

EFFECTS OF THE INHALATION OF DIESEL EXHAUST, KANTO LOAM DUST, OR DIESEL EXHAUST WITHOUT PARTICLES ON IMMUNE RESPONSES IN MICE EXPOSED TO JAPANESE CEDAR (*CRYPTOMERIA JAPONICA*) POLLEN

Kazuhito Maejima, Kumiko Tamura, Toru Nakajima

Japan Automobile Research Institute, Ibaraki, Japan

Yoshifumi Taniguchi

Hayashibara Biochemical Laboratories, Inc., Okayama, Japan

Saburo Saito

Institute of DNA Medicine, Jikei University School of Medicine, Tokyo, Japan

Hiroshi Takenaka

Department of Oto-Rhino-Laryngology, Osaka Medical College, Osaka, Japan

To assess the potential enhancement by air-pollutants of immune responses in mice, especially with regard to allergen-specific immunoglobulin E (IgE) antibody production, female BDF₁ mice (60 mice in each group) were exposed to diesel exhaust (particles, 3.24 mg/m³; nitrogen dioxide, 1.0 ppm: DE group), Kanto loam dust (particles, 3.29 mg/m³; nitrogen dioxide, 0.01 ppm: KLD group), diesel exhaust without particles (particles, 0.01 mg/m³; nitrogen dioxide, 1.1 ppm: DEG group), or clean air (pollen and control groups) for 16 h/day, 5 days/wk for 24 wk, as well as to Japanese cedar pollen (JCP) (around 550,000 grains of JCP/m³) for 2 days/wk in the same period. The control group was exposed to clean air alone throughout the experiment. The mean values for Japanese cedar pollen allergens (JCPAs)-specific immunoglobulin E (IgE) antibody titers in mice sera measured by enzyme-linked immunosorbent assay (ELISA) in the DE, KLD, and DEG groups were higher than that for the pollen alone group, but not significantly, after both 12 and 24 wk of exposure time. The percentages of animals expressing more than the minimum ELISA titer of JCPAs-specific IgE antibodies in each group were 22% (DE and pollen groups) and 27% (KLD and DEG groups) of the totals at wk 12, and no statistical differences were observed among the groups. However, at wk 24 in the DE, KLD, and DEG groups the responders comprised 73%, 63%, and 67%, respectively, significantly higher than the 33% for the pollen alone group. No significant differences were observed among the DE, KLD, and DEG groups. A slight dose-dependent increase of proliferative responses of mouse cervical lymph node cells to JCPAs in both DE and

Received 9 April 2001; sent for revision 30 April 2001; revision received 25 May 2001; accepted 29 May 2001.

We are grateful to Dr. Seido of the Yamanashi Forestry and Forest Products Research Institute for the collection of Japanese cedar pollen.

Address correspondence to Dr. Kazuhito Maejima, Energy and Environment Research Division, Japan Automobile Research Institute, 2530 Karima, Tsukuba-shi, Ibaraki 305-0822, Japan. E-mail: kmae@jari.or.jp

KLD groups was observed, but not in the DEG group. Remarkable decrease of interferon- γ and significant increase of interleukin-4 in the nasal lavage fluid were apparent after DE or DEG exposure, but not in the KLD group. These results suggest that these air pollutants (DE, KLD, and DEG) enhance the production of IgE antibodies in mice, with similar adjuvant activities in each case. Furthermore, in the early phase of exposure in which sensitization occurred with exposure to pollen, the fine particles and gas components are considered to have exhibited different enhancing mechanisms in mice as follows: (1) The fine particles augmented production of IgE antibodies through activation of T lymphocytes, and (2) the gas components exhibited almost no action on T lymphocytes, but directly induced disorders of the cytokine network and augmented the production of IgE antibodies.

Pollen allergy has become a global problem from the standpoint of quality of life (Abramson et al., 1997; Charpin et al., 1993; D'Amato et al., 1998; Seaton et al., 1996). Japanese cedar (*Cryptomeria japonica*) pollinosis, reported for the first time in 1964 by Horiguchi and Saito, has also attracted concern as a serious health problem in Japan, because of the rapid increase in patient numbers. The recent incidence of Japanese cedar pollinosis (JCPsis) is reported to be 10–20% in adults and 5–10% in children (Okuda & Shida, 1998). JCPsis is a typical immediate-type allergic disease with symptoms such as sneezing, rhinorrhea, nasal congestion, and lacrimation, and production of Japanese cedar pollen (JCP) allergens-specific immunoglobulin E (IgE) antibodies is important for its onset (Okuda & Shida, 1998).

Several epidemiological and animal experimental studies have suggested that air pollutants emitted from automobiles, especially diesel exhaust particles (DEP), enhance JCPsis (Bice et al., 1985; Fujimaki et al., 1995, 1997; Ishizaki et al., 1987; Lovik et al., 1997; Muranaka et al., 1986; Nilsen et al., 1997; Takafuji et al., 1987). In animal experiments, combinations of JCP allergens (JCPAs) or ovalbumin and DEP administered to the nasal cavity or the peritoneal cavity resulted in antigen-specific IgE antibody production in mice. However, there are few reports on whether this is characteristic of exposure to DEP or whether other respirable air pollutants may also exert the same effects. Given the necessity for sophisticated facilities, the literature regarding verification by inhalation exposure experiments is, not surprisingly, limited.

The present study was designed to elucidate the effects of simultaneous inhalation of JCP and diesel exhaust (DE), Kanto loam dust (KLD), or diesel exhaust gas (diesel exhaust without particles, DEG) on the onset of JCPsis in mice. KLD was used as a comparative fine particle against DEP because it is a fine soil dust found in the actual atmosphere in the Kanto region, including Tokyo (Maejima et al., 1997).

MATERIALS AND METHODS

Japanese Cedar Pollen

Japanese cedar pollen (JCP) collected in Chiba and Yamanashi prefectures from 1995 to 1998 was mixed and used for the experiment. Many male flowers of Japanese cedar collected in vinyl bags were dried and

taped for this purpose; then JCP were refined with a fine mesh to remove impurities and stored at -80°C until use. Finally, around 56 kg JCP was collected until the start of the exposure experiment.

Of the JCPAs extracted from JCP, two major allergens have been isolated and characterized as Cry j 1 and Cry j 2 (Yasueda et al., 1983). JCPAs (75 μg of Cry j 1 and 25 μg of Cry j 2/100 μg) was provided kindly by Hayashibara Biochemical Laboratories, Okayama, Japan, and were used for the measurement of JCPAs-specific IgE and IgGs (IgG1 and IgG2a) antibodies in mice sera, and assay of proliferative responses of mouse cervical lymph node cells.

Mice

Female BDF₁ mice (SPF), aged 5 wk (17–19 g body weight) at the commencement, were obtained from CLEA Japan, Inc., and divided into 5 experimental groups of 60 mice each. During the exposure period, the mice were housed in stainless-steel cages in inhalation chambers maintained at $24 \pm 1^{\circ}\text{C}$ and $70 \pm 10\%$ relative humidity with artificial lighting for 12 h/day. The rate of air circulation was maintained at 10 times/h. All animals were provided with a normal diet (CE-2, CLEA Japan, Inc.) and water ad libitum during the experiment. For convenience, each experimental group was designated according to the tested materials inhaled as follows; DE (DE/pollen), KLD (KLD/pollen), DEG (DEG/pollen), pollen (air/pollen), and control (air alone).

This study was performed with the approval of the Japan Automobile Research Institute Ethics Committee for Use of Experimental Animals.

Inhalation Exposure System and Conditions

A schematic illustration of the inhalation exposure apparatus is shown in Figure 1. Five vertical flow-type inhalation chambers, with an approximate capacity of 3 m³ each, were used for exposure to pollen or other tested materials. Diesel exhaust was generated by a diesel engine (engine type, direct injection; displacement 6925 cm³) conforming to the latest regulations of exhaust gas in a steady-state operation mode (engine speed, 1200 rpm; torque 38.2 kg-m) using standard light oil containing 0.04 wt% sulfur. The generated diesel exhaust was directed into a dilution tunnel, where it was diluted about 20-fold by volume with conditioned fresh air. The diluted DE was supplied into the inhalation chamber of the DE group. One of these chambers had a filter in its upper parts for removing DEP from the diluted DE, and the diluted DE without particles was supplied into the inhalation chamber of the DEG group. KLD generated by a dust feeder (Sibata, model DF-3, Japan) was supplied into the inhalation chamber of the KLD group. JCP generated by other dust feeders was fed into all the inhalation chambers except for the control group. Mice were exposed to DE, KLD, or DEG for 16 h/day, 5 days/wk for 24 wk, with pollen exposure (around 550,000 grains JCP/m³) for 16 h/day, 2 days/wk for 24 wk, and receiving conditioned fresh air at all other times,

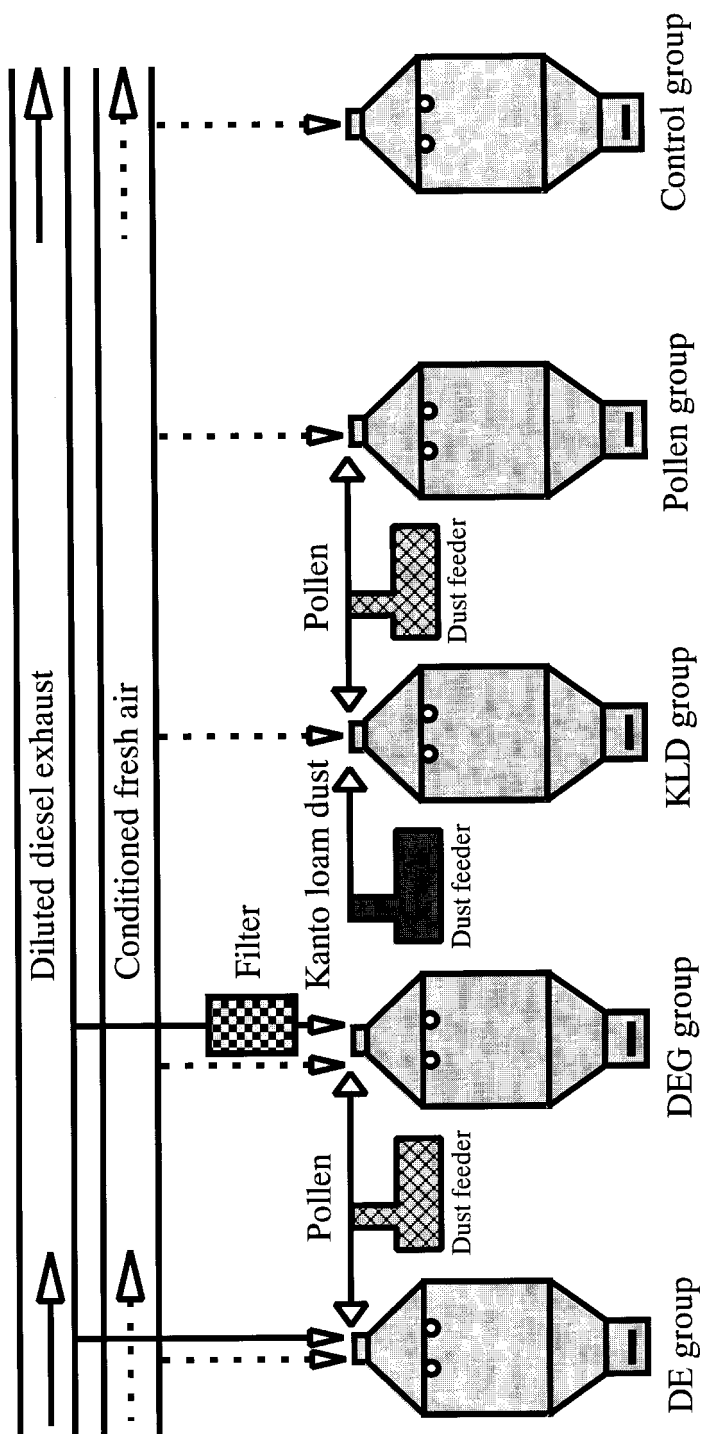


FIGURE 1. Inhalation exposure system.

like the mice of the control group. Thus, simultaneous inhalation exposure of pollen and tested materials in the DE, KLD, and DEG groups was for 2 days/wk. In a first preliminary experiment conducted before the present study (Maejima et al., 2000), 10 female BDF₁ mice were exposed to concentrations of around 500,000 or 3,000,000 pollen grains/m³ for 2 or 4 h/day, 2 days/wk for 30 wk. No elevation of JCPAs-specific IgE antibody titers in sera was recognized under these exposure conditions. In a second experiment, the JCPAs-specific IgE antibody titers of 4 of 10 mice exposed to around 500,000 pollen grains/m³ for 16 h/day, 2 days/wk for 20 wk were increased clearly with prolongation of daily exposure time to 4–8 times those used previously. The sensitization caused by pollen was concluded from these results to be more related to exposure time than concentration of pollen. Therefore, a period of 16 h and a pollen concentration of around 500,000 grains/m³ were used for the present study.

Daily mass concentrations of particles in the inhalation chambers were measured by the method of filter sampling and gravimetry. Atmospheric concentrations of key components in the inhalation chambers were monitored continuously with an automatic analyzer for automobile exhaust (Horiba, model MEXA-8120M, Japan), and pollen concentrations in the inhalation chamber were measured hourly with a particle counter (Rion, model KC-20, Japan). The average exposure concentration of JCP was 547,600 grains/m³, and the major size range of 20–30 μm accounted for 91% of the total pollen. The 50% mass median aerodynamic diameters of DEP and KLD were 0.2–0.3 and 1.25–2.5 μm, respectively (Figure 2). The average percentage of the dichloromethane-soluble organic fraction of DEP was 13.1. The concentrations of the principal ingredients of DEP and KLD are shown in Table 1. The main components of KLD were SiO₂ (37%), Fe₂O₃ (20%), and Al₂O₃ (29%). KLD of Japanese Industrial Standard Test

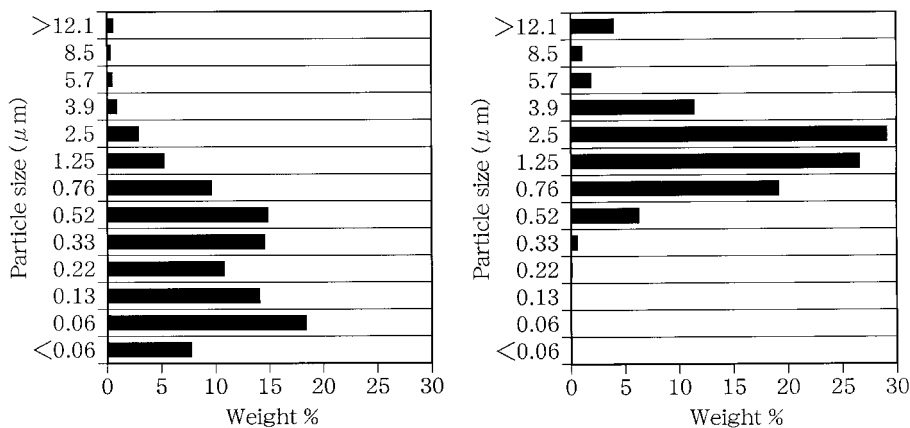


FIGURE 2. Particle size distribution of diesel exhaust particles (left) and Kanto loam dust (right) in the inhalation chamber.

TABLE 1. Concentrations of the principal ingredients of Kanto loam dust and diesel exhaust particles

Element	Unit	Kanto loam dust	Diesel exhaust particles
Aluminum	(wt%)	14	0.4
Boron	(wt%)	0.024	0.05
Calcium	(wt%)	0.22	0.036
Total chromium	(wt%)	0.01	<0.003
Iron	(wt%)	12	0.21
Total phosphorus	(wt%)	0.024	0.005
Lead	(wt%)	0.003	<0.001
Zinc	(wt%)	0.019	0.025
Nickel	(wt%)	0.005	<0.004
Total carbon	(wt%)	3.9	91.4
Total sulfur	(wt%)	0.032	0.39
Total silica	(wt%)	18.5	0

Powders No. 11 was obtained from the Association of Powder Process Industry and Engineering, Kyoto, Japan. The atmospheric concentrations of key components in the inhalation chambers measured during the experiment are summarized in Table 2. The concentration of particles in the DE and KLD groups was approximately 3.2 mg/m³. The concentrations of nitrogen oxides and nitrogen dioxide in the DE and DEG groups were approximately 30 ppm and 1 ppm, respectively.

Assays of Serum Anti-JCPAs IgE and IgGs (IgG1, IgG2a) Antibody ELISA Titers, Proliferative Response of Cervical Lymph Node Cells to JCPAs, and Nasal Rubbing Movements

Sixty mice in each group were individually bled from tail veins at wk 12, and then blood of 30 mice in each group was individually drawn from the heart under ether anesthesia at wk 24. Sera obtained from the mice in each group were stored at -80°C until measurement of JCPAs-specific IgE and IgGs antibody ELISA titers.

Details of the measurement methods for JCPAs-specific IgE and IgGs antibody ELISA titers, proliferative response to JCPAs by cervical lymph node cells, and nasal rubbing movements were as described previously (Maejima et al., 1997, 2000). Briefly, for JCPAs-specific IgE antibodies, wells of micro-titer plates were coated with 100 µl of a commercial anti-mouse IgE rat monoclonal antibody diluted to 20 µg/ml with 0.1 M NaHCO₃ (pH 8.2) at 37°C for 3 h, and then the plates were washed 5 times with phosphate-buffered saline (PBS) containing 0.05% Tween. The wells were then successively incubated at room temperature with the following reagents, washing 5 times between each pair of steps: 250 µl lactoprotein liquid for blocking at 4°C overnight; 100 µl of 10-fold dilutions of exposed or control mice sera; 100 µl biotinylated JCPAs (1 µg/ml); 100 µl streptavidin–peroxidase diluted 1:1,000; 100 µl substrate solution containing 16 mg of *o*-phenylenediamine-

TABLE 2. Mean concentrations with standard deviations of the key components of the exposure atmospheres measured in the inhalation chambers during the experiment

Group	Particles (mg/m ³)	NOx (ppm)	NO (ppm)	SO ₂ (ppm)	NO ₂ (ppm)	CO (ppm)	CO ₂ (%)
DE	3.24 ± 0.33	30.4 ± 3.1	29.4 ± 3.0	0.04 ± 0.03	1.0 ± 0.2	8.2 ± 0.8	0.50 ± 0.05
DEG	0.01 ± 0.01	28.3 ± 1.5	27.2 ± 1.5	0.02 ± 0.02	1.1 ± 0.1	7.9 ± 0.5	0.47 ± 0.03
KLD	3.29 ± 0.44	0.04 ± 0.01	0.03 ± 0.01	ND	0.01 ± 0.01	0.7 ± 0.2	0.09 ± 0.02
Pollen	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	ND	0.01 ± 0.01	0.6 ± 0.3	0.09 ± 0.02
Control	0.002 ± 0.002	0.04 ± 0.01	0.02 ± 0.01	ND	0.01 ± 0.01	0.6 ± 0.2	0.08 ± 0.02

Note. ND, not detected.

2HCl and 0.005% hydrogen peroxide in 100 ml substrate buffer (4.7 g citric acid and 7.3 g Na_2HPO_4 /L distilled water, pH 5.0). The enzyme reaction was stopped with 100 μl of 4 N H_2SO_4 , and absorbance was read at 490–650 nm with a microplate reader. Each antibody titer was expressed as the absorbance obtained by subtracting the absorbance of control mice serum from that of exposed mice serum. The minimum positive titers of IgE and the IgGs were set as 0.001, and the mean values of antibody titers calculated with data for all the animals were compared among the groups at wk 12 and 24. The percentages of animals with positive JCPAs-specific IgE or IgG1 antibody titers greater than 0.001 were also compared among the groups.

JCPAs-specific proliferative responses of lymphocytes in the lymph node in the vicinity of the nasal cavity were examined. Briefly, for cervical lymph node cells (CLNCs), the cervical lymph nodes of 10 mice in each group were pooled. CLNCs were obtained by passage through a stainless steel mesh, counted, and 6×10^5 cells/well were cultured for 3 days in a CO_2 incubator with various doses of JCPAs (0, 0.4, 2, 10 $\mu\text{g/ml}$) in the presence of mitomycin C (50 $\mu\text{g/ml}$)-treated syngeneic spleen cells as antigen-presenting cells (6×10^5 cells/well) in 96-well flat-bottomed plates in RPMI-1640 culture medium containing 10% fetal bovine serum, 5 mM HEPES, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 100 U/ml penicillin, and 100 $\mu\text{g/ml}$ streptomycin. After 3 days of cell culturing, the CLNCs were subsequently radiolabeled with [^3H]thymidine for 16 h. The uptake of radio-labeled thymidine was counted with a β -scintillation counter, and the results were expressed as counts per minute (cpm). The mean values and standard deviations of cpm were calculated from values for three wells of each microtiter plate.

Nasal rubbing movements in mice, as a parameter of allergic rhinitis, were counted for 5 min after nasal challenge, with 500 μg pollen grains suspended in phosphate-buffered saline, at both exposure time points.

For both measurement of proliferative responses of CLNs to JCPAs and nasal rubbing movements at wk 12 and 24, 10 mice per group were used.

Assays of Cytokines in Nasal Lavage Fluids

For the measurement of cytokines in nasal lavage fluids, a catheter was inserted into the pharynx and fixed; then 3.5 ml sterile saline solution was infused by syringe and the effluent was collected from exposed and control animals. Nasal lavage fluid from 10 mice per group at wk 12 and 24 was passed through a microfilter, and around 3 ml filtrate was collected in tubes and stored at -80°C until use for cytokine analysis. The concentrations of interferon- γ (IFN- γ) and interleukin 4 (IL-4) in nasal lavage fluids were analyzed with enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Camarillo, CA).

Histopathological Examination of Nasal Cavity

Details of histopathological preparations of nasal cavity were as described previously (Maejima et al., 1997). Briefly, after blood was drawn,

the brain and the mandibulum were removed from the heads of the mice and the maxillopharyngeal region was fixed in 4% paraformaldehyde/0.1 M phosphate buffer (pH 7.4) for several days. Specimens from 10 mice in each group at 12 and 24 wk were decalcified by treatment with 5% formic acid and 10% neutral-buffered formalin and processed routinely for embedding in paraffin. Sections were stained with hematoxylin and eosin, toluidine blue, and immunostaining for IgE.

Statistics

Data were analyzed by Student’s *t*-test or the chi-square test to compare individual groups. The level for significance was set at *p* < .05.

RESULTS

Anti-JCPAs IgE and IgGs Antibody Titers in Sera

The mean values with standard deviations for JCPAs-specific IgE and IgGs antibody titers in each group at wk 12 and 24 are shown in Table 3. Individual differences were marked in all groups at 12 and 24 wk after the start of exposure.

The mean values for JCPAs-specific IgE antibody titers in the DE, KLD, or DEG groups were higher than in the pollen alone group, but without statistical significance at both exposure time points. Changes in IgG1 antibody titer were similar to those for IgE, but in this case, statistical significance was found for DE and DEG at wk 12 and DE at wk 24, as compared to the pollen alone. The mean values for IgG2a antibody titers of DE, KLD, and DEG groups were significantly lower than for the pollen group at wk 24.

The percentages of animals expressing more than the minimum ELISA titer for JCPAs-specific IgE antibodies (positive rates for JCPAs-specific IgE antibodies) in each group are shown in Figure 3. Values for DE, KLD, DEG, and pollen groups at wk 12 were 22%, 27%, 27%, and 22%, respectively, with no significant intergroup differences. However, at wk 24 in the DF,

TABLE 3. Mean values with standard deviations of JCPAs-specific IgE, IgG1, and IgG2a antibody ELISA titers in each group at 12 or 24 wk after the start of exposure

Group	Time	IgE	IgG1	IgG2a
Pollen	12 wk	0.003 ± 0.022	0.006 ± 0.014	0.017 ± 0.024
	24 wk	0.007 ± 0.032	0.015 ± 0.043	0.034 ± 0.047
KLD	12 wk	0.011 ± 0.053	0.019 ± 0.112	0.007 ± 0.012
	24 wk	0.035 ± 0.103	0.048 ± 0.139	0.008 ± 0.016 ^a
DE	12 wk	0.008 ± 0.03	0.025 ± 0.05 ^a	0.013 ± 0.024
	24 wk	0.032 ± 0.071	0.125 ± 0.285 ^a	0.008 ± 0.017 ^a
DEG	12 wk	0.006 ± 0.025	0.029 ± 0.065 ^a	0.008 ± 0.018
	24 wk	0.028 ± 0.078	0.083 ± 0.263	0.011 ± 0.021 ^a

^aStatistically significantly different (*p* < .05) from the pollen group.

KLD, and DEG groups the responders comprised 73%, 63%, and 67%, respectively, significantly higher percentages than that in the pollen group (33%). No significant differences in the values were observed among the DE, KLD, and DEG groups. The percentages of animals with positive IgG1 antibodies in the DE and DEG groups were significantly higher than for the pollen group at both exposure time points.

Proliferative Response of Cervical Lymph Node Cells to JCPAs

Almost no proliferative response of cervical lymph node cells to JCPAs was seen in any groups at wk 12. However, a weak dose-dependent increase in the DE and KLD groups was observed at wk 24 (Figure 4).

Cytokines in Nasal Lavage Fluids

The mean values with standard deviations for IL-4 and IFN- γ in nasal lavage fluid for each group at wk 12 and 24 are shown in Figure 5. A remarkable decrease of IFN- γ in the DE and DEG groups as compared to the pollen group was observed at wk 12, while IL-4 in the DE and KLD groups was increased significantly.

Nasal Rubbing Movements and Histopathological Findings

The number of nasal rubbing movements in each group did not reveal any exposure-related influence. No apparent remarkable histopathological

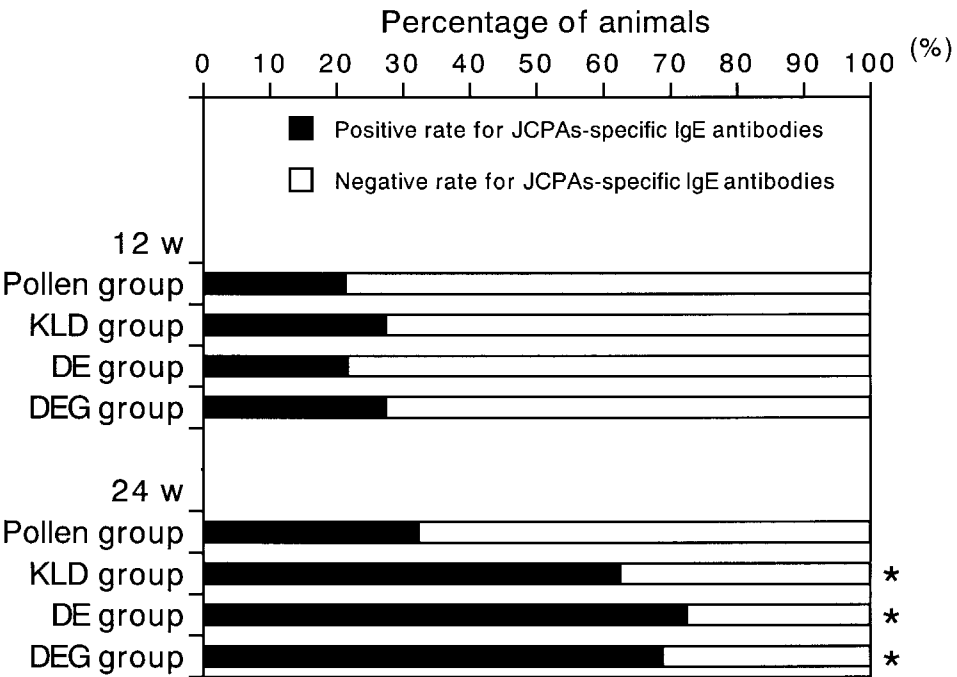


FIGURE 3. Percentages of animals expressing more than the minimum ELISA titer for IgE antibodies in each group. Asterisks indicate significant differences from the pollen group, $p < 0.05$.

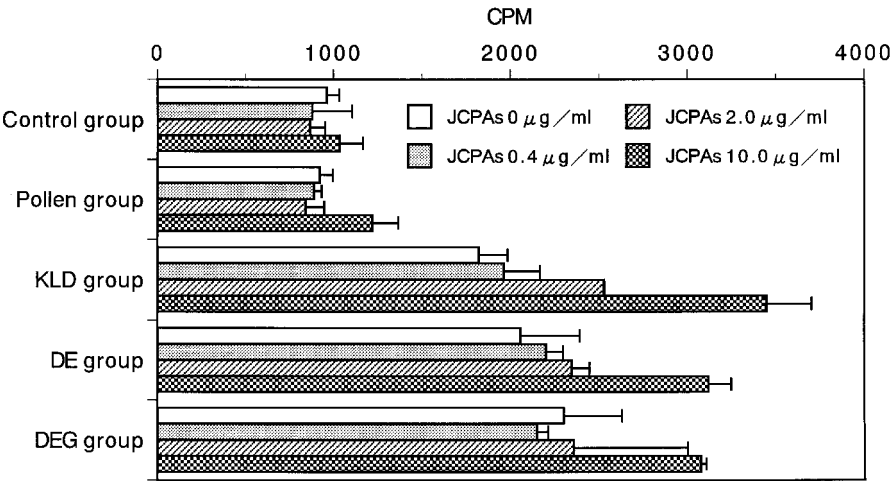


FIGURE 4. Proliferative responses to JCPAs of cervical lymph node cells pooled from each of 10 mice per group at 24 wk after the start of exposure. Columns and bars represent means $\pm$ SD, calculated from values for three wells of microtiter plates.

alterations such as hypertrophy, infiltration of inflammatory cells, or recruitment of mast cells were noted in the mucosal membranes of the nasal cavities of the exposed animals as compared with the control case (Figures 6 and 7).

DISCUSSION

In the present study, a time-dependent increase in the average JCPAs-specific IgE antibody ELISA titer in serum was observed in mice exposed only to pollen, and the DE, KLD, and DEG caused this to be augmented. Thus, the positive rate for JCPAs-specific IgE antibodies was significantly

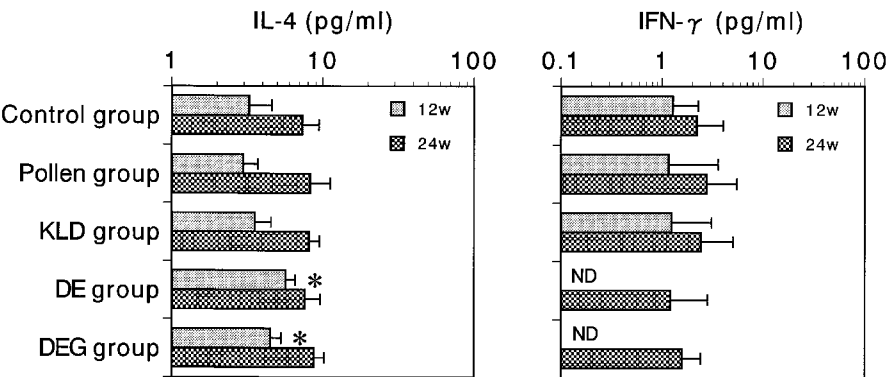


FIGURE 5. Concentrations of IL-4 and IFN- γ in nasal lavage fluid at 12 and 24 wk after the start of exposure. Columns and bars represent means $\pm$ SD for each of 10 mice per group. Asterisks indicate significant differences from the control group, $p < .05$. ND, not detected.

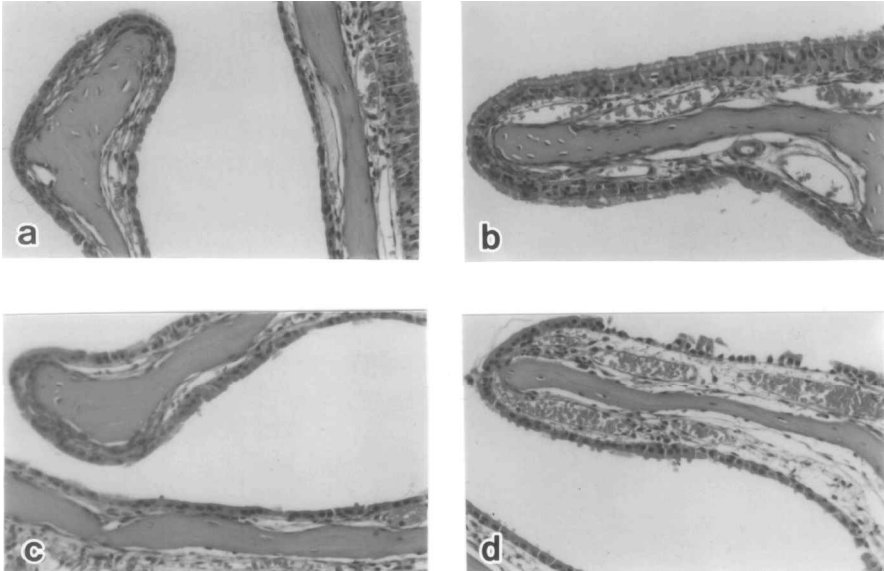


FIGURE 6. The mucosal membrane of the nasal cavity at 24 wk after the start of exposure (H&E, original magnification $\times 200$). (a) pollen alone, (b) Kanto loam dust and pollen, (c) diesel exhaust and pollen, and (d) diesel exhaust without particles and pollen.

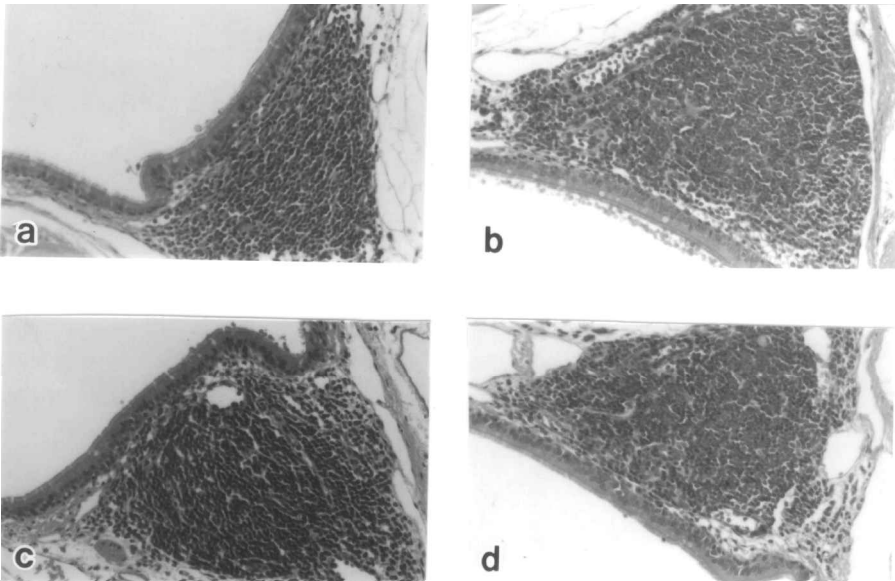


FIGURE 7. The nasal-associated lymphoid tissue (NALT) at 24 wk after the start of exposure (H&E, original magnification $\times 200$). NALT is the lymphoid tissue beneath the mucous membrane of the anterior orifice of the nasopharyngeal duct. (a) Pollen alone, (b) Kanto loam dust and pollen, (c) diesel exhaust and pollen, and (d) diesel exhaust without particles and pollen.

higher in the groups exposed to the tested materials than in the pollen-alone group after 24 wk of exposure, with no differences among the three additional treatments. Based on the findings obtained under the present experimental conditions, it can be concluded that JCPAs-specific IgE antibodies are induced in mice by exposure to pollen, and DE, KLD, and DEG promote their production, while the degree of adjuvant activity was almost the same in all three cases. However, we believe that the immune response in the mice observed in the present study was an early-phase response, based on the lower IgE antibody titers in the sera, the weak proliferative response of cervical lymph nodes to JCPAs, and/or the lack of observations of histopathological abnormality in the nasal cavity.

T-helper lymphocytes of mice are of two different subsets, Th1 and Th2. Several cytokines produced by Th2 type cells, such as interleukin 4 (IL-4), promote IgE or IgG1 antibody production, whereas those from Th1 type cells, such as interferon- γ (IFN- γ), enhance generation of IgG2a (Takahashi et al., 1996). In the present study, not only increases of IgE or IgG1 antibodies in sera, but also decreases of IgG2a antibody in the DE, KLD, and DEG groups were observed at both exposure time points, as compared with the pollen group, and tendencies for decrease in the Th1 cytokine, IFN- γ , and increase in the Th2 cytokine, IL-4, in the nasal lavage fluid of the DE and DEG groups was observed in the early exposure period. Moreover, dose-dependent increase in the proliferative responses of cervical LNCs to JCPAs in the DE and KLD groups was noted at wk 24, which was lacking in the DEG group, suggesting linkage to particles. Since the shift from Th1 to Th2 type cytokines was limited to the DE and DEG groups, it presumably was due to gas components. Thus, different promoting mechanisms can be speculated upon. Specifically, the fine particles appear to enhance production of IgE antibodies owing to activation of T lymphocytes, while gas components exhibit almost no action on T lymphocytes, but directly induce disorders of the cytokine network and increase local generation of IgE antibodies.

Since the number of antigen-specific T lymphocytes is considered to be small during the primary immune response, there is a possibility that IL-4 and IFN- γ from cells other than T lymphocytes were involved in the stimulation by DE and DEG. In the case of human beings, lung mast cells and blood basophils are reported to produce IL-4, thereby generating IgE antibodies without the necessity of T lymphocytes (Gauchat et al., 1993). According to the hypothesis of Barnes and Liew, (1995), IFN- γ secreted by Th1 type cells stimulates airway epithelial cells and induces iNOS (inducible NO-synthase), and consequently production of a large amount of NO. This then suppresses IFN- γ , and proliferated Th2 type cells generate IL-4, which in turn suppresses Th1 type cells and NO production by macrophages, but not epithelial cells, whereby activation of Th2 type cells is maintained. In addition to such a mechanism, if NO contained in DE exerts an action directly on epithelial cells, this also could stimulate production of IgE antibodies.

On the other hand, the similar results from comparison of the positive rates for JCPAs-specific IgE antibodies between the DE and KLD groups suggest an influence of the different amounts of DEP and KLD adhering to the surface of pollen in the air, shown in Figure 8. Behrendt et al. (1997) reported an associated significant increase of protein release from pollen, with dose and time dependence, indicating direct actions of substances adsorbed to airborne particles on structural elements of the pollen. The nature of the particles may be related to enhancement of IgE antibody production, explaining differences between KLD and DEP. The major element of KLD is silicon, considered to promote production of antigen-specific antibodies (Fujimaki et al., 1984, 1986; Mancino et al., 1983; Mancino & Ovary, 1980; Maejima et al., 1997). Accordingly, there is a possibility that silicon in the Kanto loam dust may have played an important role in the increase of the JCPAs-specific IgE antibodies in our mice.

In the early phase of exposure when the pollen exhibited the effect of sensitization, the mechanism shown in Figure 9 may have been working. Whatever the case, the experimental results for DEP only, and the results using Kanto loam dust and diesel exhaust without particles allow speculation that deserves further investigation.

In the present study, JCP was given to mice by whole-body exposure, which may be complicated by oral administration of allergens due to animals licking their fur. However, this may also occur in the case of Japanese monkeys (*Macaca fuscata*) and dogs kept outdoors in which natural occurrence of JCPsis has been reported (Nakamura et al., 1994; Sasaki et al., 1995). Although the chance of humans taking in pollen orally are very low as compared with animals, whole-body exposure that includes oral administration was here considered to be the most practical method of sensitization.

In Japan, JCP counts in the atmosphere are commonly made on the basis of numbers of pollen grains deposited on a glass slide in a collector

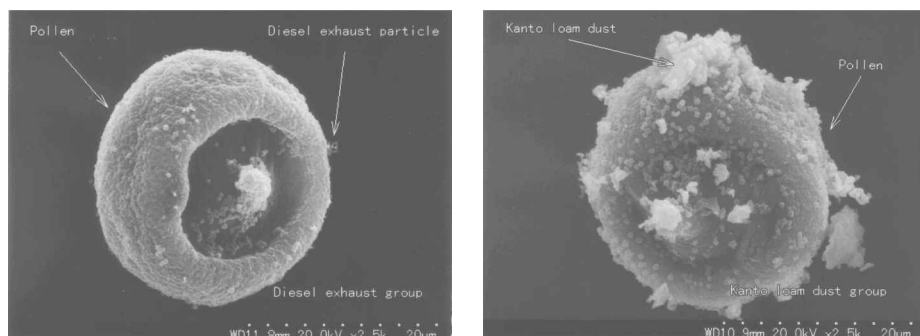


FIGURE 8. Scanning electron micrographs of pollen surfaces collected on adhesive tapes placed on the tops of the animal cages (left, DE group; right, KLD group). Large quantities of KLD were seen with around 65% pollen grains observed in the inhalation chamber of the KLD group, whereas at around 35% pollen grains there were limited particles in the inhalation chamber of the DE group.

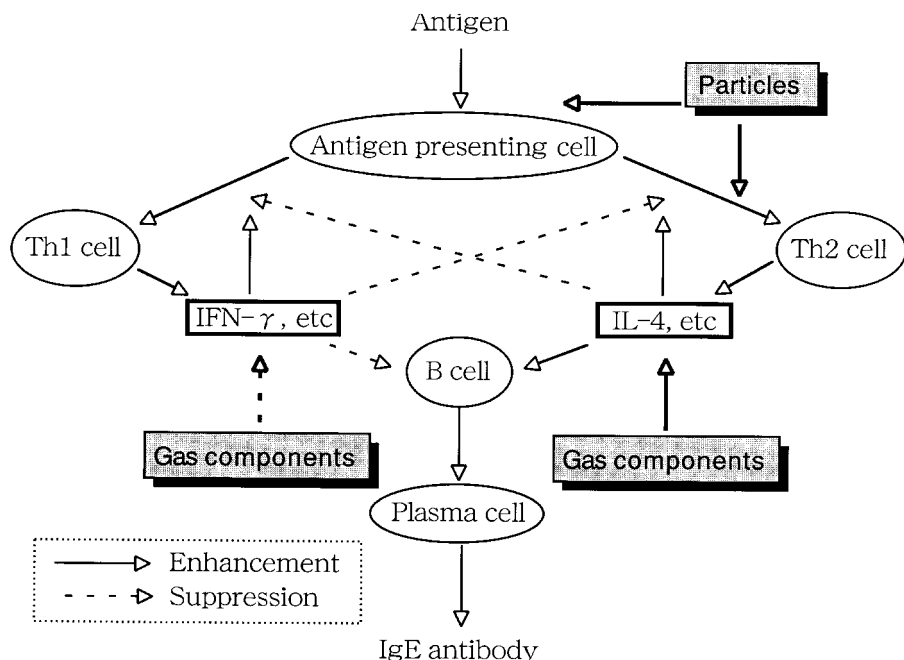


FIGURE 9. A possible model for the interaction between antibody production and cytokinetic pathways, and air pollutants in mice.

and expressed in values per day per square centimeter. Although it is difficult to estimate the relation between the exposure pollen concentration (pollen grains/m³) in the present study and the actual pollen concentration in the air in the suburbs of typical Japanese cities (pollen grains/day/cm²), the concentration used in this experiment (around 550,000 pollen grains/m³) is estimated to be several hundred times the concentration in the atmosphere on maximum scattering days (Dejima et al., 1992). Humans suffer from pollinosis if chronically exposed to an environment with pollen concentrations much lower than that in the present experiment. Regarding the exposure concentration of diesel exhaust particles and Kanto loam dust (about 3.2 mg/m³), the former is about 100–200 times that found by motor vehicle exhaust monitoring stations in Japan. Although the actual KLD concentration in the air in suburbs of typical Japanese cities is not clear, it is very unlikely that human beings would be exposed to KLD at the levels employed here. Therefore, it should be understood that the exposure conditions used in the present study were peculiar, and our experimental data from small rodents may not be easily extrapolated to the human case.

In conclusion, the results obtained from the present study suggest that fine particles may enhance the production of IgE antibodies in mice exposed to pollen. However, the nature of the particles could also be important. Furthermore, the findings suggest that gas components of DE may

be important factors in adjuvant activity regarding production of IgE antibodies in mice. In order to determine the dose response, further experiments with various concentrations of DE are now necessary.

REFERENCES

- Abramson, M., Kutin, J. J., Bailey, M., Raven, L., Dubster, K., Rolland, J., Czarny, D., and Walters, E. H. 1997. Nasal allergies and hayfever among young adults in Melbourne, Australia. *Allergol. Int.* 46:213–219.
- Barnes, P. J., and Liew, F. Y. 1995. Nitric oxide and asthmatic inflammation. *Immunol. Today* 16: 128–130.
- Behrendt, H., Becker, W. M., Fritzsche, C., Sliwa-Tomczok, W., Tomczok, J., Friedrichs, K. H., and Ring, J. 1997. Air pollution and allergy: Experimental studies on modulation of allergen release from pollen by air pollutants. *Int. Arch. Allergy Immunol.* 113:69–74.
- Bice, D. E., Mauderly, L., Jones, R. K., and McClellan, R. O. 1985. Effects of inhaled diesel exhaust on immune responses after lung immunization. *Fundam. Appl. Toxicol.* 5:1075–1086.
- Charpin, D., Hughes, B., Mallea, M., Sutra, J.-P., Balansard, G., and Vervloet, D. 1993. Seasonal allergic symptoms and their relation to pollen exposure in southeast France. *Clin. Exp. Allergy* 23:435–439.
- D'Amato, G., Spieksma, F. T. M., Liccardi, G., Jager, S., Russo, M., Kontou-Fili, K., Nikkels, H., Wuthrich, B., and Bonini, S. 1998. Pollen-related allergy in Europe. *Allergy* 53:567–578.
- Dejima, K., Saito, Y., and Shoji, H. 1992. A relationship between the annual onset day of Japanese cedar pollinosis and pollen dispersion. *J. Allergol.* 41:1405–1412 (in Japanese with English summary).
- Fujimaki, H., Ozawa, M., Imai, T., Kubota, K., and Watanabe, N. 1984. Adjuvant effects of aluminum silicate on IgE and IgG1 antibody production in mice. *Int. Arch. Allergy Appl. Immunol.* 75:351–356.
- Fujimaki, H., Hirano, S., Takenaka, S., Murakami, M., and Watanabe, N. 1986. Enhanced IgE antibody production in mice injected with fly ash. *Int. Arch. Allergy Appl. Immunol.* 80:127–131.
- Fujimaki, H., Saneyoshi, K., Nohara, O., Shiraishi, F., and Imai, T. 1995. Intranasal instillation of diesel exhaust particulate and antigen in mice modulated cytokine production in cervical lymph node cells. *Int. Arch. Allergy Appl. Immunol.* 108:268–273.
- Fujimaki, H., Saneyoshi, K., Shiraishi, F., Imai, T., and Endo, T. 1997. Inhalation of diesel exhaust enhances antigen-specific IgE antibody production in mice. *Toxicology* 116:227–233.
- Gauchat, J. F., Henchoz, S., Mazzel, G., Aubry, J. P., Brunner, T., Blasey, H., Life, P., Talabot, D., Flores-Romo, L., Thompson, J., Kishi, K., Butterfield, J., Dahinden, C., and Bonnefoy, J. Y. 1993. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 365:340–343.
- Horiguchi, S., and Saito, Y. 1964. Japanese cedar pollinosis in Nikko Tochigi. *Jpn. J. Allergol.* 13:16–18 (in Japanese).
- Ishizaki, T., Koizumi, K., Ikemori, R., Ishiyama, Y., and Kushibiki, E. 1987. Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. *Ann. Allergy* 58:265–270.
- Lovik, M., Hogseth, A. K., Gaarder, P. I., Hagemann, R., and Eide, I. 1997. Diesel exhaust particles and carbon black have adjuvant activity on the local lymph node response and systemic IgE production to ovalbumin. *Toxicology* 121:165–178.
- Maejima, K., Tamura, K., Taniguchi, Y., Nagase, S., and Tanaka, H. 1997. Comparison of the effects of various fine particles on IgE antibody production in mice inhaling Japanese cedar pollen allergens. *J. Toxicol. Environ. Health* 52:231–248.
- Maejima, K., Tamura, K., Taniguchi, Y., and Saito, S. 2000. Study on the inhalation exposure conditions of pollen for inducing the production of Japanese cedar (*Cryptomeria japonica*) pollen-specific IgE antibody in mice. *Jpn. J. Palynol.* 46:155–161.

- Mancino, D., and Ovary, Z. 1980. Adjuvant effects of amorphous silica and aluminium hydroxide on IgE and IgG1 antibody production in different inbred mouse strains. *Int. Arch. Allergy Appl. Immunol.* 61:253–258.
- Mancino, D., Buono, G., Cusano, M., and Minucci, M. 1983. Adjuvant effects of a crystalline silica on IgE and IgG1 antibody production in mice and their prevention by the macrophage stabilizer poly-2-vinylpyridine *N*-oxide. *Int. Arch. Allergy Appl. Immunol.* 71:279–281.
- Muranaka, M., Suzuki, S., Koizumi, K., Takafuji, S., Miyamoto, T., Ikemori, R., and Tokiwa, H. 1986. Adjuvant activity of diesel-exhaust particulate for the production of IgE antibody in mice. *J. Allergy Clin. Immunol.* 77:616–623.
- Nakamura, S., Minezawa, M., Gotoh, S., Yokota, A., Hashimoto, M., and Nigi, H. 1991. Naturally occurring pollinosis in the Japanese monkey (*Macaca fuscata*). In *Primate today*, ed. E. Ehara, pp. 643–646. Amsterdam: Elsevier Science Publishers B.V.
- Nilsen, A., Hagemann, R., and Eide, I. 1997. The adjuvant activity of diesel exhaust particles and carbon black on systemic IgE production to ovalbumin in mice after intranasal instillation. *Toxicology* 124:225–232.
- Okuda, M., and Shida, T. 1998. Clinical aspects of Japanese cedar pollinosis. *Allergol. Int.* 47:1–8.
- Sasaki, Y., Kitagawa, H., Fujioka T., Kitoh, K., Iwasaki, T., Sakaguchi, M., and Inouye, S. 1995. Hypersensitivity to Japanese cedar (*Cryptomeria japonica*) pollen in dogs. *J. Vet. Med. Sci.* 57:683–685.
- Seaton, A., Soutar, A., and Mullins, J. 1996. The increase in hay fever: Pollen, particulate matter and SO₂ in ambient air. *Q. J. Med.* 89:279–284.
- Takafuji, S., Suzuki, S., Koizumi, K., Tadokoro, K., Miyamoto, T., Ikemori, R., and Muranaka, M. 1987. Diesel-exhaust particulate inoculated by the intranasal route have an adjuvant activity for IgE production in mice. *J. Allergy Clin. Immunol.* 79:639–645.
- Takahashi, S., Fossati, L., Iwamoto, M., Merino, R., Motta, R., Kobayakawa, T., and Izui, S. 1996. Imbalance towards Th1 predominance is associated with acceleration of lupus-like autoimmune syndrome in MRL mice. *J. Clin. Invest.* 97:1597–1604.
- Yasueda, T., Yui, Y., Shimizu, T., and Shida, T. 1983. Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J. Allergy Clin. Immunol.* 71:77–86.