

A POLLEN MODEL IN THE RAT FOR TESTING ADJUVANT ACTIVITY OF AIR POLLUTION COMPONENTS

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*During the last decades, the prevalence of allergy has increased worldwide. Allergic rhinitis ("hay fever") and asthma are two of the most common allergic diseases. A possible cause for increased allergy to pollen is air pollution. The increase of industrialization and the number of diesel engines associated with diesel exhaust particles (DEP) in the air parallel the increase in allergic airway diseases. To investigate the adjuvant effect of DEP in pollen allergy, Brown Norway (BN) rats were sensitized intranasally or intratracheally with timothy grass pollen (*Phleum pratense*) with or without DEP (3 mg/ml). Intranasal sensitization (200 μ l, 10 mg/ml) was performed daily for 5 consecutive days and intratracheal sensitization (200 μ l, 10 mg/ml) was performed once. Challenge with pollen was performed at day 21 similarly to the sensitization protocol. Blood samples were taken at day 28 after the first sensitization. The binding of DEP to pollen grains was studied by scanning electron microscopy and the inflammatory response in the lung was studied by light microscopy. Immunoglobulin E (IgE) and IgG₁ responses against pollen grains were measured by digoxigenin (DIG) enzyme-linked immunosorbent assay (ELISA). Scanning electron microscopy revealed a mixture of free DEP and DEP associated with pollen grains. Both intranasal and intratracheal routes of administration of pollen grains induced inflammatory reactions in the lung with an influx of macrophages, eosinophilic granulocytes, and granuloma formation. Pollen grains were localized in the alveoli after both intranasal and intratracheal administration and were surrounded by macrophages. The number and localization of pollen grains were similar for both routes of administration. After coexposure with DEP, DEP-loaded macrophages were found around the pollen. Localization, inflammatory reaction, and integrity of pollen were similar to those seen without DEP. At day 28, specific IgE and IgG₁ antibodies were found in serum of rats immunized intranasally or intratracheally. IgE antibody response was higher in rats immunized with pollen grains and DEP than in rats immunized with pollen only (dilution mean $\pm$ SEM: 59.4 ± 4.6 vs. 27 ± 5.1). The IgG₁ antibody response was much higher compared to the IgE response (factor of 10^4), but the level of IgG₁ antibodies was only slightly increased by DEP (dilution mean $\pm$ SEM: $24.2 \pm 2.0 \times 10^4$ vs. $16.1 \pm 2.1 \times 10^4$). In conclusion, the intranasal application of pollen in the BN rat is a suitable and elegant method to evoke inflammatory reactions in the lung and pollen-specific IgE responses measured by DIG ELISA. Finally, this model gives similar results on adjuvant activity of DEP found in the ovalbumin models presented previously.*

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During the last 30 yr, the prevalence of allergies, asthma, and eczema has increased from 4% to 20% in the United States, United Kingdom, Japan, and Finland (Haahtela et al., 1990; Rusznak et al., 1997; Ninan & Russell, 1992; Omran & Russell, 1996; Burr et al., 1989). Epidemiological studies suggest that the increase in the prevalence of allergic disease such as asthma may be associated with air pollution (Rusznak et al., 1997). The increase of industrialization and the number of diesel engines associated with diesel exhaust particles (DEP) in the air parallel the increase in allergic airway diseases (Peterson & Saxon, 1996). Moreover, DEP enhance the development of clinical symptoms among already sensitized subjects (Corbo et al., 1993). It has been shown that the incidence of allergic rhinitis caused by pollen is higher in areas with air pollution than in nonpolluted areas, while the pollen counts in both areas were almost the same (Ishizaki et al., 1987).

DEP are a major component of ambient air pollution and particulate respirable particles. A large part of DEP are $<1\ \mu\text{m}$ in diameter, and certain fractions can be carried deep into the lungs when inhaled. Several studies in human and animal models have suggested that DEP may act as an adjuvant for pollen allergy, either by influencing the cytokine production (Diaz Sanchez et al., 1996, 1997; Diaz Sanchez, 1997), by enhancing the immunoglobulin E (IgE) response (Diaz Sanchez, 1997) or the pollen-specific IgE (Maejima et al., 1997; Muranaka et al., 1986; Diaz Sanchez et al., 1996, 1997), by modulating the immunoglobulin isotype switching (Diaz Sanchez et al., 1994; Takenaka et al., 1995), or by acting as vectors for submicron fragments of pollen grains that would otherwise be too small to deposit in human airways (Knox et al., 1997; Behrendt et al., 1991, 1992, 1997).

Experiments have shown that specific IgE responses in mice immunized with ovalbumin (OA) mixed with DEP were stronger than when mice were immunized with OA or DEP only (Nilsen et al., 1997; Fujimaki et al., 1994, 1995, 1997; Takafuji et al., 1987, 1989; Muranaka et al., 1986). A few studies carried out in the mouse with Japanese cedar pollen and DEP resulted in a comparable increase of IgE using passive cutaneous anaphylaxis (PCA) (Fujimaki et al., 1994; Maejima et al., 1997; Muranaka et al., 1986). In most studies OA has been used to mimic an allergic/asthmatic reaction of humans in animal models. The purpose of the study was to present an animal model in which a more relevant allergen was used to study the interaction between allergens and air pollutants. We have examined two relevant routes of administration of pollen grains and studied the inflammatory responses and antibody formation by means of a sensitive digoxigenin (DIG) enzyme-linked immunosorbent assay (ELISA). To validate this model we have tested the adjuvant activity of DEP, which has been many times presented with its adjuvant activity in ovalbumin models in the mouse.

MATERIALS AND METHODS

Animals

Six- to 8-wk-old male Brown Norway (BN) rats were obtained from Harlan-Olac (Zeist, The Netherlands). The animals were housed in Macrolon type II and III cages. Food pellets and tap water were available *ad libitum*, and all animals were allowed to adapt for at least 1 wk before the experiments started.

Pollen Grains

Pollen grains from timothy grass (*Phleum pratense*) and lyophilized extracts from this pollen were obtained from ALK Benelux, Houten, The Netherlands.

Diesel Exhaust Particles

Diesel exhaust particles (DEP) were kindly provided by Dr. P. Scheepers (Department of Toxicology, Faculty of Medical Sciences, Catholic University Nijmegen, Nijmegen, The Netherlands). Large amounts of DEP were collected from a 4-kW light-duty diesel engine (Yanmar L90E, single cylinder) fueled with commercial-quality Shell diesel fuel purchased at a local gas station in Delft, The Netherlands. The DEP were collected on glass-fiber membrane filters at the Faculty of Chemical Technology and Materials Sciences of the Delft University of Technology. DEP were collected at steady-state conditions at a filter temperature of $70 \pm 10^\circ\text{C}$ (50% load, 3000 rpm). The loaded membrane filters were stored at -20°C in the dark until further treatment. The content of 1-nitropyrene amounted to approximately $1.5 \mu\text{g/g}$ DEP as determined in a gas chromatography–mass spectroscopy (GC-MS) analysis according to the method described in Scheepers et al. (1995). In the scanning electron microscopical study (described later) the particles appear to be 25–35 nm, but were frequently aggregated to particles of variable size. In a *Limulus* amebocyte lysate (LAL) test (Limusate, Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands), endotoxin is measured as described by the manufacturer, and DEP suspensions were below the detection level ($<0.025 \text{ ng/ml}$). To prepare a DEP/pollen suspension, DEP were weighed, suspended in phosphate-buffered saline (PBS, 3 mg/ml), and ultrasonicated with a Branson sonifier 250. Next, pollen grains were added to this suspension (final concentration 10 mg/ml PBS) and the suspension was homogenized by vigorous shaking.

Sensitization and Challenge of the Animals

For intranasal sensitization, rats (3 rats/group) were slightly anesthetized with halothane (2%). At the moment of awakening, 0.1 ml pollen (10 mg pollen/ml PBS) or pollen/DEP suspension (10 mg pollen and 3 mg

DEP/ml PBS) was applied into each nostril. For intranasal sensitization and challenge, this route of application was repeated for 5 consecutive days (days 0–4 and days 21–25, respectively).

For intratracheal instillation, rats (4 rats/group) were anesthetized by an intraperitoneal injection with a mixture of ketamine (20 mg/kg body weight, Kombivet, Etten-Leur, the Netherlands), Rompun (2.5 mg/kg body weight, Bayer Leverkusen, Germany), and atropine (0.05 mg/kg body weight, Eurovet, Bladel, The Netherlands) (KRA). Thereafter, for sensitization (day 0) and for challenge (day 21), 0.2 ml pollen (10 mg pollen/ml PBS) or pollen (10 mg/ml)/DEP (3 mg/ml) suspension was intratracheally administered with an intratracheal cannula.

Serum Samples

Blood samples were obtained prior to sensitization and at day 28 (3 and 7 days after the last intranasal and intratracheal challenge, respectively). After collection, the blood was kept at 4°C for about 2–4 h for clotting. The blood samples were centrifuged for 15 min at 3000 rpm at 4°C. The serum was removed and stored in small aliquots at –20°C until use.

ELISA for Specific Anti-Timothy Pollen IgE and IgG

The enzyme-linked immunosorbent assay (ELISA) was performed in flat-bottomed 96-well polystyrene microtiter plates with high binding capacity (MaxiSorp number 439454, Nunc, Denmark, or number 655092, Greiner, Germany).

All incubations were performed with 100- μ l aliquots. After adding a solution, the plates were covered and shaken. Each incubation was followed by extensive washing—6 cycles on an automatic plate washer with PBS containing 0.1% Tween-20. The incubations at 37°C were performed in a thermostatically controlled water bath (the plates floated on the water).

A pollen extract–digoxigenin (DIG) solution was prepared by labeling the pollen extract with DIG using a reagent kit (see Labeling Proteins with DIG). Pollen extract has a molecular mass of approximately 70 kD, and a molecular reaction mix of 1:10 was used. The protein concentration was measured according to Lowry, and a Western blot was performed to verify the labeling between extract and DIG.

Microwells were coated overnight at 4°C with monoclonal mouse anti-rat IgE (03-9700, Zymed, San Francisco, CA) with goat anti-rat IgG₁ (Jackson Immuno Laboratories, West Grove, PA) in Na₂CO₃ buffer (0.1 M; pH 9.6). Blocking reagent [PBS containing 0.1% (v/v) Tween-20 and 1% (w/v) bovine serum albumin (BSA)] was added and incubated for 1 h at 37°C. Rat serum samples (diluted) were incubated for 1 h at 37°C in PBS. The binding of IgE was measured by incubating a pollen extract–DIG solution (0.01 μ g/ml in blocking reagent) for 1 h at 37°C, followed by an incubation of anti-DIG Fab fragments poly-labeled with peroxidase (PO)

(number 1633716; Boehringer Mannheim, Germany; 50 U/ml; 1:1250) and an incubation for 20 min at 20°C in the dark with tetramethylbenzidine/dimethyl sulfoxide (TMB/DMSO) substrate. The enzyme reaction was stopped by adding 100 μ l 2 M H_2SO_4 , and the optical density was read at 450 nm on a multichannel photometer. Specificity of the reaction was controlled by deleting essential steps or replacing the DIG/pollen extract by a DIG/house dust mite extract. In all cases, extinction values declined to the range of the preimmunization values.

Labeling Proteins with DIG

Pollen extract was labeled with digoxigenin 3-*O*-succinyl- ϵ -amino-caproic acid *N*-hydroxysuccinimide ester (DIG-NHS) using a DIG antibody labeling kit (number 1367200, Boehringer Mannheim, Germany). Non-reacted DIG-NHS was separated on a Sephadex *G-25 column. The labeled protein was collected in fractions of 1 ml. After determining the protein concentration according to Lowry, the fractions with the highest protein concentration were pooled and the protein concentration was determined. The solution had a protein concentration of 70 mg extract/ml PBS.

Scanning Electron Microscopy

To study the binding of DEP to pollen, 3 mg DEP/ml PBS was suspended and ultrasonificated. To this ultrasonified suspension, 10 mg dry pollen was added. To remove the excess and unbound DEP, this suspension was washed three times in PBS followed by centrifugation. Dehydration was performed in a graded ethanol series, followed by two 5-min treatments in 100% hexamethyldisilazane (HMDS, number 804324, Merck). After the final HMDS treatment the sample was air-dried on a glass slide. After being air-dried, the slides were coated with a thin layer of gold using a sputter coating unit (Polaron, Watford, UK) and examined in a scanning electron microscope (PSEM 525, Philips, Eindhoven, The Netherlands).

Histopathology

Lungs were in situ fixed in 4% phosphate-buffered formaldehyde and embedded in paraplast. Five-micrometer sections were prepared and routinely stained with hematoxylin and eosin (H&E) and with May Grünwald Giemsa. The histopathological lesions were semiquantitatively and blinded scored from minimal ($\pm$) to marked (+++). In this score the incidence and the severity of the lesions were incorporated; for example, for granuloma $\pm$ indicates a few, incidental small granulomas; +++ indicates >15 medium/large granuloma.

Statistical Analysis

The serum dilutions determining antibodies to pollen allergens were compared after sensitization and challenge with pollen grains alone and after pollen grains in combination with DEP. The serum dilution was

determined by an arbitrarily chosen 60% of the extinction value obtained after sensitization and challenge of pollen alone (60% of the extinction value for intranasal immunization is 0.5 and for the intratracheal immunization 0.9). The statistical differences between groups were determined using Student's two-sample *t*-tests. The data were calculated as means $\pm$ SEM (standard error of the mean). The result was considered significant when a *p* value of $<.05$ was found.

RESULTS

Scanning Electron Microscopy (SEM)

Results of the SEM investigations show that timothy pollen grains have a diameter of about 30 μm (see Figure 1a). In a suspension of pollen grains and ultrasonified DEP, only a very small part of the DEP appeared to be associated with pollen (Figure 1, b and c).

Histopathology

The histological changes in the lung are summarized in Table 1. Control animals showed a few microgranuloma, a minimal focal perivascular and peribronchial mononuclear cell infiltrate, and minimal alveolitis.

In all exposed animals, pollen grains were present, mostly in moderate quantities, and were diffusely spread in all lobes. The localization of pollen grains was alveolar, irrespective of the administration of DEP (Figure 2, a and b). However, the number of pollen grains increased when the challenge of pollen grains was combined with DEP. Nearly all pollen were surrounded by alveolar macrophages. In all exposed groups, many relatively large granulomas were present, consisting of pollen grains, giant cells, fibroblasts, macrophages, and eosinophilic granulocytes (Figure 2, a and b). After intranasal application of pollen grains, DEP seem to increase, and after intratracheal application, DEP seem to reduce this granuloma formation. Diffusely there was a moderate to marked peribronchiolar and perivascular inflammation of eosinophils and mononuclear cells. There was a slight tendency to a stronger perivascular infiltrate in the groups treated intranasally in comparison with the groups treated intratracheally, despite the presence of fewer pollen grains. Alveolitis, hypertrophic mucous cells, and fibrosis were found, and the bronchus associated lymphoid tissue (BALT) showed several large, discrete germinal centers.

The animals who received DEP (intratracheally or intranasally) showed heavily loaded macrophages. Pollen grains were enclosed by these macrophages (Figure 2b). Moreover, DEP were noticed in the alveoli, in alveolar septa, and in granulomas. The animals challenged with pollen grains and DEP had larger quantities of DEP in the lungs than animals only sensitized with pollen grains and DEP. No increased perivascular infiltrate was observed in combination with DEP. Differences between the intranasal route and intratracheal route were not statistically significant.

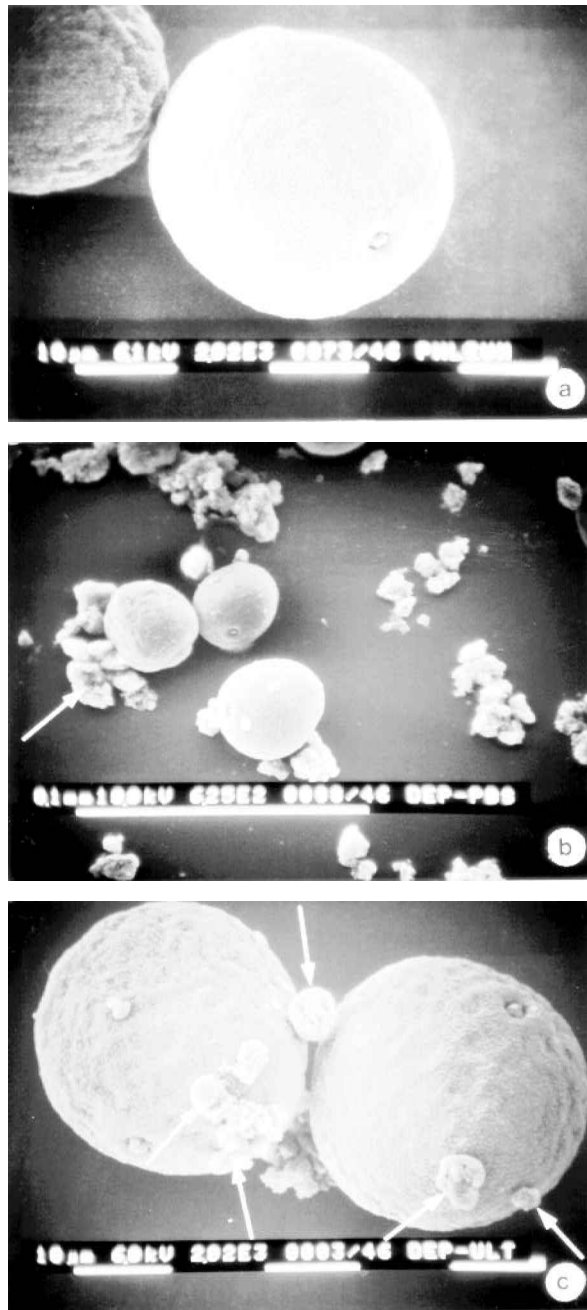


FIGURE 1. (a) Scanning electron micrograph of a timothy grass (*Phleum pratense*) pollen grain. (b) In a DEP/pollen suspension, DEP adhere to the pollen (arrow). Free DEP are also seen. (c) At higher magnification, DEP particles are visible on the surface of two pollen grains. Magnification $\times 1500$ (a and c), $\times 440$ (b).

TABLE 1. Histological Changes in Lungs of Rats Exposed to Pollen Grains and DEP

Treatment	Nontreated	Immunization route							
		Intranasal				Intratracheal			
Immunization									
Sensitization pollen	—	Pollen	Pollen	Pollen + DEP	Pollen + DEP	Pollen	Pollen	Pollen + DEP	Pollen + DEP
Challenge pollen	—	Pollen	Pollen	Pollen + DEP	Pollen + DEP	Pollen	Pollen	Pollen	Pollen + DEP
Number of animals examined	3	3	3	3	3	4	4	3	3
Quantity of pollen in sections	—	+	++	++	++	++	+++	++	+++
Granuloma	±	++	+++	+++	+++	+++	++	++	++
Perivascular (peribronch.) infiltrate	±	+++	+++	+++	+++	++	++	++	++
Alveolitis	±	+	+	+	+	+	+	+	+
Fibrotic foci	—	++	++	++	++	++	++	++	++
Hypertrophy bronchiolar epithelium	—	+++	+++	+++	+++	++	++	++	++
BALT with discrete germinal centers	—	2 ^a	3 ^a	3 ^a	3 ^a	4 ^a	3 ^a	2 ^a	3 ^a

Note. Ratings: — = none, ± = minimal, + = slight, ++ = moderate, +++ = marked.

^aNumber of rats stimulated.

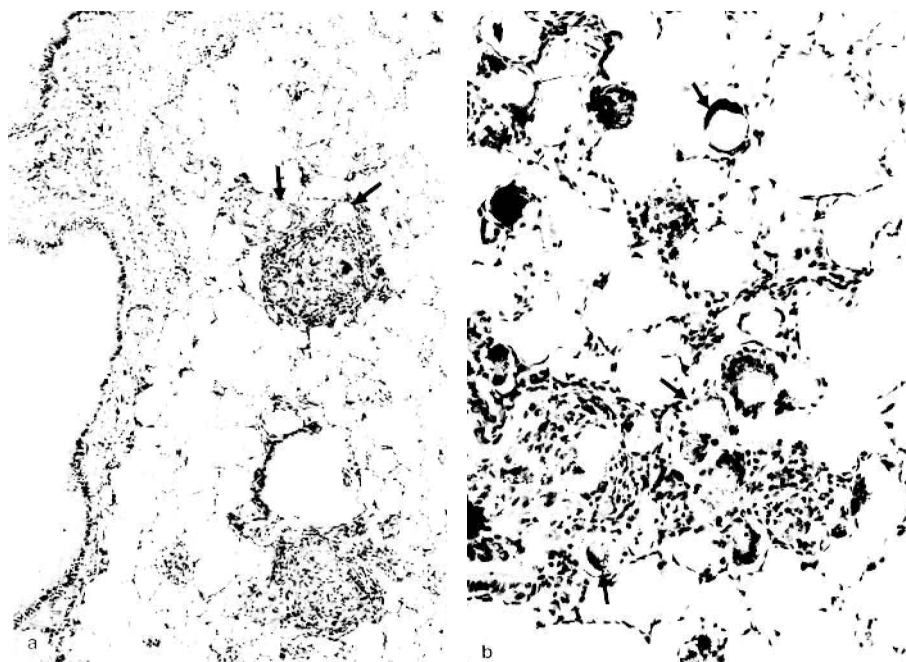


FIGURE 2. (a) Lung of rat, immunized and challenged with pollen grains. Two moderate-sized granulomas, situated in the alveolar region, are present enclosing some pollen grains (arrows). Bronchiole (left) shows slightly hypertrophied mucous epithelium. H&E, 110 $\times$. (b) Alveolar region of lung of rat, immunized and challenged with pollen grains and DEP. All pollen grains, lying free in alveoli, are completely enclosed by alveolar macrophages, packed with DEP (arrows). Also a granuloma is present with a few incorporated pollen. H&E, 220 $\times$.

Effect of DEP on IgE and IgG₁ Production

Serum prior to sensitization showed in the DIG ELISA for IgE low extinction values at a dilution of 1:2 (below 0.25). At day 28, 3 days after the latest intranasal challenge of pollen grains, the extinction values of all sera were increased at a dilution of 1:2 (0.8 ± 0.08) up to a dilution of 1:128 (0.35 ± 0.01). Combining the sensitization and/or challenge with DEP (groups pollen/pollen + DEP and pollen + DEP/pollen + DEP), a significant increase in extinction values was found compared to the extinction values of rats treated with pollen grains alone. Similar results were found after intratracheal administration of pollen grains combined with DEP. To express the differences in serum dilution, the arbitrarily chosen 60% of the mean extinction level obtained after immunization with pollen grains alone was used for comparison. In Figure 3 it is shown that after intranasal application of pollen grains and DEP a significant increase of the dilution is found ($p < .001$ and $.01$) for the groups with pollen/pollen + DEP and pollen + DEP/pollen + DEP, respectively, compared to the group with pollen grains alone. Intratracheal administration of pollen grains and DEP resulted in a significant elevation of the titer of groups with pollen/pollen +

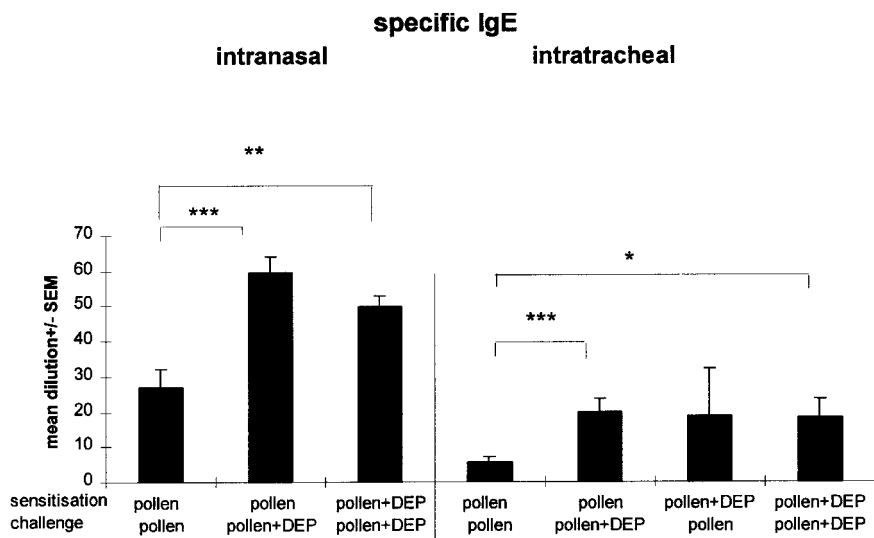


FIGURE 3. Serum dilution of grass-pollen-specific IgE after intranasal and intratracheal immunization. Rats ($n = 3/\text{group}$) were sensitized 5 times (days 1–5) and challenged at days 21–25 with pollen only, sensitized with pollen and challenged with pollen and DEP, or sensitized and challenged with pollen grains and DEP. The serum titer was determined by 60% of the extinction value obtained after sensitization and challenge of pollen only. Comparison was between dilutions after sensitization and challenge with pollen grains only and in combination with DEP. Significant differences are indicated as * $p < .05$, ** $p < .01$, and *** $p < .001$.

DEP and pollen + DEP/pollen + DEP ($p < .001$ and $.05$), respectively, compared to the pollen/pollen group.

The DIG ELISA for IgG₁ revealed much higher extinction values for antibodies to pollen grains. After intranasal administration, a 1:100 dilution of the serum developed an extinction value between 2.5 and 3.0, and the extinction declined to preexposure levels with a dilution of 1:500,000. The extinction value after the intratracheal administration reached maximally 2.5 and became also comparable with the preexposure values at a dilution of 1:100,000. To express the differences in serum dilution, the arbitrarily chosen 60% of the mean extinction level obtained after immunization with pollen grains alone was used for comparison (Figure 4). When the challenge or sensitization procedure was combined with DEP, an increase of the pollen-specific IgG₁ antibodies was found. This increase was statistically significantly after intratracheal sensitization with pollen grains and challenge with pollen grains coexposed with DEP compared to intratracheal sensitization and challenge with pollen grains only.

DISCUSSION

This study shows that in the BN rat, pollen grains induce a pattern comparable to an allergic state such as the production of IgE antibodies,

and an increase in the number of macrophages and eosinophilic granulocytes in the lung. Moreover, DEP show an adjuvant effect on IgE and IgG₁ production to timothy grass pollen grains after intranasal and intratracheal exposure of BN rats. Finally, the intranasal application of pollen in the BN rat is a suitable and elegant method to evoke inflammatory reactions in the lung and pollen-specific IgE responses measured by DIG ELISA.

Most of the studies in which adjuvant activity of particulate matter is examined use the ovalbumin model, in which an allergic reaction is provoked. In these studies, DEP have already shown adjuvant activity only (Nilsen et al., 1997; Fujimaki et al., 1994, 1995, 1997; Takafuji et al., 1987, 1989; Muranaka et al., 1986). Ovalbumin is a very efficient antigen after administration by inhalation and by intranasal application. However, it is not the most relevant antigen to induce an IgE response in a model to study effects of air pollution. More realistic antigens for studying the association between air pollution and allergy are pollen grains or house dust mite antigens. In our study we have chosen grass pollen, which plays a major role in airway allergy.

The interaction between DEP and pollen grains has been extensively studied (Knox et al., 1997; Behrendt et al., 1991, 1992, 1997). Pollen grains not only carry allergens, but also incorporate pollutants when re-

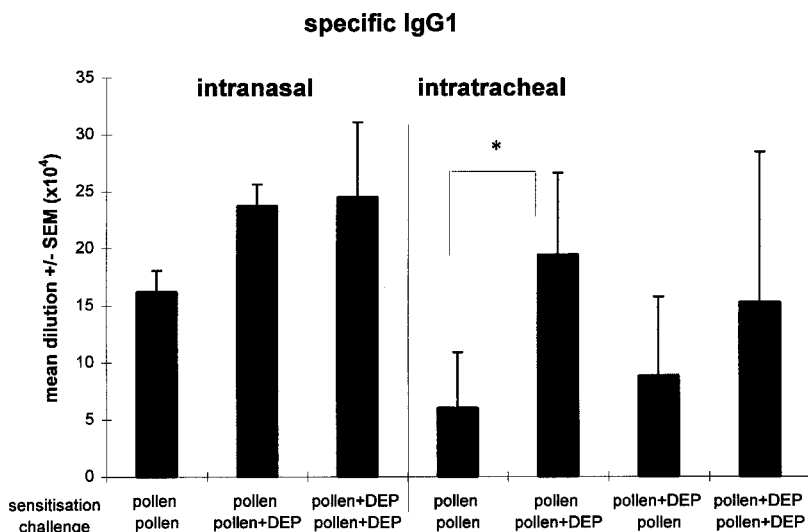


FIGURE 4. Serum dilution of grass-pollen-specific IgG₁ after intranasal and intratracheal immunization. Rats ($n = 4$ /group) were sensitized at day 1 and challenged at day 21 with pollen grains only, sensitized with pollen grains and challenged with pollen grains and DEP, sensitized with pollen grains and DEP and challenged with pollen grains, or sensitized and challenged with pollen grains and DEP. The serum titer was determined by 60% of the extinction value obtained after sensitization and challenge of pollen only (not shown). Comparison was between dilutions after sensitization and challenge with pollen grains only and in combination with DEP. Significant difference is indicated as $*p < .05$.

leased into a polluted atmosphere. Pollen grains can accumulate lead, cadmium, mercury, and sulfur, and DEP aggregates have been also identified in pollen grains (Behrendt et al., 1997). In our experiment, an excess of DEP was incubated with pollen grains (10 mg pollen grains vs. 3 mg DEP) and examined by SEM. After thoroughly washing, pollen grains show only a relative small surface area covered by DEP. The adherence of DEP to pollen appears to be relatively small in our study, as seen on pollen grains from polluted areas by Behrendt et al. (1997). Whether or not our pollen grains released allergens into the buffer fluid was not examined.

Microscopic examination revealed pollen grains in the alveolar air space after intranasal administration. Once present, they were surrounded by macrophages. Moreover, a severe inflammatory response developed, including granuloma formation around pollen grains and a perivascular influx of eosinophilic granulocytes. The combination of DEP and pollen grains showed DEP-loaded macrophages surrounding pollen grains and a similar inflammatory response. Allergic diseases of the respiratory tract are commonly induced by pollen. So far, no literature is available to indicate that these pollen grains induce a similar inflammatory response in human as shown in our study with BN rats. It is known that in the BN strain microgranulomas occur spontaneously (Ohtsuka et al., 1997). Granuloma formation is a type IV reaction, which may not be expected in the BN rats as this rat strain is a high producer of IgE. However, in studies (not shown) we have noted (at day 21) that the granuloma formation due to pollen grains and specific antibodies occurs only after challenge and not after primary sensitization. This may indicate that these granulomas are initiated by an immunological process.

So far, in most studies in which pollen grains were administered to mice and rats, the levels of IgE to pollen allergens have been determined by passive cutaneous anaphylaxis (PCA) (Tamura et al., 1986; Fujimaki et al., 1994; Maejima et al., 1997; Muranaka et al., 1986). In our study we successfully measured specific IgE and IgG₁ after intranasal and intratracheal sensitization and challenge with pollen grains using a DIG ELISA. Five successive intranasal sensitizations and challenges induced almost similar specific IgE pollen levels compared to single intratracheal sensitization and intratracheal challenge. Our results are in line with previous studies (Tamura et al., 1986; Fujimaki et al., 1994; Maejima et al., 1997), in which it was shown that administration of DEP (intraperitoneally or intranasally) increases the IgE level compared to immunization with Japanese cedar pollen grains alone. In addition, our study is also in line with those using a combination of ovalbumin and DEP only (Nilsen et al., 1997; Fujimaki et al., 1994, 1995, 1997; Takafuji et al., 1987, 1989; Muranaka et al., 1986). We found extreme high specific IgG₁ levels, similar to those observed by Maejima et al. (1997); however, we found only a statistical increase in IgG₁ antibody production when the pollen grains were intranasally coexposed with DEP.

In our study we found that DEP intranasally or intratracheally administered was most effective in increasing the pollen-grain-specific IgE and IgG₁ responses when DEP were challenged at day 21. This is in agreement with the observations of Diaz Sanchez and co-workers (Diaz Sanchez, 1997), who demonstrated a differential effect of DEP on the B-cell response. In nonallergic persons, DEP increase the nasal total IgE production and increase the production of cytokine production in a non-Th1, non-Th2-type fashion. Furthermore, these authors showed that in ragweed-allergic subjects DEP reduced the Th1-type cytokines and increased the Th2-type and other cytokines, while the allergen-specific IgE, but not the total IgE, was markedly increased. So it seems that DEP induce increased production of IgE. However, when memory cells are triggered by specific allergens, memory B cells are preferentially stimulated by DEP. More studies are needed to investigate the role of DEP in the initiation of memory cells.

In conclusion, timothy pollen grains induce an allergic state in the BN rats after intranasal and intratracheal administration. It was shown that DEP have an adjuvant activity on the IgE antibody production in this model. Using pollen as an allergen, the interaction of allergen and particulate matter and the local inflammatory reactions on pollen can be studied.

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