

Questa fotocopia viene fornita
per motivi strettamente personali
di studio e/o ricerca

COMPARISON OF THE EFFECTS OF VARIOUS FINE
PARTICLES ON IgE ANTIBODY PRODUCTION IN MICE
INHALING JAPANESE CEDAR POLLEN ALLERGENS

Kazuhito Maejima, Kumiko Tamura

Japan Automobile Research Institute, Ibaraki, Japan

Yosifumi Taniguchi

Hayashibara Biochemical Laboratories, Inc., Okayama, Japan

Sumi Nagase

Nagase Institute, Ibaraki, Japan

Hisako Tanaka

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

The adjuvant effects of various fine particles [Kanto loam dust, fly ash, carbon black, diesel exhaust particles (DEP), and aluminum hydroxide (alum)] on immunoglobulin E (IgE) antibody production in female BDF₁ mice were examined. In experiment 1, animals both received 25 µg of each particle intranasally and were exposed to aerosolized Japanese cedar pollen allergens (JCPA) for 30 min/d at 1-wk intervals for the first 8 wk. This was followed by exposure for 30 min every 3 wk for the next 9 wk. As parameters of allergic rhinitis, measurements were made of JCPA-specific IgE and IgG antibody titers, the protein-adsorbing capacity of each type of particle, and nasal rubbing movements. The increases in anti-JCPA IgE and IgG antibody production in mice treated with aerosolized JCPA plus respective particles were significantly greater than that found with aerosolized JCPA alone. This was associated with no marked differences in the other allergic rhinitis parameters. In experiment 2, after the administration of particles as in experiment 1, about 160,000 grains of Japanese cedar pollen (JCP, native dry pollen) were dropped onto the tip of the nose of mice twice a week for 16 wk. Six weeks after the first immunization, the anti-JCPA IgE antibody titers of groups treated with the respective particles were greater than 1:20, whereas those of mice treated with JCP alone were 1:10. No significant differences in the anti-JCPA IgE and IgG antibody productions, nasal rubbing counts, or histopathological changes were observed after 18 wk. These results suggested the nature of the particles, their capacity to adsorb antigens, and/or their size may not be related to enhancement of IgE antibody production nor symptoms of allergic rhinitis. However, IgE antibody production seemed to occur earlier in mice treated with particles than in mice immunized with allergens alone.

In Japan, the incidence of allergic rhinitis caused by Japanese cedar (*Cryptomeria japonica*) pollen has increased markedly in the last 30 yr (Inouye et al., 1986a). Inouye et al. (1986b) reported that about 30% of the general population aged 20–39 yr residing in the Tokyo area tested immunoglobulin E (IgE) antibody-positive for Japanese cedar

Received 27 August 1996; sent for revision 1 October and 13 November 1996; accepted 5 March 1997.

Address correspondence to Dr. Kazuhito Maejima, Health Effect Research Laboratory, Japan Automobile Research Institute, 2530 Karima, Tsukuba-shi, Ibaraki 305, Japan. E-mail: kmae@jari.or.jp

pollen allergens (JCPA). Two major allergens have been isolated and characterized as Cry j I and Cry j II (Yasueda et al., 1983; Sakaguchi et al., 1990). In another study using a periodical health examination of 892 university students, the percentage of subjects with JCPA-specific IgE antibody was 27% and the incidence of those with Japanese cedar pollinosis was 12% (Nakamura, 1990). In Japan, Japanese cedar pollinosis is considered to be a frequently occurring immediate-type allergic disease with symptoms such as sneezing, rhinorrhea, nasal congestion, and lacrimation.

Several epidemiological studies have suggested that the rapid increase of subjects with pollinosis by comparison of the incidences of pollinosis in different parts of the Nikko district of Japan is related to air pollution (Kaneko et al., 1980; Mori, 1995; Ishizaki et al., 1987). Results showed that the incidence of pollinosis among residents alongside main roads with heavy traffic was higher than that of residents in districts with less traffic. Since the pollen counts in these areas were almost the same and there were no significant differences in the age or sex distributions of the subjects examined in these environments, air pollution was concluded to be a possible enhancing factor.

Muranaka et al. (1986) reported that the IgE antibody responses to ovalbumin (OA) in mice immunized by intraperitoneal injection of OA mixed with diesel exhaust particles (DEP) were higher than with OA alone. Moreover, Suzuki and Kanoh (1993) reported that pyrene, which is abundant in the neutral fraction extracted from DEP, produced an adjuvant effect on IgE antibody production in mice immunized by intraperitoneal injection of OA or JCPA.

These investigations suggest that air pollutants emitted from automobiles, especially DEP, enhance pollinosis. However, there are few reports on whether the enhancing effect on IgE antibody production is characteristic of DEP or whether other respirable particles may also exert the same effect. Therefore, in the present study, the potential enhancing effects of various kinds of fine particles [Kanto loam dust, fly ash, carbon black, DEP, and aluminum hydroxide (alum)] on IgE antibody production in mice exposed to aerosolized JCPA or Japanese cedar pollen (JCP) were examined.

MATERIALS AND METHODS

Mice

Female BDF₁ mice aged 10 wk (22–23 g body weight) were obtained from CLEA Japan, Inc. During the experiment, the animals were housed 6 to a plastic cage with wood shavings in an air-conditioned room (temperature $23 \pm 1^\circ\text{C}$; relative humidity $65 \pm 10\%$), with artificial lighting for 12 h/d from 7 a.m. to 7 p.m. They were provided with a normal diet (CE-2, CLEA Japan, Inc.) and water ad libitum throughout the experiment.

Allergens and Particles

The crude JCPA used in this study (experiment 1) was kindly provided by Hayashibara Biochemical Laboratories, Okayama, Japan. One liter of 0.125 M ammonium bicarbonate was added to 50 g of Japanese cedar pollen from Akita prefecture, and crude JCPA was extracted by shaking the mixture for 2 h at 4°C and then centrifugation for 30 min at $10,000 \times g$. The contents of total protein and of two major allergens (Cry j I and Cry j II) in the supernatant were measured by the method of Bradford (1976) and enzyme-linked immunosorbent assay (Sawatani et al., 1993), respectively. The amount of protein in the crude JCPA was 65 µg containing 17.4 µg of Cry j I and 1.73 µg of Cry j II per milliliter. The crude JCPA was dialyzed against distilled water before administration to mice by inhalation. The native dry JCP used in this study (experiment 2) was collected in Chiba prefecture.

Kanto loam dust (dust for industrial testing no. 11), fly ash (no. 10), and carbon black (no. 12) were obtained from the Association of Powder Process Industry and Engineering, Kyoto, Japan. DEP were collected in the dilution tunnel used for an inhalation experiment in our institute (Nakajima & Suzuki, 1995). Aluminum hydroxide (alum) used as a positive control of these particles was prepared by the method of Levine and Vaz (1970). The characteristics of these particles are shown in Table 1.

Immunization Schedules

Experiment 1 Six groups of 6 mice were immunized 8 times at 1-wk intervals, followed by once in 3 wk or the next 9 wk. Mice were anesthetized with ether, and 25 µg of particles in 10 µl of phosphate-buffered saline solution (PBS, pH 7.2) was dropped onto the

TABLE 1. Characteristics of particles

	Particle size (µm)	Main components (%)	Surface area (m ² /g)
Kanto loam dust	1.6–2.3	SiO ₂ (34–40) Al ₂ O ₃ (26–32)	1.9
Fly ash	4.8–5.7	SiO ₂ (>45) Al ₂ O ₃ (>20)	3.9
Carbon black	0.03–0.2	Carbon (100)	20.4
DEP	0.3–0.5	Carbon (40) SO ₄ ²⁻ (20) Soluble organic fraction (40) ^a	2.4
Aluminum hydroxide (alum)	0.26–2.2	Al(OH) ₃ (100)	6.2

^aThe main components in the soluble organic fraction were pyrene, 1-nitropyrene, phenanthrene, and benz[a]anthracene.

nostrils of each mouse. The groups were then exposed to crude JCPA aerosol generated with a glass nebulizer at a total protein concentration of 1 µg/L for 30 min/d (Maejima et al., 1995). Over 80% of the aerosolized JCPA droplets were less than 1.0 µm in diameter. Another group of six mice was exposed to aerosolized JCPA without administration of particles.

Nasal rubbing movements in mice, a parameter of allergic rhinitis (Saito et al., 1994), were counted for 5 min after nasal challenge for 1 wk after the final immunization. The allergen used for nasal challenge was 500 µg of pollen grains suspended in PBS and administered by the same route as particles.

Mice in each group were bled from tail vein at 3-wk intervals after the first immunization. One week after measurement of nasal rubbing movements, blood was drawn from the heart of mice under ether anesthesia. Sera obtained from the mice in each group were pooled and stored at -80°C until use.

Experiment 2 Six groups of 6 mice were immunized twice a week for 16 wk. Doses of 25 µg of the respective particles in PBS were administered to 5 groups of mice by the same procedure as in experiment 1. After the administration of particles, 8 units of JCP were dropped onto the tip of the nose of each mouse by micropipet. One unit of JCP contained about 20,000 pollen grains counted by microscopy. Other experimental procedures and measurements were as described for experiment 1.

Assays of Serum Anti-JCPA IgE and IgG Antibody Titers

Anti-JCPA IgE antibody titers were measured by the passive cutaneous anaphylaxis (PCA) reaction using rat dorsal skin with a 24-h sensitization period by the method of Watanabe and Ovary (1977). Briefly, 0.1-ml samples of serial twofold dilutions of the test sera were injected intracutaneously into the back of male Sprague-Dawley rats weighing 500–700 g (CLEA Japan, Inc.) and 24 h after this passive sensitization the rats were challenged intravenously with Sugi basic protein (SBP) in 1 ml of 1% Evan's blue dye. The dose of antigens used for challenge was 130 µg of SBP containing 100 µg of Cry j I and 30 µg of Cry j II. SBP purified from JCP from Chiba prefecture by the methods of Yasueda et al. (1983) and Taniguchi et al. (1989) was also provided by Hayashibara Biochemical Laboratories. The test was carried out in duplicate and the PCA titer was expressed as the reciprocal of the maximal dilution giving a spot of 5 mm in diameter.

Enzyme-linked immunosorbent assay (ELISA) of anti-JCPA IgG antibody titers was carried out by a modification of the method of Imaoka et al. (1993). Briefly, the wells of microtiter plates (Immulon 2, Dynatech) were coated with 100 µl SBP (1 µg/ml) and incubated overnight at 4°C. Then the plates were washed 5 times with PBS

containing 0.05% Tween 20 using a microplate washer (model 1550, Bio-Rad Laboratories). The wells were then successively incubated at room temperature with the following reagents, washing 5 times between each pair of steps: 200 μ l of Block Ace (Dainihon Seiyaku, Osaka, Japan) for blocking; 100 μ l of serial 10-fold dilutions of the test and normal sera; 100 μ l of biotinylated rabbit anti-mouse IgG (Zymed) diluted 1:20,000; 100 μ l of streptavidin-peroxidase (Sigma) diluted 1:1000; 100 μ l of substrate solution containing 16 mg of o-phenylenediamine-2HCl and 0.005% hydrogen peroxide in 100 ml of substrate buffer (4.7 g citric acid and 7.3 g Na_2HPO_4 /L distilled water, pH 5.0). Absorbance was read at 490 nm with a microplate reader (model 3550, Bio-Rad Laboratories). IgG titers were expressed as reciprocals of the number of dilutions of test sera exhibiting twofold higher absorbances than the same dilution of normal serum.

Protein-Adsorbing Capacities of the Particles

Samples of 1 or 10 mg of each type of particle were added to 2 ml of crude JCPA dialyzed against PBS. Particles in the crude JCPA were dispersed with ultrasonic waves and a vortex mixer for 1 min each. After standing at room temperature for 30 min, the preparations were centrifuged at $30,000 \times g$ for 1 h at 4°C. The amount of protein adsorbed to each type of particle was estimated from the protein concentration in the supernatant as measured with protein assay kit (Bio-Rad Laboratories) based on the dye-binding assay method of Bradford (1976).

Histopathological Examinations

Three untreated mice were added to the six already mentioned groups as controls for histopathological examination. After blood was drawn, the brain and the mandibulum were removed from the heads of these mice and the maxillopharyngeal region was fixed with 4% paraformaldehyde/0.1 M phosphate buffer (pH 7.4) for several days. Specimens were decalcified by treatment with 5% formic acid and 10% formalin for several days. Three frontal sections were made as described by Nagano et al. (1988); that is, an anterior section was made at the posterior edge of the incisor teeth, a middle one at the level of the incisive papilla, and a posterior one at the anterior edge of the molar teeth. These tissue blocks were dehydrated and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin, and toluidine blue.

Immunostaining for IgE After digestion of the sections with 0.05% protease type VIII (Sigma, St. Louis, MO)/0.05 M Tris-HCl buffer, pH 7.6, at 37°C for 30 s, the sections were immersed in 5% normal goat serum for 20 min at room temperature and subsequently incubated with anti-mouse IgE monoclonal antibody (kindly provided by Dr.

N. Watanabe; Department of Tropical Medicine, Jikei University School of Medicine) at 4°C overnight. The sections were then washed and treated with horseradish peroxidase-labeled anti-rat IgG F(ab)₂ (Organon Teknika Corporation, Durham, NC) for 2 h at room temperature. IgE was then detected with 0.02% 3,3'-diaminobenzidine (DAB; Dojindo, Kumamoto, Japan)/0.05 M Tris-HCl buffer, pH 7.6, with 0.005% hydrogen peroxide. Sections were counterstained with methyl green.

Statistics

Data were analyzed by two-way analysis of variance (ANOVA) followed by Student's *t*-test to compare individual group. The level of significance was established at $p < .05$.

RESULTS

Experiment 1

Anti-JCPA IgE and IgG Antibody Productions and Nasal Rubbing Movements The anti-JCPA IgE antibody titers in each group are shown in Figure 1. The anti-JCPA IgE antibody (PCA) titers in mice immunized with aerosolized JCPA plus alum or fly ash were 1:5 and 1:10, respectively, in wk 3 after the first immunization, whereas those in the other groups were below 1:5. In wk 6 after the first immunization, the titers of all groups immunized with the respective particles were higher than 1:80, whereas that of the group treated with aerosolized JCPA alone was lower. Mice immunized with aerosolized JCPA plus carbon black, DEP, alum, fly ash, or Kanto loam dust exhibited peak levels with PCA titers of 1:320, 1:640, 1:640, 1:640, and 1:1280, respectively, in wk 18 after the first immunization. No increase in the PCA titer was observed throughout the experiment in mice inhaling aerosolized JCPA alone.

The changes in the anti-JCPA IgG antibody titers in each group are shown in Figure 2. Previously it was found that most of anti-JCPA IgG antibody in mice was the anti-JCPA IgG₁ antibody type (data not shown). Therefore, the anti-JCPA IgG antibody rather than the anti-JCPA IgG₁ antibody was measured. The anti-JCPA IgG antibody titer in mice immunized with aerosolized JCPA plus DEP was 1:1000 in wk 3 after the first immunization, whereas those in the other groups were below 1:100. In wk 6 the ELISA titers of groups immunized with aerosolized JCPA plus the respective particles were all greater than 1:1000 except for treated with aerosolized JCPA alone. The mice immunized with aerosolized JCPA plus fly ash, Kanto loam dust, alum, carbon black, and DEP exhibited ELISA titers of 1:100,000, 1:500,000, 1:500,000, 1:500,000, and 1:500,000, respectively, in wk 18 after the first immu-

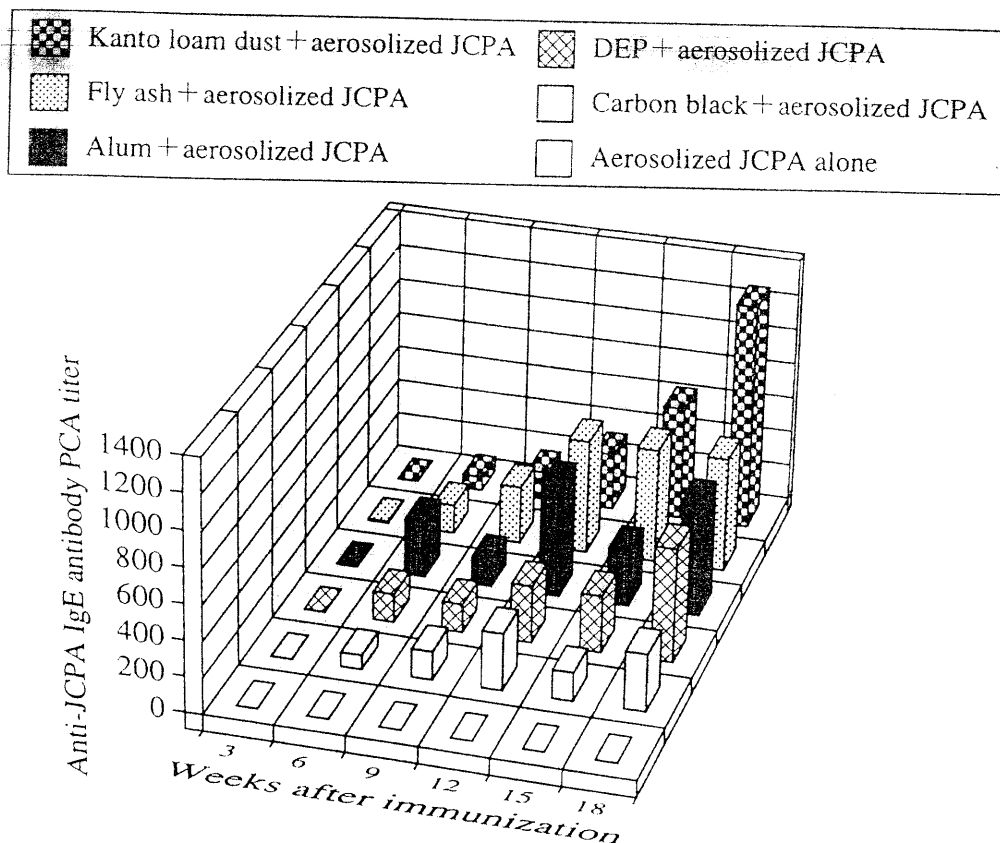


FIGURE 1. Anti-JCPA IgE antibody production in mice in response to intranasal administration of aerosolized JCPA with or without 25 μ g of particles. PCA titers were measured on pooled sera from six mice in each group.

nization. No increase in the ELISA titer was observed throughout the experiment in mice treated with aerosolized JCPA alone.

The mean nasal rubbing counts of mice immunized with aerosolized JCPA plus the respective particles were higher than those inhaling aerosolized JCPA alone (Figure 3). The nasal rubbing counts of mice immunized with aerosolized JCPA plus Kanto loam dust, fly ash, or DEP were significantly higher than those of animals immunized with aerosolized JCPA alone. There was no marked difference in nasal rubbing between mice immunized with aerosolized JCPA plus carbon black and alum from JCPA alone.

Amounts of Protein Adsorbed to Particles The concentration of protein in crude JCPA adsorbed to Kanto loam dust, fly ash, carbon black, DEP, and alum was 4.2, 4.7, 7.1, 14.8, and 70 μ g/mg, respectively.

Histopathological Findings No apparent remarkable histological alterations were noted in the mucosal membrane of the nasal cavity

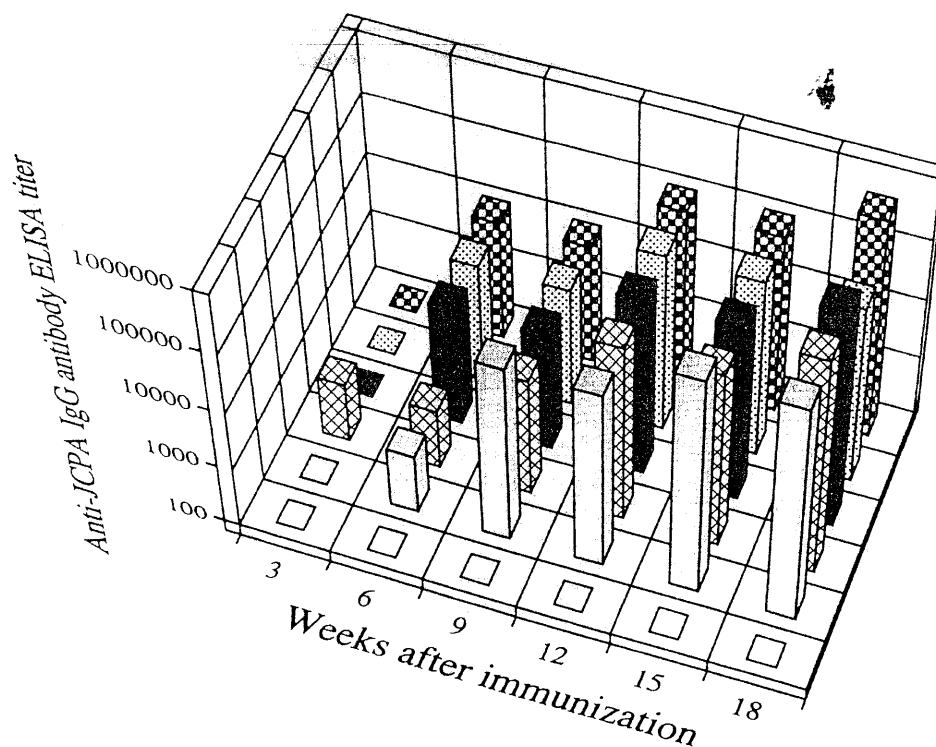


FIGURE 2. Anti-JCPA IgG antibody production in mice in response to intranasal administration of aerosolized JCPA with or without 25 μ g of particles. ELISA titers were measured on pooled sera from six mice in each group. Legend is presented in Figure 1.

of mice treated with aerosolized JCPA alone as compared to control. However, thickening of the epithelial layer and hypertrophy of goblet cells was observed in the respiratory epithelium of mice treated with aerosolized JCPA and the respective particles. The thickness of the epithelium of these mice was nearly twice that of the controls (Figure 4). Slight infiltration of eosinophils and neutrophils was noted in the subepithelial regions. There were no marked alterations in the olfactory and the squamous epithelia of the nasal cavity. No apparent differences in histological findings were noted among the five groups treated with aerosolized JCPA plus the respective particles.

The lymphoid tissue beneath the mucous membrane of the anterior orifice of the nasopharyngeal duct, which is known as nasal-associated lymphoid tissue (NALT), does not develop in control mice. Mice that had inhaled aerosolized JCPA alone or with the respective particles showed high densities of lymphoid cells, well-developed high endothelial venules (HEV), and presence of germinal centers (GC) in NALT. There were no marked differences in the grades of development of lymphoid tissue in the respective groups.

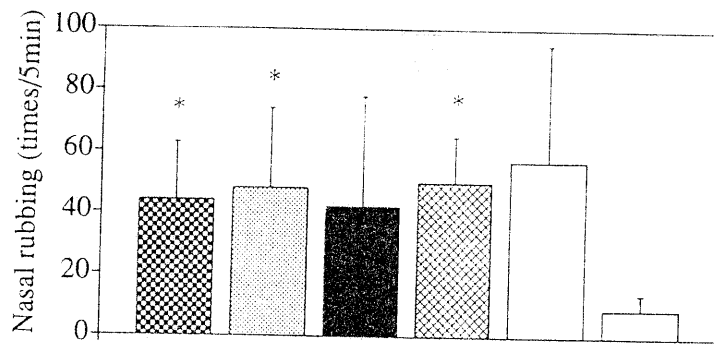


FIGURE 3. Nasal rubbing after JCPA challenge. Columns and bars represent means $\pm$ SD for groups of six mice. Asterisks indicate significant differences from the group treated with aerosolized JCPA alone ($p < .05$). Legend is presented in Figure 1.

A few subepithelial mast cells with granules were found in control mice and mice treated with aerosolized JCPA alone. In contrast, degranulated mast cells were frequently observed in subepithelial regions and the intraepithelial layer of the wall of the nasal cavity of mice treated with aerosolized JCPA plus the respective particles.

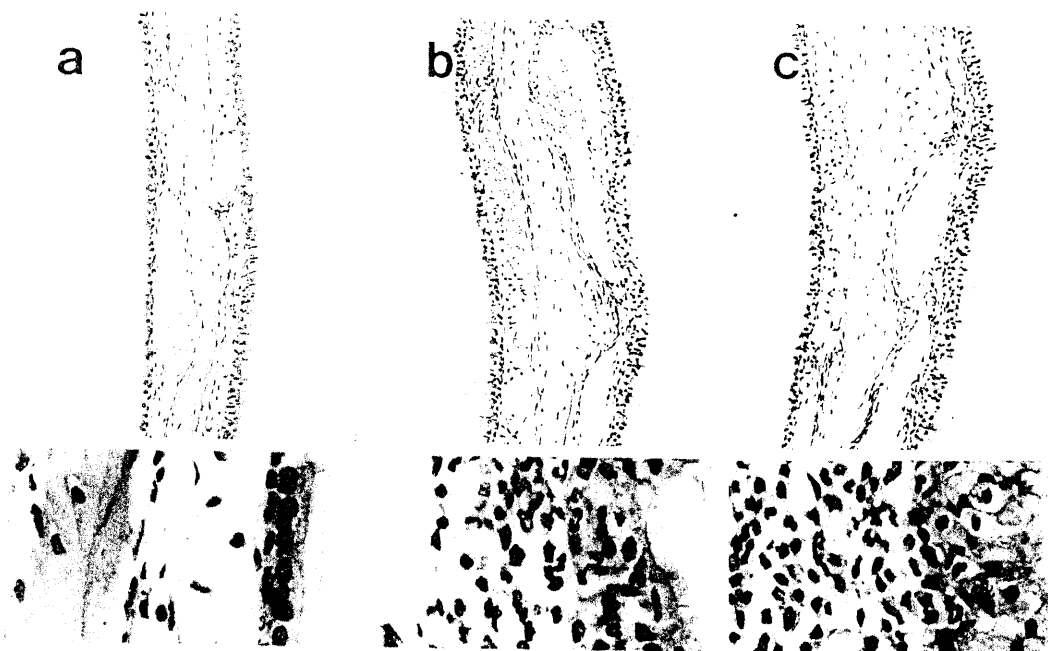


FIGURE 4. Thickening of the respiratory epithelium of the turbinate in mice treated with aerosolized JCPA (original magnification $\times 40$). The high magnified photographs in lower part of each column show subepithelial leukocyte infiltration (original magnification $\times 100$): (a) untreated control, (b) mouse treated with aerosolized JCPA and DEP, (c) mouse treated with aerosolized JCPA and alum.

There were no IgE-positive cells in the wall of the nasal cavity of control or mice treated with aerosolized JCPA alone. On the other hand, immunostaining of IgE was noted in the follicular dendritic cells of the GC of NALT, plasma cells around NALT, and the cell membrane of subepithelial and intraepithelial mast cells in mice treated with aerosolized JCPA plus the respective particles (Figure 5). As shown in Table 2, the distributions and grades of IgE-positive cells were similar in the different groups. Most IgE-bound mast cells showed complete degranulation. Intraepithelial IgE-bound mast cells were restricted to the respiratory epithelium from the anterior to the posterior of the nasal cavity.

Experiment 2

Anti-JCPA IgE and IgG Antibody Productions and Nasal Rubbing Movements The changes of the anti-JCPA IgE antibody titers in each group are shown in Figure 6. The PCA titers in mice immunized with JCP plus Kanto loam dust or alum were 1:5 and 1:10, respectively, in wk 3 after the first immunization, whereas those in the other groups were below 1:5. In wk 6, the titers of all groups were higher than 1:10. The mice immunized with JCP alone and JCP plus Kanto loam dust, fly ash, carbon black, DEP, and alum exhibited peak levels with PCA titers of 1:80, 1:80, 1:80, 1:160, 1:160, and 1:320, respectively, at 18 wk after the first immunization.

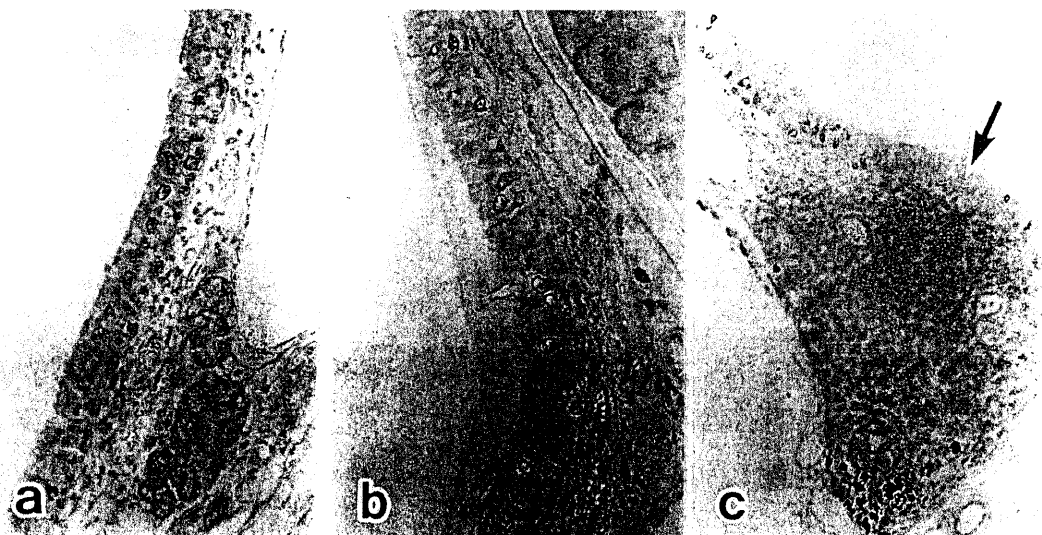
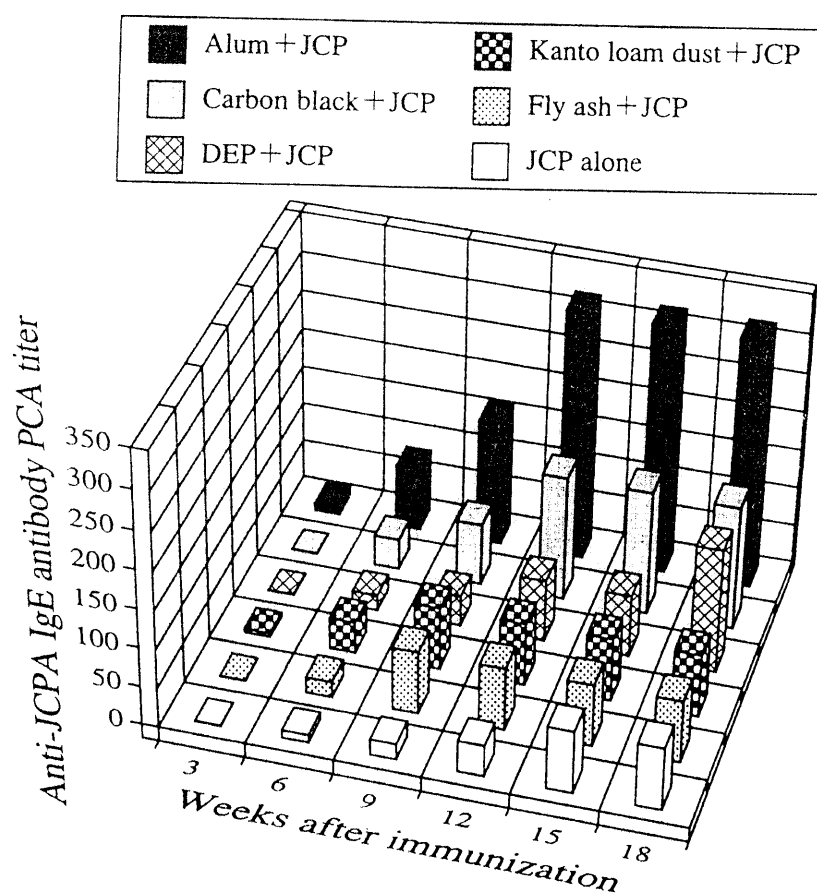


FIGURE 5. Immunostaining of IgG. A and b show IgE-bound mast cells in the epithelial layer. (a) Mouse treated with aerosolized JCPA and DEP. (b) Mouse treated with aerosolized JCPA and alum (original magnification $\times 80$). (c) Reactive product showing IgE in plasma cells accumulated beside the basal portion of NALT and on the surface of lymphocytes beneath the epithelial layer (arrow) (original magnification $\times 40$).

TABLE 2. Distribution and average histological grading of IgE⁺ cells in the nasal cavity of mice treated with aerosolized JCPA

	GC	Peri-NALT plasma cells	Intramucosal IgE ⁺ mast cells		
			Anterior	Middle	Posterior
No treatment	0	0	0	0	0
Aerosolized JCPA alone	0	0	0	0	0
Kanto loam dust + aerosolized JCPA	0.7	1.1	1.4	1.6	1.2
Fly ash + aerosolized JCPA	0.3	0.9	2.0	1.5	2.4
Carbon black + aerosolized JCPA	0.3	0.8	2.2	1.3	1.6
DEP + aerosolized JCPA	0.2	1.5	1.8	3.0	2.2
Alum + aerosolized JCPA	0.5	1.0	2.8	2.2	2.3

Note. Histological grading: very slight, 0.5; slight, 1.0; moderate, 2.0; marked, 3.0

**FIGURE 6.** Anti-JCPA IgE antibody production in mice in response to intranasal administration of JCP with or without 25 μ g of particles. PCA titers were measured on pooled sera from six mice in each group.

The changes of the anti-JCPA IgG antibody titers in each group are shown in Figure 7. The ELISA titer in mice immunized with JCP plus carbon black was 1:1000 at 3 wk after the first immunization, whereas those in other groups were below 1:100. Six weeks after the first immunization, the ELISA titers of groups immunized with JCP plus the respective particles were greater than 1:1000, while that of the group treated with JCP only was lower. In all groups, the ELISA titers were 1:10,000 at 18 wk after the first immunization. No significant differences in nasal rubbing movements were observed in the immunized groups with and without particle treatments.

Histopathological Findings Thickening of the respiratory epithelium, hypertrophy of goblet cells, dilatation of subepithelial vessels, hypertrophy of NALT, and development of HEV were noted in all groups treated with JCP or JCP plus the respective particles (Figure 8). Furthermore, mucus secretion in the nasal cavity and subepithelial infiltration of eosinophils and neutrophils were observed in the mice treated with JCP plus the respective particles. In these groups, degran-

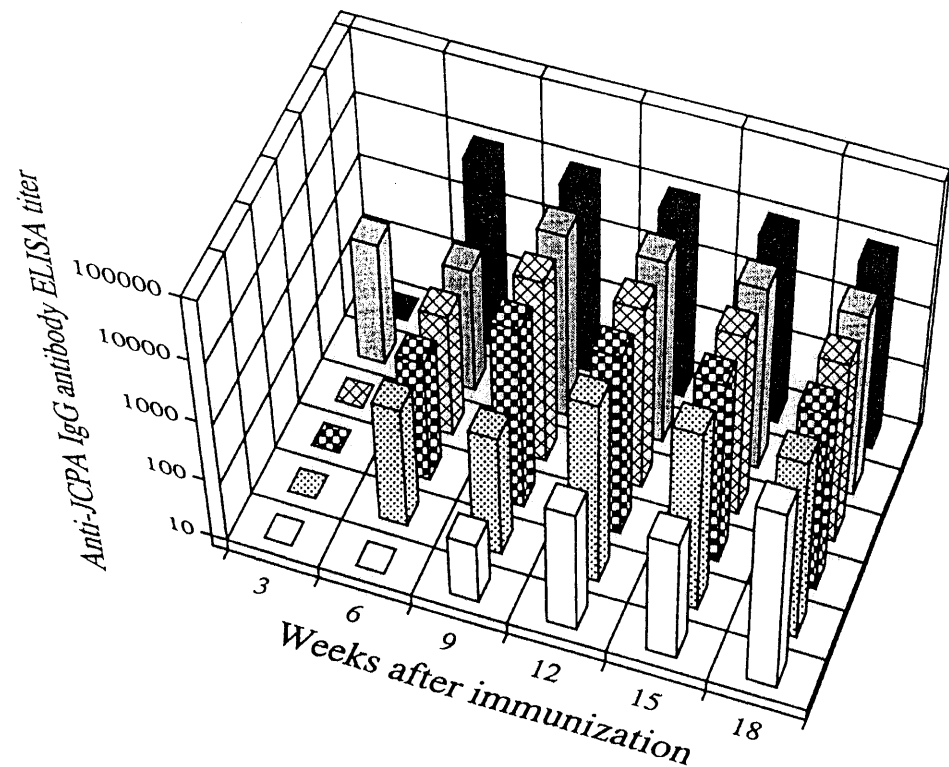


FIGURE 7. Anti-JCPA IgG antibody production in mice in response to intranasal administration of JCP with or without 25 μ g of particles. ELISA titers were measured on pooled sera from six mice in each group. Legend is presented in Figure 6.

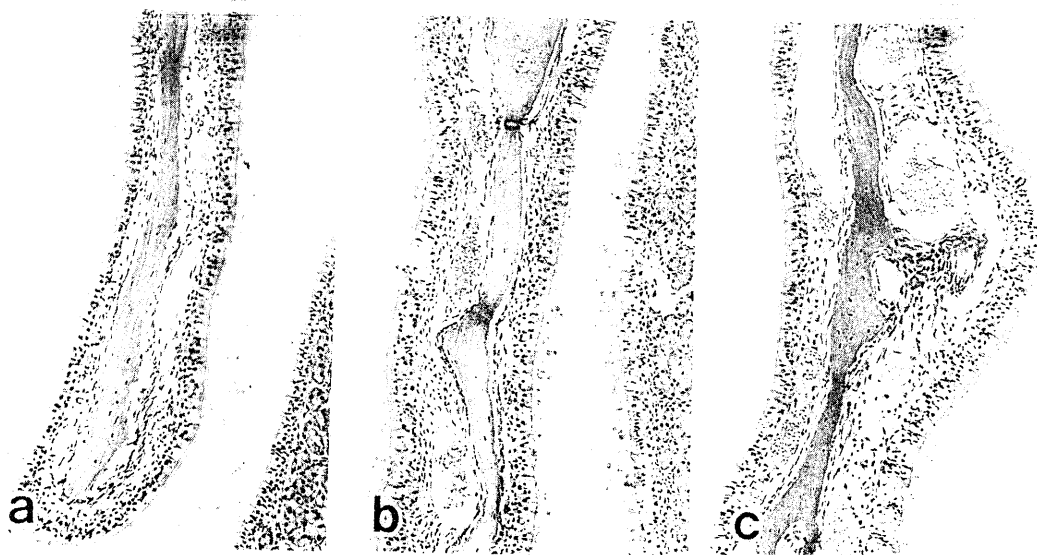


FIGURE 8. Thickening of the respiratory epithelium, infiltration of eosinophils and neutrophils and dilatation of vessels in the turbinate of mice treated with (a) JCP alone, (b) JCP and carbon black, and (c) JCP and fly ash. (original magnification $\times 40$).

ulation of mast cells was not complete, with cells frequently containing a few granules.

IgE-positive plasma cells were located in the basal portion of NALT in mice treated with JCP alone. In mice treated with JCP plus the respective particles, however, IgE-positive plasma cells were widely distributed beneath the respiratory epithelium (Figure 9, a and b). The lymphocytes in NALT displayed surface IgE, and IgE were also detected in the GC (Figure 9c). IgE-bound mast cells were found widely not only in the epithelial layer and subepithelial region of the respiratory epithelium but also in the olfactory epithelium (Figure 10). The distribution and grading of IgE positive cells are presented in Table 3.

DISCUSSION

Since World War II, the Japanese government has been promoting a program for planting Japanese cedars to improve forest resources. As a result, the area of artificial forests containing Japanese cedar in the 1980s was about twice that in 1950s. Flowering and seed bearing of these trees became active about 30 yr after initial planting (Yokoyama & Kanazashi, 1990). The primary cause of the rapid increase of subjects with pollinosis may be due to a rise in the amount of pollen as allergens, as high exposure to pollen is known to be a risk factor for developing hay fever (Charpin et al., 1993).

There are several reports on the effect of properties of particles on

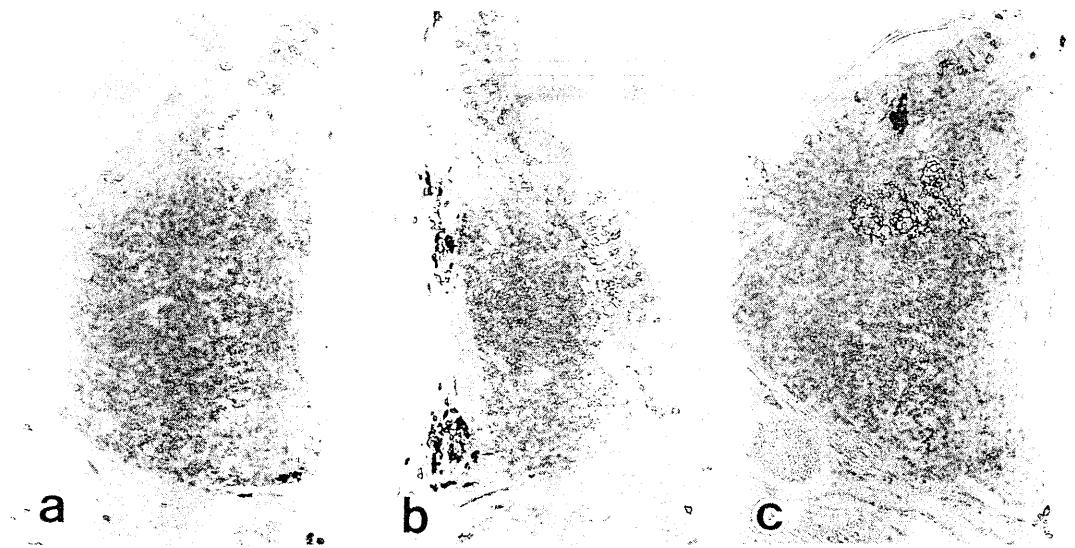


FIGURE 9. IgE staining of NALT IgE-positive plasma cells accumulated beside the basal portion of NALT in mice treated with (a) JCP alone or (b) JCP and carbon black. The surface IgE of lymphocytes (a-c) and IgE-positive germinal center (c) are noted (original magnification $\times 40$).

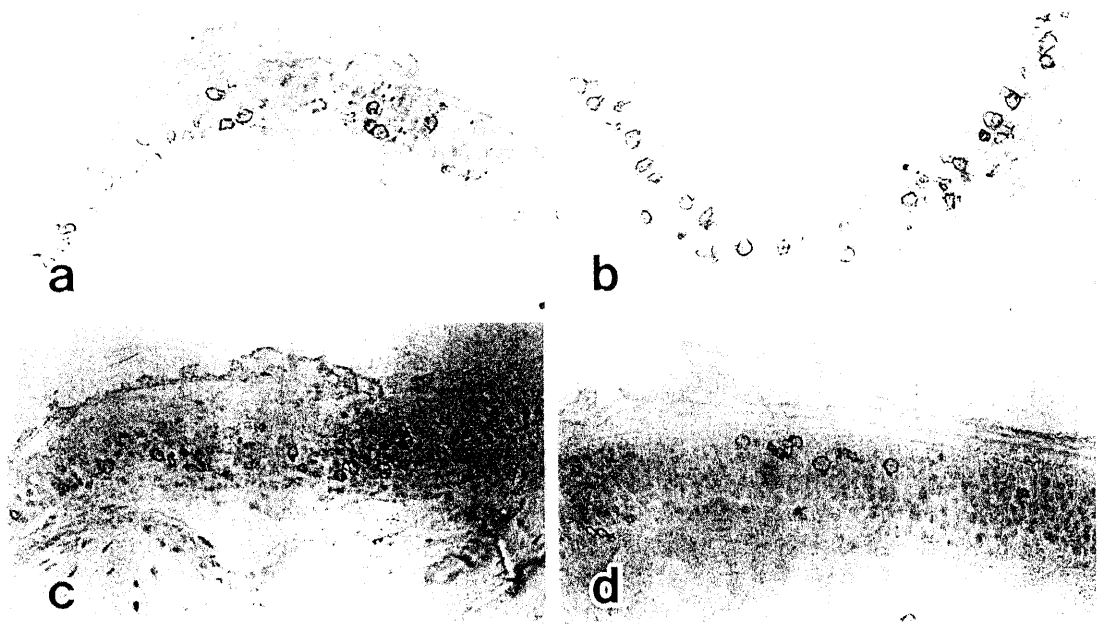


FIGURE 10. IgE-bound mast cells observed in the respiratory epithelium of mice treated with (a) JCP alone, (b) JCP plus DEP, or (c) JCP plus Kanto loam. IgE-bound mast cells are also noted in the olfactory epithelium in a mouse treated with (d) JCP and Kanto loam. (original magnification $\times 80$).

TABLE 3. Distribution and average histological grading of IgE⁺ cells in the nasal cavity of mice treated with JCP

	GC	Peri-NALT plasma cells	Intramucosal IgE ⁺ mast cells		
			Anterior	Middle	Posterior
No treatment	0	0	0	0	0
JCP alone	0	0.9	0.8	0.9	1.0
Kanto loam dust + JCP	0	1.5	1.5	2.8	2.5
Fly ash + JCP	0	2.0	1.8	2.5	2.8
Carbon black + JCP	0.3	1.5	2.3	2.3	2.5
DEP + JCP	0.5	1.8	1.6	2.5	2.0
Alum + JCP	0	1.8	1.5	3.0	1.8

Note. Histological grading: very slight, 0.5; slight, 1.0; moderate, 2.0; marked, 3.0.

the IgE response in mice. Takafuji et al. (1989) collected suspended particulate matter (SPM) from urban atmospheres using a high-volume air sampler. The sampler roughly separated the airborne particles into fractions of two sizes, SPM-1 (>7.0 μm) and SPM-4 (1.1–2.0 μm). The SPMs consisted mainly of carbon, quartz, calcite, rock salt, calcium sulfate, chlorite, cristobalite, and feldspar. They found that the IgE antibody responses of mice immunized with 0.25 μg of OA plus 25 μg of particles by the intranasal route were higher than those of animals immunized with OA alone. No significant difference in the adjuvant activities of the SPM-1 and SPM-4 fractions was reported, although the capacity of SPM-4 to adsorb protein (OA) was approximately seven times greater than SPM-1. From these results, it was suggested that particle size and the capacity to adsorb protein do not affect anti-OA IgE antibody production in mice. Fujimaki et al. (1984) treated mice intratracheally with 0.2 mg fly ash or 0.2 mg aluminum silicate mixed with 10 μg OA. No significant difference between the IgE titers in these groups was observed, although a significant difference between the abilities of fly ash and aluminum silicate to adsorb the antigens was reported. In the present study, the anti-JCPA IgE and IgG antibody production in animals immunized with aerosolized JCPA was enhanced by the respective particles, but no remarkable differences in the magnitudes of the anti-JCPA antibody responses, nasal rubbing counts, or histopathological changes in the nasal cavity of mice treated with different types of particles were found. These data suggest that the components of particles, their capacities to adsorb antigens, and their sizes may not be related to the observed rise in IgE production and increased incidence in allergic rhinitis symptoms.

Tamura et al. (1986) reported that daily inhalation of JCP induced an IgE antibody responses in female BDF₁ mice. In their experiment, airway sensitization to JCP was performed by applying JCP daily to

the tip of the nose for three 5-d periods with 2-d intervals between treatments. Treatments were continued for about 1 min/d until the mice had difficulty in breathing due to ingestion of pollen into the nasal cavity. The anti-JCPA IgE PCA titers in mice were 1:40 in wk 4 after the final immunization. In the present study, similar results were observed in mice immunized with JCP alone. However, the quantity of pollen grains taken into the nasal cavity of mice is uncertain. It is of interest that no remarkable differences were observed in the final PCA titers, nasal rubbing counts, or histopathological changes in the six groups. It should be noted that the particles accelerated IgE antibody production in mice immunized with JCP.

Kanto loam dust is soil dust obtained from the Kanto area including Tokyo, while fly ash is an environmental pollutant produced by burning powdered coal. The main components of both of these particles are Si and Al, and they were thus used as typical soil dusts in this study. Mancino et al. (1983) reported that the adjuvant effect of silica was related to the damage induced in macrophages. Our results may be in agreement with this possibility, although the effects of Kanto loam dust and fly ash on macrophages were not investigated. A predominant component of DEP is elemental carbon (see Table 1). Carbon black is almost equal in composition to elemental carbon. SPM consists mainly of DEP, soil dust, and secondary aerosols in urban districts of Japan. Secondary aerosols are formed by chemical reactions in the atmosphere. The relative contributions of these particles to SPM are 30–40%, 15–25%, and 10–15%, respectively (Koyama, 1990; Yoshizumi et al., 1989). Therefore, in view of the composition of SPM in terms of DEP, it would appear that DEP may be a significant component involved in enhanced IgE production.

The method of administration of allergens in experiment 1 may not seem realistic, as the allergens were applied by spray to the animals. In experiment 2, allergens were applied directly onto the nose, which more closely resembles inadvertent exposure in humans. However, the concentration of particles could not be determined. Although both methods of administration of allergens in experiments 1 and 2 have merits and demerits, the results were similar. Almost all previous animal studies involved the relationship between the particles administered by intraperitoneal, intratracheal, and intranasal routes and IgE antibody production. Inhalation studies are necessary to assess the effects of air pollutants on the onset of pollinosis. Studies on the effect on IgE antibody production in mice from automobile exhaust with native dry JCP administered by inhalation are now being carried out.

REFERENCES

- Bradford, M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.

- 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 south-east France. *Clin. Exp. Allergy* 23:435-439.
- 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.
- Imaoka, K., Sakaguchi, M., and Inouye, S. 1993. Antibody responses against Japanese cedar pollen allergen (Cry j I) in different strains of rats. *Exp. Anim.* 42:61-65 (in Japanese with English summary).
- Inouye, S., Sakaguchi, M., Mori, H., Miyamura, K., Ujiya, S., Shigehara, S., and Noguchi, Y. 1986a. Seroepidemiology of Sugi (Japanese cedar) pollinosis: Increase of IgE antibody positive rate in recent years. *Igaku no Ayumi* 138:285-286 (in Japanese).
- Inouye, S., Sakaguchi, M., Hori, T., and Watanabe, T. 1986b. A seroepidemiological study of *Cryptomeria japonica* (Japanese cedar) pollinosis: Measurement of IgE antibody. *Igaku no Ayumi* 136:959-960 (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.
- Kaneko, S., Shimada, K., Horiuchi, H., Endo, A., Kodama, M., Shinoda, N., Imai, T., and Takiguchi, K. 1980. Nasal allergy and air pollution. *Oto-Rhino-Laryngol. Tokyo* 23:270-281 (in Japanese).
- Koyama, T. 1990. Regional differences of atmospheric particulate in Kanagawa prefecture (2)—Relationship between chemical composition of suspended particulates and emission sources. *Bull. Kanagawa Prefectural Environmental Center* 12:1-14 (in Japanese with English summary).
- Levine, B. B., and Vaz, N. M. 1970. Effect of combination of inbred strain, antigen dose on immune responsiveness and reagin production in the mouse. *Int. Arch. Allergy Appl. Immunol.* 39:156-171.
- Maejima, K., Tamura, K., and Nagase, S. 1995. Automobile exhaust and allergy—Examinations of the measurement of IgE antibodies in mice and inhalation method of antigen. *J. Jpn. Auto. Res. Inst.* 17:288-290 (in Japanese with English summary).
- 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.
- Mori, A. 1995. Sensitization and onset of Japanese cedar pollinosis in children. *Allergy* 44:7-15 (in Japanese with English summary).
- Muranaka, M., Suzuki, S., Koizumi, K., Takafuji, S., Miyamoto, T., Ikemori, R., and Tokiwa, H. 1986. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J. Allergy Clin. Immunol.* 77:616-623.
- Nagano, K., Enomoto, M., Yamanouchi, K., Aiso, S., and Katagiri, T. 1988. Toxicologic pathology of upper respiratory tract. *J. Toxicol. Pathol.* 1:115-127 (in Japanese with English summary).
- Nakajima, T., and Suzuki, T. 1995. Automobile emission and chronic obstructive disease in small animals—Experimental design and concentration of key components. *J. Jpn. Auto. Res. Inst.* 17:20-23 (in Japanese with English summary).
- Nakamura, S. 1990. Investigation into the frequency of cases with Japanese cedar pollinosis among the students and staff members in our university. *Jpn. J. Allergol.* 39:476-482 (in Japanese with English summary).
- Saito, H., Asakura, K., and Kataura, A. 1994. The in vivo effects of anti-CAM antibody on OA sensitized rats. *Jpn. J. Allergol.* 43:579-584 (in Japanese with English summary).
- Sakaguchi, M., Inouye, S., Taniai, M., Ando, S., Usui, M., and Matuhashi, T. 1990. Identification of the second major allergen of Japanese cedar pollen. *Allergy* 45:309-312.
- Sawatani, M., Yasueda, H., Akiyama, K., Shida, T., Taniguchi, Y., Usui, M., Ando, S., Kurimoto, M., and Matuhashi, T. 1993. Immunological and physicochemical properties of

- Cry j II, the second allergen of Japanese cedar pollen (*Cryptomeria japonica*). *Allergy* 42:738-747.
- Suzuki, T., and Kanoh, T. 1993. The adjuvant activity of pyrene in diesel exhaust on IgE antibody production in mice. *Jpn. J. Allergol.* 42:963-968.
- Takafuji, S., Suzuki, S., Koizumi, K., Ohashi, H., Muranaka, M., and Miyamoto, T. 1989. Enhancing effect of suspended particulate matter on IgE antibody production in mice. *Int. Arch. Allergy Appl. Immunol.* 90:1-7.
- Tamura, S., Kobayashi, T., Kikuta, K., Nakagawa, M., Sakaguchi, M., and Inouye, S. 1986. IgE antibody responses against Japanese cedar pollen in the mouse. *Microbiol. Immunol.* 30:883-891.
- Taniguchi, Y., Ikegawa, H., Usui, M., Ando, S., Kurimoto, M., Sakaguchi, M., Inouye, S., and Matuhashi, T. 1989. Biological and immunological properties of Sugi basic protein-pullulan conjugate: I. Suppressive effect on IgE antibody production and on IgE mediated reactions. *Int. Arch. Allergy Appl. Immunol.* 89:136-142.
- Watanabe, N., and Ovary, Z. 1977. Antigen and antibody detection by in vivo methods: A reevaluation of passive cutaneous anaphylactic reactions. *J. Immunol. Methods* 14:381-390.
- Yasueda, H., 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.
- Yokoyama, T., and Kanazashi, T. 1990. A change in the area of Sugi forests as a source of pollen source. In *IgE production and environmental factors*, eds. M. Muranaka and K. Taniguchi, pp. 67-79. Tokyo: Medical Tribune, Inc. (in Japanese with English summary).
- Yoshizumi, K., Watanabe, T., Ishii, K., Yoshioka, H., Asakura, K., Tamura, T., Kan, M., and Yoshino, N. 1989. The source apportionment evaluation of ambient particulate matter in the Tokyo metropolitan area by a receptor oriented model. *Ann. Rep. Tokyo Metropolitan Res. Inst. Environ. Protect.* 3-10 (in Japanese).