

Fine particles of widely different composition have an adjuvant effect on the production of allergen-specific antibodies

Berit Granum ^{a,*}, Per Ivar Gaarder ^b, Else-Carin Groeng ^a,
Rita-Bente Leikvold ^a, Ellen Namork ^a, Martinus Løvik ^a

^a Department of Environmental Medicine, National Institute of Public Health, PO Box 4404 Nydalen, N-0403 Oslo, Norway

^b Department of Immunology and Transfusion Medicine, Ullevål University Hospital, N-0407 Oslo, Norway

Received 2 March 2000; received in revised form 17 October 2000; accepted 18 October 2000

Abstract

Diesel exhaust particles (DEP) are reported to increase the specific IgE response to allergens, and results from our laboratory suggest that the particle core of DEP contribute to this adjuvant activity. The purpose of the present study was to explore further the adjuvant effect of particles per se, that is particles by themselves. NIH/Ola mice were given two intraperitoneal injections with ovalbumin (OVA; 10 µg) alone or OVA in combination with PSP, polytetrafluoroethylene (teflon), titanium dioxide (TiO₂) or amorphous silica particles (2.8×10^{10} – 2.8×10^{12}). Blood samples were drawn 7 days after the last injection, and serum levels of allergen-specific and total IgE and IgG2a were measured. All types of particles gave increased levels of allergen-specific IgE and IgG2a. Similar results were obtained after intranasal or intratracheal instillation with OVA plus PSP or silica. Our results indicate that fine particles of widely different composition may have an adjuvant effect on the production of allergen-specific antibodies. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Adjuvants; Allergy; IgE; Mice; Ovalbumin; Particles

1. Introduction

In recent decades, an increased prevalence of allergic conditions has been observed in developed countries (Howarth, 1998). The causes for this trend are not known, but components of air pol-

lution may play a role. In industrialised countries, the exposure to some components of air pollution (SO₂, large soot particles) has declined during the last decades. However, there is an increased emission of oxides of nitrogen and fine particles (diameter < 2.5 µm). Combustion of diesel fuels has been estimated to contribute about 20–38% of the particle load in the air over Great Britain (Quality of Urban Air Review Group, 1993; Anderson, 1997; Murphy et al., 1998).

* Corresponding author. Tel.: +47-22-042322; fax: +47-22-042686.

E-mail address: berit.granum@folkehelsa.no (B. Granum).

There are several studies reporting that diesel exhaust particles (DEP) can augment allergy-related immune responses (Muranaka et al., 1986; Fujimaki et al., 1995; Diaz-Sanchez et al., 1997; Løvik et al., 1997). In a previous study in BALB/c mice, both DEP and carbon black (CB) were found to have an adjuvant effect on the production of allergen-specific IgE (Løvik et al., 1997). The IgE adjuvant effect of CB was, however, slightly lower than the effect from the same dose weight of DEP. Both DEP and CB have a non-extractable carbon core. In contrast to DEP, CB has a very low amount of chemical substances adsorbed to the carbon core. These results, therefore, indicate that although some of the adjuvant effect of DEP may be due to the adsorbed chemicals, the non-extractable carbon core causes much of the observed adjuvant effect (Suzuki et al., 1993; Løvik et al., 1997; Tsien et al., 1997). In a previously reported experiment, we studied further the adjuvant effect of particles per se, that is particles by themselves, by giving NIH/Ola mice intranasal, intratracheal or intraperitoneal administration of ovalbumin (OVA) plus well-characterised particles, namely polystyrene particles (PSP). Mice given OVA plus PSP produced higher levels of allergen-specific IgE than mice given OVA alone did (Granum et al., 2000c).

The aim of the present study was to elucidate further the adjuvant effect of particles per se in NIH/Ola mice, using well-characterised particles such as polytetrafluoroethylene (teflon), titanium dioxide (TiO₂) and silica particles, in addition to PSP. In a previous study, we found that the total number and surface area of particles were better parameters than the total mass of particles when investigating the adjuvant capacity of PSP (Granum et al., 2000b). Since PSP, teflon, TiO₂ and silica particles have different densities (1.05, 1.50, 3.90 and 1.96 g/ml, respectively), on an equal mass basis, each particle dose would differ considerably in both the number of particles and total surface area. We, therefore, chose to give mice particle doses based on the number of particles. Specific IgE antibodies was used as an indicator of an allergy-related (Th2-dependent) immune response, while specific IgG2a antibodies was used as an indicator of a non-allergic (Th1-dependent) response (Finkelman et al., 1990).

2. Materials and methods

2.1. Mice

Specific pathogen-free 6–8 week old inbred female NIH/OlaHsd mice were obtained from Harlan UK Limited, Oxon, UK. They were randomly allocated into groups of 8 mice each, and rested for 1–2 weeks before entering the experiment. The mice were housed on Beekay GLP Bedding (B&K Universal AS, Nittedal, Norway) in type III macrolon cages placed in filter cabinets, and they were fed pelleted food (RM1, SDS, Essex, UK) and tap water ad libitum. Each mouse was marked by ear punching. The room had a 12 h/12 h light/dark cycle (30–60 lux in cages), and air temperature and moisture were within recommended limits (20 ± 1°C and 40–50%, respectively). The experiments were performed in conformity with the laws and regulations controlling experiments with live animals in Norway.

2.2. Allergen and particles

OVA (chicken egg albumin, grade VII, cat. no. A-7641, Sigma, St Louis, MO) was dissolved (1 mg/ml) in Hank's Balanced Salt Solution (HBSS; Gibco BRL, Paisly, Scotland). PSP (cat. no. 07304, Polysciences Europe GmbH, Eppelheim, Germany) had a diameter of 0.2 µm (S.D.: 0.01 µm) and were provided as 2.5% aqueous suspensions. PSP instilled intratracheally had a diameter of 0.1 µm (cat. no. 00876). Teflon (cat. no. 44 509-6, Aldrich Chemical Company, Inc., Milwaukee, WI) had a diameter ranging from 0.05 to 0.5 µm, and they were provided as 60 wt% dispersion in water containing 6 wt% of octyl phenoxy polyethoxyethanol (Triton). Triton (cat. no. T-6878, Sigma), used to explore possible adjuvant effect from this chemical, was diluted to a concentration of 6 wt% in deionised, sterile water. Both PSP and teflon are man made, and they belong to a class of covalent compounds of high polymers (Waser et al., 1982a). PSP consist of polymerised styrene (CH₂=CHC₆H₅). Due to the polymerisation process and the use of an initiator, every polystyrene chain has a sulphate group at both ends (Polysciences, personal communication).

Teflon consists of polymerised tetrafluoroethylene ($\text{CF}_2=\text{CF}_2$) and has fluoride ions on the surface (Waser et al., 1982b).

TiO_2 (cat. no. 89490, Fluka Chemie AG, Buch, Switzerland) and amorphous silica particles (stock code S0002005PN, Bangs Laboratories, Inc., Fishers, IN) were provided as dry powders, and they were $0.2\ \mu\text{m}$ in diameter according to the supplier. Both TiO_2 and silica particles were suspended in deionised, sterile water before use. Silica particles were included because silicates make up a large part of ambient inorganic mineral particles in urban areas in industrialised countries (Takafuji et al., 1989; Ormstad et al., 1997; Williams, 1999). We used amorphous (non-crystalline) silica particles that are made by a precipitation reaction of silicon dioxide. Thus, these particles have hydroxyl or silanol ($\text{Si}-\text{OH}$) surface groups (Bangs Laboratories, personal communication). TiO_2 particles were chosen because, with the particle diameter used in this study, they are known to be of low toxicity and have a low in vivo solubility (Oberdörster et al., 1992). The TiO_2 particles used are mineral particles that have an anatase structure (tetragonal–ditetragonal dipyramidal crystal system; The Barthelmy Bit Home Page, 1999).

2.3. Transmission electron microscopy

Particles of silica, TiO_2 and teflon were diluted in distilled water. Droplets ($5\ \mu\text{l}$) of the suspensions were adsorbed to glow discharged carbon grids for 1 min, negatively stained with 0.5% phosphotungstic acid (NaPT , pH 7.0) for another min, drained with filter paper and air dried. The specimens were inspected in a transmission electron microscope (JEM-1010, Jeol Ltd, Tokyo, Japan) at 100 keV. The TEM micrographs showed that these particles varied in size and shape (Fig. 1 shows TiO_2 and silica). However, when calculating the number of particles in a given amount of TiO_2 and silica suspensions, we used the diameter defined from the suppliers, namely $0.2\ \mu\text{m}$. According to the supplier, the teflon particles ranged from 0.05 to $0.5\ \mu\text{m}$ in diameter. We calculated the size distribution and could, thereby, estimate the approximate number of particles in a given amount of the teflon suspension. Since the purpose of this study was to investigate qualitative, and not quantitative, differences in the adjuvant effects of the various particles, we chose to give mice particle doses based on an approximately equal number of particles. Thus, we did not calculate the total surface area of each particle dose.

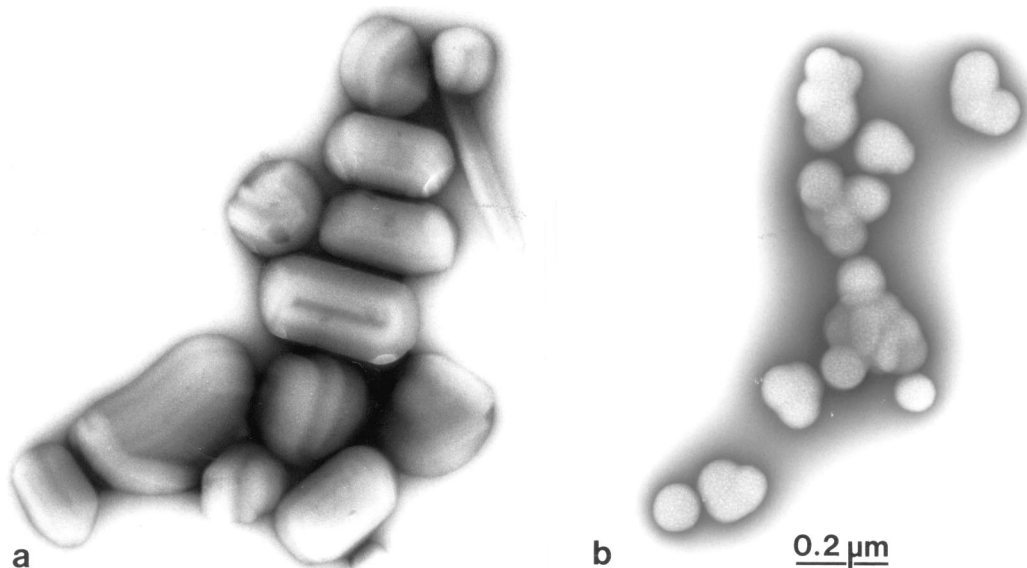


Fig. 1. Electron micrographs of negatively stained preparations of (a) TiO_2 particles and (b) silica particles. $\times 60\ 000$.

Table 1

The table summarises the design of the experiments using intraperitoneal injection^a

Exp. 1		Exp. 2		Exp. 3	
Group	Days 0 and 16	Group	Days 0 and 17	Group	Days 0 and 16
A	–	A	–	A	–
B	OVA	B	OVA	B	OVA
C	Low TiO ₂ +OVA	C	Low silica+OVA	C	Med silica
D	Med TiO ₂ +OVA	D	Med silica+OVA	D	Med silica+OVA
E	High TiO ₂ +OVA	E	High silica+OVA	E	Med TiO ₂
F	Low teflon+OVA	F	Med TiO ₂ +OVA	F	Med TiO ₂ +OVA
G	Med teflon+OVA	G	Low teflon+OVA	G	High teflon
H	High teflon+OVA	H	Med teflon+OVA	H	High teflon+OVA
I	High PSP+OVA	I	High teflon+OVA	I	Triton
		J	High PSP+OVA	J	Triton+OVA

^a The mice were exsanguinated 7 days after the last injection. Low: 2.8×10^{10} particles; med: 2.8×10^{11} particles; high: 2.8×10^{12} particles.

2.4. Collection of blood samples

Before the start of each experiment, the mice were bled once. Blood samples were drawn from the distal part of the lateral femoral vein (Aaberge et al., 1992). After removing hair from the area with a scalpel blade, the vein was punctured with a 21-G needle (Terumo Europe n.v., Leuven, Belgium) and 200 µl blood samples were collected into heparinised capillary tubes (Vitrex, Herlev, Denmark). At the end of each experiment, the mice were anaesthetised with CO₂, and blood was obtained by heart puncture. Serum or heparin plasma from all blood samples was stored at –20°C until analysed.

2.5. Experimental design

In experiment 1, NIH/Ola mice were given intraperitoneal injections on days 0 and 16 with 10 µg of OVA alone or OVA in combination with TiO₂, teflon or PSP (see Table 1 for details). One group of mice was left untreated. On day 23, the mice were exsanguinated. We have previously investigated the effect of different doses of PSP on the production of antibodies. Therefore, we used only one dose of PSP in the present study (Granum et al., 2000b).

Experiment 2 was performed to verify the findings in the first experiment, and in addition ex-

plore the adjuvant effect of silica particles. NIH/Ola mice were given intraperitoneal injections on days 0 and 17 with 10 µg of OVA alone or OVA in combination with silica, TiO₂, teflon or PSP (see Table 1 for details). One group of mice was left untreated. The mice were exsanguinated on day 24.

In experiment 3, we explored the effect from the different particles alone. NIH/Ola mice were given intraperitoneal injections, on days 0 and 16, with OVA alone, silica, TiO₂ or teflon alone or OVA plus the different particles (see Table 1 for details). The mice were exsanguinated on day 23. We have in a number of previous experiments found that PSP alone do not influence the levels of either allergen-specific or total IgE and IgG2a (Granum et al., 2000c). PSP were, therefore, left out in this experiment. Since the teflon suspension contained Triton, mice were given OVA plus the same amount of Triton as in the final dilution of the teflon suspension (0.85 mg/injection), or Triton alone to explore possible adjuvant effects from this chemical.

We also explored the adjuvant effect from silica and PSP after intranasal and intratracheal instillations. Due to practical considerations, such as the volume and the viscosity of the test solution that could be used, mice were given different particle doses in these experiments. Before each instillation, NIH/Ola mice were anaesthetised with an

intravenous injection with diprivan (1–3 mg/mouse, 10 mg/ml, Zeneca Ltd, Macclesfield, Cheshire, UK). In experiments using intranasal instillation, mice were given, via the nostrils, 30 μ l of a solution containing 3 μ g of OVA alone, OVA plus 1.7×10^{11} particles of silica or PSP, or PSP alone. The instillations were given once a week for 3 weeks. The instillation protocol was repeated three times with 1–2 week intervals. The mice were exsanguinated 8 days after the last instillation. In experiments using intratracheal instillation, mice were given 100 μ l of the test solution via a blunt needle mounted on a 1-ml syringe (Codan Medical ApS, Rødby, Denmark), introduced perorally into the trachea. Groups of mice were given 5 μ g of OVA alone, OVA plus 1×10^{12} particles of silica or PSP, or particles alone on days 0 and 14. The mice were exsanguinated on day 22. One group of mice was left untreated as a negative control.

2.6. Mouse IgE and IgG2a antibodies to OVA

The procedures have been described in detail previously (Løvik et al., 1997; Granum et al., 2000c). In short, micro-titre plates coated with a monoclonal rat anti-mouse IgE or rat anti-mouse IgG2a were allowed to capture IgE or IgG2a antibodies, respectively, in mouse sera. Then followed by incubation with, in succession, biotinylated OVA, pre-formed complexes of streptavidin and biotinylated alkaline phosphatase, and finally with *p*-nitrophenyl phosphate diluted in diethanolamine buffer. Dilutions of a mouse serum pool containing defined amounts of IgE anti-OVA (0.05–3.2 arbitrary U/ml) and IgG2a anti-OVA (0.002–0.64 arbitrary U/ml) were included on every plate for standard curve production. Optical density was measured at 405 nm.

2.7. Total mouse serum IgE and IgG2a

The procedures have been described in detail previously (Løvik et al., 1997; Granum et al., 2000c). In short, microplates coated with a monoclonal rat anti-mouse IgE or IgG2a were allowed to capture IgE and IgG2a antibodies, respectively, in mouse sera, followed by incubation with biotin

labelled rat anti-mouse IgE or IgG2a. The micro-titre plates were then incubated with alkaline phosphatase labelled avidin (for the detection of total IgE) or pre-formed complexes of avidin and biotinylated phosphatase (for detection of IgG2a antibodies), followed by *p*-nitrophenyl phosphate in a diethanolamine buffer. Mouse IgE or IgG2a isotype standards were used to construct the standard curves (1–1000 ng/ml and 0.01–2 μ g/ml, respectively). Optical density was measured at 405 nm.

2.8. Statistics

Statistical analysis was performed with Sigma-Stat[®] Statistical Analysis System for Windows Version 2.03 (Jandel Scientific, Erkrath, Germany). SigmaStat performs normality and equal variance tests on all data. Kruskal–Wallis One Way Analysis of Variance on Ranks was used to analyse for differences among the treatment groups in each experiment. When the difference among the treatment groups were greater than would be expected by chance ($P \leq 0.05$) the data were further analysed using Student's *t* test for pairwise comparison when normality and equal variance tests for the different groups to be compared were passed. The Mann–Whitney Rank Sum Test was used when some of the test samples were outside the detection range, or when normality or equal variance tests failed. Limit for statistical significance was set at $P = 0.050$.

3. Results

3.1. All particle types tested had an adjuvant effect on the production of allergen-specific IgE and IgG2a after intraperitoneal injection in NIH/Ola mice

Mice given OVA plus the highest dose of silica or TiO₂ particles (2.8×10^{12}) became sick right after the first injection and had to be taken out of the experiments. In experiment 1, NIH/Ola mice given intraperitoneal injections with OVA in combination with TiO₂ (2.8×10^{11}), teflon (2.8×10^{11} , 2.8×10^{12}) or PSP (2.8×10^{12}) had significantly

higher levels of allergen-specific IgE compared to mice given OVA alone (Fig. 2a). There were no statistically significant differences between mice given OVA in combination with the lowest dose of TiO₂ or teflon (2.8×10^{10}) and mice given OVA alone. Regarding allergen-specific IgG2a, the mice showed an antibody response pattern similar to IgE anti-OVA (Fig. 2b). In experiment 2 (Table 2), OVA plus silica (2.8×10^{10} , 2.8×10^{11}), teflon (2.8×10^{11} , 2.8×10^{12}) or PSP (2.8×10^{12}) gave significantly higher levels of allergen-specific IgE compared to OVA alone. Mice given OVA plus TiO₂ (2.8×10^{11}) or teflon (2.8×10^{10}) had higher,

but not significantly higher, levels of IgE anti-OVA compared to mice given OVA alone. Except for mice given OVA plus the lowest dose of silica (2.8×10^{10}), all mice given OVA plus particles had significantly higher levels of IgG2a anti-OVA compared to mice given OVA alone.

The level of total IgE showed response patterns similar to the level of allergen-specific IgE. However, there were statistically significant differences in the level of total IgE between mice given OVA alone and mice given OVA plus 2.8×10^{12} teflon or 2.8×10^{11} silica particles only. Except for mice given OVA plus PSP, there were no statistically

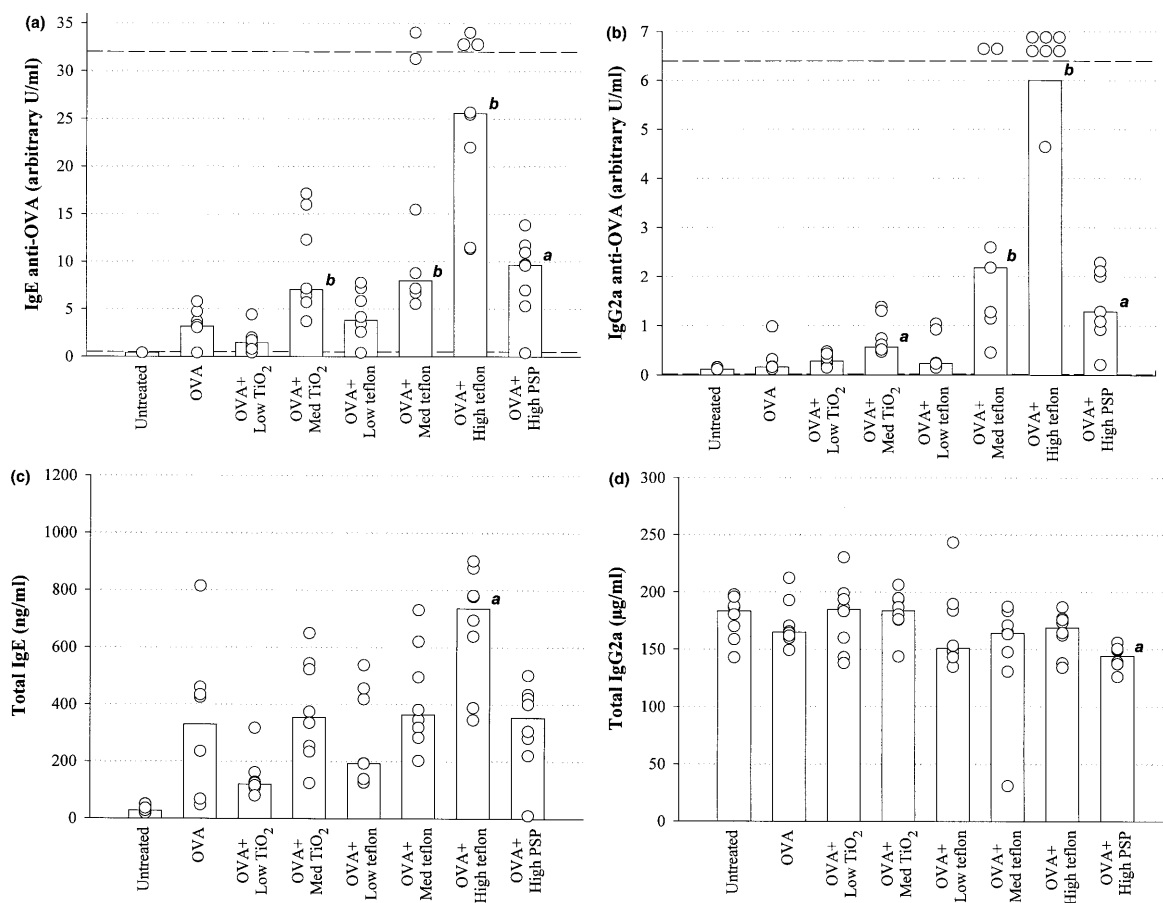


Fig. 2. Fig. 2a–d show the levels of IgE anti-OVA, IgG2a anti-OVA, total IgE and total IgG2a, respectively, in untreated NIH/Ola mice, mice given intraperitoneal injections with OVA alone and OVA plus TiO₂, teflon or PSP. Individual (○) and median (columns) values for each group of mice are shown. A statistically significant difference between mice given OVA and mice given OVA plus particles is denoted by (^a $P \leq 0.01$; ^b $P \leq 0.001$). The black dashed lines denote lower and upper quantitative detection-limits for measurements of allergen-specific IgE and IgG2a. Low: 2.8×10^{10} particles; med: 2.8×10^{11} particles; high: 2.8×10^{12} particles.

Table 2

Median values and 5 and 95 percentiles of antibody levels in groups of untreated mice, mice given intraperitoneal injections with OVA alone and in mice given OVA in combination with silica, TiO₂, teflon or PSP^b

Treatment ^a	IgE anti-OVA (arbitrary U/ml)		IgG2a anti-OVA (arbitrary U/ml)	
	Median	5 and 95 percentiles	Median	5 and 95 percentiles
Untreated	<0.9	–	0.1	0.08, 0.14
OVA	2.0	<0.9, 8.9	0.1	0.09, 0.58
Low silica + OVA	6.5 ^d	3.2, 43.3	0.2	0.1, >6.4 ^f
Med silica + OVA	11.9 ^d	5.2, 30.2	>6.4 ^e	0.5, >6.4
Med TiO ₂ + OVA	4.0	<0.9, 40.8	1.1 ^c	0.3, >6.4
Low teflon + OVA	2.6	<0.9, 41.4	1.3 ^d	0.2, >6.4
Med teflon + OVA	7.7 ^d	3.4, 20.8	4.5 ^d	0.2, >6.4
High teflon + OVA	20.8 ^c	<0.9, 58.0	>6.4 ^e	0.4, >6.4
High PSP + OVA	8.1 ^d	3.0, 13.5	2.6 ^c	0.5, >6.4

^a Low: 2.8×10^{10} particles; med: 2.8×10^{11} particles; high: 2.8×10^{12} particles.

^b A statistically significant difference between mice given OVA and mice given OVA plus particles is denoted by

^c $P \leq 0.05$.

^d $P \leq 0.01$.

^e $P \leq 0.001$.

^f The upper detection limit for measurement of allergen-specific IgG2a was 6.4 arbitrary U/ml.

significant differences in the levels of total IgG2a between mice given OVA alone and mice given OVA plus particles. Fig. 2c-d show the levels of total IgE and IgG2a from experiment 1.

Both intranasally and intratracheally instilled silica plus OVA and PSP plus OVA gave significantly higher levels of IgE anti-OVA and IgG2a anti-OVA compared to mice given OVA alone (Table 3). The levels of total IgE showed the same response pattern as allergen-specific IgE. Mice given PSP alone intranasally or silica alone intratracheally had slightly (although significantly) lower levels of total IgG2a compared to mice given OVA alone (results not shown).

3.2. Mice given OVA plus Triton had increased levels of allergen-specific IgE and IgG2a compared to mice given OVA alone

In experiment 3, all groups of mice given particles plus OVA had significantly higher levels of allergen-specific IgE and IgG2a compared to mice given OVA alone (Table 4). Also Triton plus OVA gave increased levels of IgE and IgG2a compared to OVA alone. There were no statistically significant differences in the levels of allergen-specific IgE or IgG2a between mice given

teflon plus OVA and mice given Triton plus OVA ($P = 0.469$). There were no detectable levels of allergen-specific IgE in mice given particles or Triton alone. Similarly, there were no statistically significant differences in the levels of IgG2a anti-OVA between untreated mice and mice given TiO₂, teflon or Triton alone. In contrast, there was a statistically significant, but very small increase in the levels of IgG2a anti-OVA in mice given silica alone compared to mice given OVA alone. The levels of total IgE showed the same response pattern as IgE anti-OVA, whereas there were no differences in the levels of total IgG2a between the different groups of mice (results not shown).

4. Discussion

Particulate matter (PM) is generated from a wide range of natural and anthropogenic sources. In urban areas in industrialised countries, the fine fraction ($< 2.5 \mu\text{m}$ in diameter) is often mainly composed of sulphur aerosols and soot particles (e.g. DEP). The coarse fraction ($2.5\text{--}10 \mu\text{m}$ in diameter) is predominantly composed of inorganic mineral material such as silicates (Takafuji

et al., 1989; Ormstad et al., 1997; Williams, 1999). PM, with a diameter less than 10 μm , has been reported to have an adjuvant effect on the production of allergen-specific IgE in mice (Takafuji et al., 1989; Ormstad et al., 1998). Since soot particles were the main component of the PM, these particles were believed to be at least partially responsible for the observed adjuvant effect in these studies. However, they could not exclude that also other particles in the PM fraction could contribute to this effect.

We have in previous studies found that PSP have an adjuvant effect on the production of allergen-specific antibodies after intranasal, intratracheal or intraperitoneal administration in NIH/Ola mice (Granum et al., 2000a,c). The aim of the present study was to explore further the adjuvant effect from particles per se using particles such as teflon, silica and TiO_2 , in addition to PSP. Mice given intraperitoneal injections with OVA plus the different particles had significantly stronger antibody responses compared to mice given OVA alone. Similar results were also found after intranasal and intratracheal instillations with OVA plus silica or PSP. In the present study, we found that fine particles (about 0.2 μm in diameter) of

widely different composition elicited an increased production of allergen-specific antibodies, indicating that also other particles than soot particles in ambient air may have an adjuvant effect. Our findings suggest that fine particles per se, regardless of chemical composition, have a capacity to augment allergen-specific antibody production.

We explored possible adjuvant effects from Triton since the teflon suspension used in this study contained this chemical. Mice given OVA plus a dose of Triton similar to the high dose of teflon suspension (850 $\mu\text{g}/\text{injection}$) had higher antibody levels than mice given OVA alone. In an additional experiment, OVA plus Triton in similar amounts as in low and medium doses of the teflon suspension (8.5 and 85 $\mu\text{g}/\text{injection}$, respectively) did not give an increased level of allergen-specific antibodies when compared to OVA alone (unpublished results). These results suggest that the increased antibody production after injections with OVA plus medium doses of the teflon suspension were mainly caused by the teflon particles, whereas Triton may have contributed substantially to the increased antibody production after injections with OVA plus high doses of the teflon suspension.

Table 3

Result from experiments using intranasal and intratracheal instillation: median values and 5 and 95 percentiles of antibody levels in untreated mice and mice given intranasal or intratracheal instillation with OVA alone or OVA in combination with silica or PSP^a

Treatment	IgE anti-OVA (arbitrary U/ml)		IgG2a anti-OVA (arbitrary U/ml)	
	Median	5 and 95 percentiles	Median	5 and 95 percentiles
<i>Intranasal</i>				
OVA	<0.5	<0.5, 2.4	0.1	0.09, 0.16
Silica + OVA	7.5 ^d	2.6, 29.2	0.5 ^d	0.2, 1.1
PSP	<0.5	–	0.1	0.07, 0.14
PSP + OVA	2.3 ^d	1.0, 5.6	0.3 ^b	0.1, 0.6
<i>Intratracheal</i>				
Untreated	<0.5	–	0.7	0.3, 1.1
OVA	<0.5	–	0.6	0.5, 0.9
Silica	<0.5	<0.5, 2.1	0.6	0.3, 1.1
Silica + OVA	2.2 ^d	1.1, 4.5	1.9 ^d	1.0, 5.4
PSP	<0.5	–	0.7	0.6, 0.9
PSP + OVA	2.2 ^d	0.9, 4.9	1.1 ^c	0.5, 1.3

^a A statistically significant difference between mice given OVA and mice given OVA plus particles is denoted by

^b $P \leq 0.05$.

^c $P \leq 0.01$.

^d $P \leq 0.001$.

Table 4

Median values and 5 and 95 percentiles of antibody levels in groups of untreated mice, mice given intraperitoneal injections with OVA alone, and silica, TiO₂, teflon or Triton alone or in combination with OVA^a

Treatment	IgE anti-OVA (arbitrary U/ml)		IgG2a anti-OVA (arbitrary U/ml)	
	Median	5 and 95 percentiles	Median	5 and 95 percentiles
Untreated	<0.5	–	0.1	0.06, 0.13
OVA	0.9	<0.5, 3.3	0.1	0.04, 0.18
Silica	<0.5	–	0.2	0.1, 0.3
Silica+OVA	8.7 ^c	4.0, 17.4	0.8 ^c	0.2, 1.4
TiO ₂	<0.5	–	0.1	0.07, 0.15
TiO ₂ +OVA	5.7 ^b	2.7, 14.4	0.2 ^c	0.1, 0.4
Teflon	<0.5	–	0.1	0.07, 0.15
Teflon+OVA	17.7 ^b	<0.5, 26.2	0.3 ^b	0.1, 1.9
Triton	<0.4	–	0.1	0.07, 1.12
Triton+OVA	14 ^c	7.9, 24.5	0.2	0.1, 0.5

^a A statistically significant difference between mice given OVA and mice given OVA plus particles is denoted by

^b $P \leq 0.01$

^c $P \leq 0.001$.

It has been estimated that during episodes with high levels of particulate matter (100–200 $\mu\text{g}/\text{m}^3$) in developed countries, there may be approx. 100 000 particles per ml of air (cut-off size not defined; Seaton et al., 1995). During 24 h, the human lung is exposed to between 10 000 and 20 000 l of air. A person could inhale about 1×10^{12} – 2×10^{12} particles daily. About 50% of the particles are being deposited, thus about 5×10^{11} – 1×10^{12} particles could be deposited daily. Therefore, if comparisons are made on basis of the body weight of the exposed individual, we used relatively high particle doses in experiments using intranasal and intratracheal instillation (1.7×10^{11} and 1×10^{12} particles/injection, respectively). Working in a model system, we used high particle doses to obtain optimal differences between mice given OVA alone and mice given OVA plus particles. ‘Realistic’ particle doses may be even more difficult to assess when the particles are given intraperitoneally. However, experiments using intraperitoneal injections are useful for exploring the functional effect of particles on antibody production. We are among the first to explore the effect of particles per se on antibody production (Kurup et al., 1994). Therefore, as a starting point, it was important to use experimental conditions that could provide clear responses.

Further experiments with smaller doses of particles given via the relevant route (airways) with a relevant allergen (from e.g. pollen, cat, mite) should be performed to move from our model system towards a more real life-like situation.

Administration of OVA plus particles, gave increased production of both allergen-specific IgE and IgG2a antibodies compared to administration of OVA alone. The functional effect of the presence of both specific IgE and IgG on allergy-related immune responses is somewhat unclear (Aalberse et al., 1995; Mark et al., 1995; Wittenman et al., 1996). In cases in which IgE and IgG2a antibodies have the same specificity and thus compete for the binding to the same epitopes, high levels of IgG2a would be expected to mask and neutralise the allergens. Consequently, IgG2a may prevent cross binding of membrane-bound IgE on mast cells or basophils and thus prevent the initiation of allergic immune responses.

Our findings indicate that particles per se may exert a general adjuvant activity. However, hypothetically, if Th2-polarising agents are present at the same time as the particle (e.g. chemical components adsorbed to the particle surface), particles may increase the specific IgE levels selectively (Van Zijverden and Granum, 2000).

Acknowledgements

The present work was supported by grants from the Research Council of Norway, the Norwegian Red Cross and the National Institute of Public Health to Berit Granum. The authors acknowledge Berit A. Stensby and Åse Eikeset for expert technical assistance in the laboratory. Finally, we acknowledge the biotechnicians at the Research Animal Unit for providing technical assistance with the animals.

References

- Aaberge, I.S., Michaelsen, T.E., Rolstad, A.K., Groeng, E.-C., Solberg, P., Løvik, M., 1992. SCID-Hu mice immunized with a pneumococcal vaccine produce specific human antibodies and show increased resistance to infection. *Infect. Immun.* 60, 4146–4153.
- Aalberse, R.C., van Ree, R., Danneman, A., Wahn, U., 1995. IgE antibodies to tetanus toxoid in relation to atopy. *Int. Arch. Allergy Immunol.* 107, 169–171.
- Anderson, H.R., 1997. Air pollution and trends in asthma. In: *The Rising Trends in Asthma*. Wiley, Chichester, pp. 190–207.
- Diaz-Sanchez, D., Tsien, A., Fleming, J., Saxon, A., 1997. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. *J. Immunol.* 158, 2406–2413.
- Finkelman, F.D., Holmes, J., Katona, I.M., Urban, J.F.J., Beckmann, M.P., Park, L.S., et al., 1990. Lymphokine control of in vivo immunoglobulin isotype selection. *Annu. Rev. Immunol.* 8, 303–333.
- Fujimaki, H., Saneyoshi, K., Nohara, O., Shiraishi, F., Imai, T., 1995. Intranasal instillation of diesel exhaust particulates and antigen in mice modulated cytokine productions in cervical lymph node cells. *Int. Arch. Allergy Immunol.* 108, 268–273.
- Granum, B., Gaarder, P.I., Eikeset, Å., Stensby, B.A., Løvik, M., 2000a. The adjuvant effect of particles — importance of genetic background and pre-sensitisation. *Int. Arch. Allergy Immunol.* 122, 167–173.
- Granum, B., Gaarder, P.I., Løvik, M., 2000b. IgE adjuvant activity of particles — what physical characteristics are important? *Inhal. Toxicol.* 12 (Suppl. 3), 365–372.
- Granum, B., Gaarder, P.I., Løvik, M., 2000c. IgE adjuvant effect caused by particles — Immediate and delayed effects, *Toxicology*, In press.
- Howarth, P.H., 1998. Is allergy increasing? — early life influences. *Clin. Exp. Allergy* 28 (Suppl. 6), 2–7.
- Kurup, V.P., Seymour, B.W., Choi, H., Coffman, R.L., 1994. Particulate *Aspergillus fumigatus* antigen elicit a TH2 response in BALBc mice. *J. Allergy Clin. Immunol.* 93, 1013–1020.
- Løvik, M., Høgseth, A.-K., Gaarder, P.I., Hagemann, R., 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.
- Mark, A., Björkstén, B., Granström, M., 1995. Immunoglobulin E responses to diphtheria and tetanus toxoids after booster with aluminium-adsorbed and fluid DT-vaccines. *Vaccine* 13, 669–673.
- Muranaka, M., Suzuki, S., Koizumi, K., Takafuji, S., Miyamoto, T., Ikemori, R., et al., 1986. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J. Allergy Clin. Immunol.* 77, 616–623.
- Murphy, S.A., Bérubé, K.A., Pooley, F.D., Richards, R.J., 1998. The response of lung epithelium to well characterised fine particles. *Life Sci.* 62, 1789–1799.
- Oberdörster, G., Ferin, J., Gelein, R., Soderholm, S.C., Finkelstein, J., 1992. Role of the alveolar macrophage in lung injury: Studies with ultrafine particles. *Environ. Health Perspect.* 97, 193–199.
- Ormstad, H., Gaarder, P.I., Johansen, B.V., 1997. Quantification and characterisation of suspended particulate matter in indoor air. *Sci. Total Environ.* 193, 185–196.
- Ormstad, H., Gaarder, P.I., Johansen, B.V., Løvik, M., 1998. Airborne house dust elicits a local lymph node reaction and has an adjuvant effect on specific IgE production in the mouse. *Toxicology* 129, 227–236.
- Quality of Urban Air Review Group, 1993. Second report: Diesel vehicle emissions and urban air quality. The Department of the Environment, London.
- Seaton, A., MacNee, W., Donaldson, K., Godden, D., 1995. Particulate air pollution and acute health effects. *Lancet* 345, 176–178.
- Suzuki, T., Kanoh, T., Kanbayashi, M., Todome, Y., Ohkuni, H., 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., Tadokoro, K., Ohashi, H., Muranaka, M., et al., 1989. Enhancing effect of suspended particulate matter on the IgE antibody production in mice. *Int. Arch. Allergy Appl. Immunol.* 90, 1–7.
- The Barthelmy Bit Home Page, 1999. Mineralogy database (Online). Available: <http://web.wt.net/~daba/Mineral/data/Anatase.html> (11 November, 1999).
- Tsien, A., Diaz-sanchez, D., Ma, J., Saxon, A., 1997. The organic component of diesel exhaust particles and phenanthrene, a major polyaromatic hydrocarbon constituent, enhances IgE production by IgE-secreting EBV-transformed human B cells in vitro. *Toxicol. Appl. Pharmacol.* 142, 256–263.
- Van Zijverden M., Granum B., 2000. Adjuvant activity of particulate pollutants in different mouse models, *Toxicology*, 152 (1–3), 69–77.

- Waser, J., Trueblood, K.N., Knobler, C.M., 1982a. A classification of chemical substances. In: Ricci, J., Jackson, D.C., Golden, S. (Eds.), *Chem One*, 2nd edn. McGraw–Hill, Singapore, pp. 127–142.
- Waser, J., Trueblood, K.N., Knobler, C.M., 1982b. Organic chemistry. In: Ricci, J., Jackson, D.C., Golden, S. (Eds.), *Chem One*, 2nd edn. McGraw–Hill, Singapore, pp. 643–677.
- Williams, M.L., 1999. Patterns of air pollution in developed countries. In: Holgate, S.T., Samet, J.M., Koren, H.S., Maynard, R.L. (Eds.), *Air Pollution and Health*. Academic Press, London, pp. 83–104.
- Witteaman, A.M., Stapel, S.O., Sjamsoedin, D.H.S., Jansen, H.M., Aalberse, R.C., van der Zee, J.S., 1996. Fel d 1-specific IgG antibodies induced by natural exposure have blocking activity in skin tests. *Int. Arch. Allergy Immunol.* 109, 369–375.