into both anterior nares once a week. Sneezes were counted for 20 min after the administration of OVA-saline (n = 8/group); central values and interquartiles (25th and 75th percentile); *p < 0.05 and **p < 0.01, respectively, versus filtered air; †p < 0.05 versus sneezes after first nasal OVA administration of filtered air; ‡p < 0.05 and §§p < 0.01, respectively, versus sneezes caused by the first nasal OVA administration of 0.3 mg/m³ DE; §§p < 0.01 and §§§p < 0.001, respectively, versus sneezes caused by the first nasal OVA administration of 1.0 mg/m³ DE.

DE Augmented the Nasal Secretion Induced by Repeated Nasal Administration of OVA

The effect of exposure to DE on nasal secretion induced by repeated nasal OVA administration is shown in Figure 4. OVA enhanced nasal secretion at the fifth administration in the guinea pigs exposed to filtered air by about 3-fold compared with that induced by the first administration. The sixth administration tended to enhance the sneezing response, but not significantly. Exposure to 0.3 and 1.0 mg/m³ DE augmented the number of sneezes induced by OVA from the third administration to about 17- to 31- and 16- to 42-fold, respectively, compared with the first administration. In comparison with

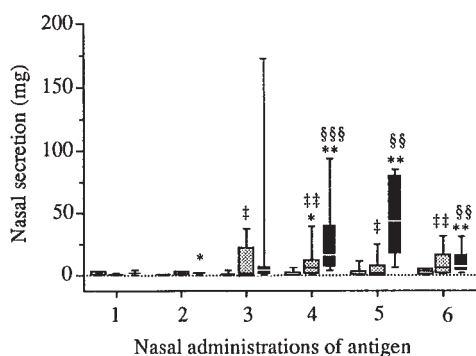


Figure 4. Effect of exposure to DE on nasal secretion induced by repeated nasal OVA administration. Guinea pigs were exposed to filtered air or to DE containing 0.3 mg/m³ or 1.0 mg/m³ of DEP (open, shaded, and filled column, respectively) for 5 wk. During the exposure, guinea pigs were administered OVA-saline into both anterior nares once each week. Nasal secretion from the nostrils was measured as an index of exocrine activity of the nasal mucosa for 20 min after the administration OVA-saline (n = 8/group); central values and interquartiles (25th and 75th percentile); *p < 0.05 and **p < 0.01, respectively, versus filtered air; †p < 0.05 and ‡p < 0.01, respectively, versus sneezes caused by the first nasal OVA administration of 0.3mg/m³ DE; §§p < 0.01 and §§§p < 0.001, respectively, versus sneezes caused by the first nasal OVA administration of 1.0 mg/m³ DE group.

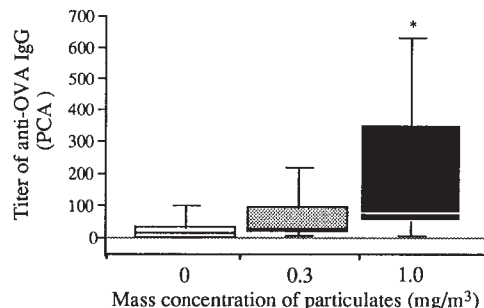


Figure 5. Effect of exposure to DE on anti-OVA IgG antibody titers in guinea pigs immunized by repeated intranasal administration of OVA. At 24 h after last administration of OVA, blood was retrieved. Serum levels of OVA-specific IgG antibodies administered were determined by homologous PCA tests. Sera were injected intradermally into guinea pigs for passive sensitization. After 4 h, these animals were injected intravenously with a saline solution containing OVA and Evans blue. A blue spot with a diameter of 8 mm or larger at the site of the intradermal serum injection 30 min after elicitation was taken as positive response (n = 8/group); central values and interquartiles (25th and 75th percentiles); *p < 0.05 versus filtered air group.

the filtered air and the 1.0 mg/m³ DE groups, DE significantly augmented the sneezing response from the third administration of OVA by about 7- to 38-fold that of the group exposed to air. The 0.3 mg/m³ DE group also significantly augmented the sneezing response at the fourth administration by about 8-fold compared with the filtered air group.

DE Enhanced the Production of Specific Anti-OVA-IgG and anti-OVA-IgE

The effects of exposure to DE on anti-OVA IgG antibody and anti-OVA IgE antibody titers, respectively, in guinea pigs immunized by repeated intranasal administration of OVA are shown in Figures 5 and 6. DE increased the anti-OVA IgG titer in a concentration-dependent manner (Figure 5). The anti-OVA IgG titer in the animals exposed to 1.0 mg/m³ of DE increased 6.8 times compared with that in animals exposed to filtered air. Although the anti-OVA IgE titer was low compared with that of anti-OVA IgG, DE also increased the anti-OVA IgE titer in a concentration-dependent manner (Figure

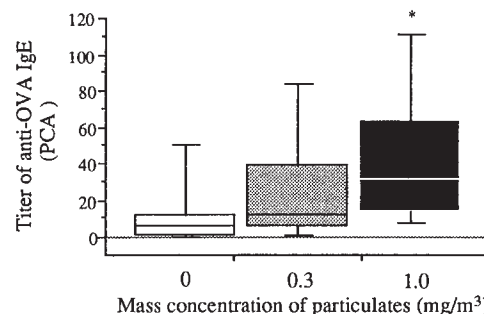


Figure 6. Effect of exposure to DE on anti-OVA IgE antibody titers in guinea pigs immunized by repeated intranasal administration of OVA. At 24 h after the last OVA administration, blood was retrieved. Serum levels of OVA-specific IgE antibodies were determined by homologous PCA tests. Sera were injected intradermally in guinea pigs for passive sensitization. After 7 d, the animals were intravenously injected with a saline solution containing OVA and Evans blue. A blue spot with a diameter of 8 mm or larger at the site of the intradermal serum injection 30 min after elicitation was taken as positive (n = 8/group); central values and interquartiles (25th and 75th percentiles); *p < 0.05 versus filtered air group.

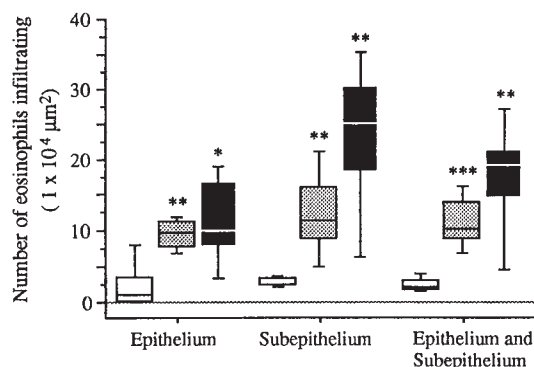


Figure 7. Effect of exposure to filtered air or DE containing 0.3 mg/m³ or 1.0 mg/m³ of DEP (open, shadowed, and filled columns, respectively) on the infiltration of eosinophils into epithelium and subepithelium induced by repeated nasal OVA administration. A tissue block was removed from the anterior nasal cavity by making two transverse cuts perpendicular to the hard palate immediately posterior to the upper incisors. Sections were stained with hematoxylin-eosin to identify eosinophils. Infiltrating eosinophils into respiratory epithelium and subepithelium were counted using a video micrometer. Results are expressed as the number of eosinophils per 10⁴ μm² (n = 8/group); central values and interquartiles (25th and 75th percentiles); *p < 0.05, **p < 0.01, and ***p < 0.001, respectively, versus filtered air.

6). The anti-OVA IgE titer in the animals exposed to 1.0 mg/m³ of DE was about three times higher than that in the animals exposed to filtered air.

Infiltration of Eosinophils into Nasal Epithelium and Subepithelium

The effect of DE exposure upon infiltration of the epithelium induced by repeated nasal OVA administration is shown in Figure 7. Exposure to DE augmented the number of eosinophils that infiltrated both the nasal epithelium and the subepithelium. Exposure to 0.3 and 1.0 mg/m³ DE augmented the total number of infiltrating eosinophils induced by OVA administration to about four and seven times the number exposed to filtered air.

DISCUSSION

Our present results showed that exposure to DE enhances the incidence of sneezing and the amount of nasal secretion induced by repeated nasal OVA administration in a concentration-dependent manner (Figures 3 and 4). This study is the first to show physiologic and histologic evidence of DE enhancement of an antigen-specific nasal allergic reaction. Many factors such as nasal mucosal responsiveness to chemical mediators released by antigen-antibody reaction such as His, anti-OVA-IgG, and -IgE, nasal epithelial permeability, infiltration of inflammatory cells into epithelium and subepithelium, and stimulation of sensory nerve endings are considered to play an important role in the augmentation of nasal allergic reaction. Chemical mediators, neuropeptides, and cytokines released into the nasal microenvironment may cause the increases in these factors. Previous studies have revealed that exposure to DE increases nasal mucosal responsiveness (7, 8), nasal epithelial permeability, and infiltration of inflammatory cells into epithelium and subepithelium (15). To our knowledge, however, no published data are available on the effects of DE exposure under the nasal allergic reaction induced by repeated nasal OVA administration, on nasal mucosal responsiveness, nasal epithelial permeability, and infiltration of inflammatory cells into epithelium and subepithelium. It has been reported that exposure to DE (10) and nasal administration of DEP (9, 11) enhances produc-

tion of anti-OVA-IgG and anti-OVA-IgE by intranasal administration of OVA in humans (11) and in mice (9). Effect of exposure of guinea pigs to DE on the production of the OVA-specific IgG and IgE remain unsolved.

We have reported that short-term (7) and relatively long-term (8) exposures to DE enhance nasal mucosal responsiveness to His. DE contains DEP and many gaseous components such as NO₂, NO, sulfur dioxide (SO₂), and formaldehyde. We have shown that DEP increases nasal mucosal responsiveness to His (6). Gaseous pollutants in DE may affect nasal mucosal responsiveness. Air pollutants such as NO₂ (16, 17), NO (18), SO₂ (19), sulfuric acid aerosol (20), and formaldehyde (21) can induce airway hyperresponsiveness.

Titers of specific anti-OVA-IgG and anti-OVA-IgE significantly increased in animals exposed in a concentration-dependent manner (Figures 5 and 6). These results indicate an adverse effect of DE on nasal allergic reaction. Exposure to DE (10) enhances antigen-specific IgE antibody production in mice through increases in interleukin-4 (IL-4) and IL-10 and a decrease in interferon-gamma production. The intranasal administration of DEP (5, 9), or exposure to extremely high concentrations of NO₂ (22) or SO₂ (23) with an allergen enhances allergen-specific IgE and IgG antibody production. The effects of exposure to NO₂ at low concentrations, NO, formaldehyde, and other gaseous chemicals on allergen-specific IgE and IgG antibody production remain to be elucidated. All models using experimental animals have their own limitations. In this model, nasal allergic reactions are mediated by predominantly allergen-specific IgG. In humans, however, titers of allergen-specific IgE and Ig class switch to IgE play important roles in allergic rhinitis. Few antibodies against cytokines of guinea pigs are available. Therefore, rhinitis model using guinea pigs has limitations when we intend to elucidate the contribution of Ig class switch to IgE and the related cytokine production to the aggravation of nasal allergic reactions induced by exposure to DE.

The enhanced permeability of the nasal airway epithelium facilitates penetration of the epithelial barrier. We have reported that nasal epithelial permeability to horseradish peroxidase (HRP) with a molecular weight of 40,000 daltons, increased in animals exposed to DE containing 1 or 3.2 mg/m³ of DEP for 28 d (15). Therefore, exposure to DE under the concentrations studied here may increase nasal epithelial permeability, which could play an important role in augmenting nasal allergic reactions. Among the components of DE, our preliminary results showed that the intranasal administration of DEP enhances nasal epithelial permeability to HRP (A. Konno, unpublished data). Exposure to NO₂ (24), SO₂ (25), or formaldehyde (26) also enhances the permeability of tracheal or pulmonary epithelium. However, little is understood about the effects of DE components on the permeability of nasal mucosal epithelium.

It can be seen in Figure 7 that exposure to DE augmented the number of eosinophils infiltrating both the nasal epithelium and subepithelium induced by nasal OVA administration. Infiltrating eosinophils may release toxic granular proteins such as major basic protein, eosinophil cationic protein and eosinophil peroxidase, which could damage or desquamate nasal epithelial cells, as observed in patients with asthma (27). Epithelial damage enhances epithelial permeability, stimulation of sensory nerve endings, and the release of chemical mediators. Therefore, eosinophilic airway inflammation plays a key role in the aggravation of allergic rhinitis (28). Stimulating the peripheral terminals of sensory nerves results in sneezing, nasal secretion, and nasal congestion. We reported that DEP induces vascular permeability in the skin (6) and the sneezing response (7). Pretreatment with capsaicin inhibits the increase

in vascular permeability and the sneezing response induced by DEP (T. Kobayashi, unpublished data). Assuming a combustion process, DE contains many gaseous irritants such as formaldehyde as well as unknown irritants that can induce sneezing, nasal secretion, and nasal congestion. Therefore, DEP and gaseous irritants could stimulate sensory nerves and induce the release of neuropeptides such as substance P and calcitonin gene-related peptide from peripheral terminals of the trigeminal nerves (29, 30). Therefore, the sneezing response and nasal secretion induced by antigen-antibody reaction may have been augmented by transmitters released by exposure to DE.

Arachidonic acid metabolites such as prostaglandin (PG) $F_{2\alpha}$ and PGE_1 (31) can potentiate the secretion induced by cholinergic stimulation. DE (32) and components of DE such as NO_2 (33, 34) and acid (35) affect the arachidonic acid metabolism. Therefore, inflammatory mediators released by exposure to DE possibly also augmented the nasal response induced by antigen-antibody reaction. Effect of exposure to DE on the release of transmitters and inflammatory mediators that correspond to the aggravation of nasal allergic reaction remains to be elucidated. Effects of DE on all potential mechanisms should be elucidated since DE contains many gaseous compounds and DEP, which absorb many toxic compounds whose effects on the potential mechanisms are unknown. Especially, it is necessary to elucidate which components, gaseous compounds, or DEP in DE are responsible for the augmentation of nasal allergic reaction.

In conclusion, exposure to DE enhances the nasal allergic reaction induced by repeated antigen administration in guinea pigs.

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