

The Adjuvant Effect of Particles – Importance of Genetic Background and Pre-Sensitisation

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Key Words

Adjuvants · Allergy · Genetics · IgE · Mice · Ovalbumin · Particles

Abstract

Background: We have previously reported that simple and well-characterised particles, such as polystyrene particles (PSP), have an IgE adjuvant effect in mice. The purpose of this study was to explore the importance of genetic background concerning the adjuvant effect of PSP in different strains of mice. **Methods:** Inbred NIH/Ola, BALB/c and C3H/HeJ mice were given two intraperitoneal injections with either PSP plus OVA or OVA alone, and then an intraperitoneal challenge with OVA alone. NIH/Ola mice were also pre-sensitised to develop a weak or strong IgE response to OVA, and then given an intraperitoneal challenge with PSP plus OVA or OVA alone. Serum levels of total and allergen-specific IgE and IgG2a were measured. **Results:** PSP had a specific IgE and IgG2a adjuvant effect in NIH/Ola mice but not in C3H/HeJ and BALB/c mice. Weakly pre-sensitised NIH/Ola mice showed the same response pattern as the naive NIH/Ola mice. In contrast, strongly pre-sensitised mice showed

an antibody response pattern similar to that of high-responder BALB/c mice. **Conclusions:** Our results indicate that the allergen responder status, either genetic or induced, is of importance for the adjuvant effect from particles. The IgE and IgG2a adjuvant effect may depend on the genetically determined susceptibility of an individual to be influenced by exposure to the adjuvant. Therefore, the allergy-enhancing effect from particle pollution may differ between individuals.

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Introduction

In recent decades, an increased prevalence of allergic conditions has been observed in developed countries [1, 2]. The development of allergic diseases depends upon both genetic predisposition and the environment. Genetic alterations, however, are not sufficient to explain the observed increase in the prevalence of allergy since these changes have occurred rapidly, over only a few decades, in very large outbred populations [3]. Thus, environmental factors, such as stimulation of the immune system by microbes, changes in diet and exposure to air pollution,

may play an important role for the increased prevalence [4–7].

There are studies reporting that diesel exhaust particles (DEP) have an adjuvant effect on the production of IgE (man and mouse) and IgG1 (mouse) [5, 8–11]. Furthermore, some studies suggest that there are murine strain differences in allergic airway inflammation, and in the production of allergen-specific immunoglobulins after intratracheal instillation of DEP plus an allergen [12,13]. DEP are complex particles that consist of a non-extractable carbon core with adsorbed chemical substances. Results from our laboratory indicate that a considerable part of the effect of DEP might be caused by the non-extractable particle core itself [8]. To study the IgE adjuvant effect of particles per se further, we gave NIH/Ola mice intranasal, intratracheal or intraperitoneal immunisations with polystyrene particles (PSP) and ovalbumin (OVA). The results from this study supported the notion that particles per se can have an IgE adjuvant effect [14]. We chose PSP as a model particle because PSP are well-characterised and simple particles.

The aim of the present study was to explore the influence of the genetic background and OVA responder status on the adjuvant effect of simple particles. In the first experiments in the present study, we investigated the influence of the genetic background on the specific IgE- and IgG2a adjuvant effect of PSP in mice not pre-sensitised with OVA. In another set of experiments, we investigated the adjuvant effect of PSP in terms of responsiveness to OVA in weakly or strongly pre-sensitised mice. As an indicator of an allergic (Th2-dependent) immune response, we used the concentration of specific IgE. The concentration of specific IgG2a was used as an indicator of a non-allergic (Th1-dependent) response [15].

Material and Methods

Mice

Specific pathogen-free 6- to 8-week-old inbred female BALB/cABom and male C3H/HeJBom mice (females of the appropriate age were not available) were obtained from Bomholtgård, Ry, Denmark. Inbred female NIH/OlaHsd mice were obtained from Harlan UK Ltd., Oxon, UK. The mice were randomly allocated to experimental groups of 7–9 mice each, and allowed to rest for 1–2 weeks before entering the experiments. The mice were housed on Beekay GLP Bedding (B&K Universal AS, Nittedal, Norway) in type III macrolon cages placed in filter cabinets, and 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 lx in cages), and air temperature and moisture were within recommended limits ($20 \pm 1^\circ\text{C}$ and 40–50%, respectively). The experiments were performed in conformity with the laws and regulations controlling

experiments with live animals in Norway. They were approved by the Experimental Animal Board under the Ministry of Agriculture in Norway.

Allergen and Adjuvants

OVA (chicken egg albumin, grade VII, A7641) was obtained from Sigma, St Louis, Mo., USA. OVA was dissolved (1 mg/ml) in Hanks Balanced Salt Solution (HBSS; Gibco BRL, Paisley, UK), and each mouse was given 10 µg OVA per injection. PSP were obtained from Polysciences Europe GmbH, Eppelheim, Germany. The particles had a diameter of 0.1 µm (standard deviation = 0.006 µm). Due to the polymerisation process and the use of an initiator, every polystyrene chain has a sulphate group at both ends. Thus, the particles have a negatively charged surface [Polysciences, pers. commun.]. PSP were provided as 2.5% aqueous suspensions with less than 0.1% sodium dodecyl sulphate. In our model using intraperitoneal injection, 0.1% sodium dodecyl sulphate was found not to have an adjuvant effect on the production of allergen-specific IgE and IgG2a antibodies [unpubl. obs.]. In the experimental procedure, the PSP and OVA solutions were mixed for 30–60 min before being administered to the mice. Under the conditions used, OVA was found to adsorb to PSP during this incubation time [unpubl. obs.]. Each mouse was given $1.89 \cdot 10^{13}$ particles (12.7 mg, 500 µl) per injection. Al(OH)₃ (Superfos Biosector A/S, Vedbaek, Denmark) was obtained at a concentration of 30 mg/ml, and was diluted in HBSS to a final concentration of 4 mg/ml. Each mouse was given 2 mg Al(OH)₃ (500 µl) per injection.

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 [16]. After shaving the area with a scalpel blade, the vein was punctured with a 21-gauge needle and 100–200 µl blood was 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.

Mouse IgE and IgG2a Antibodies to OVA

The procedures have been described in detail previously [8, 17]. In short: for detection of IgE anti-OVA, microtitre plates coated with monoclonal rat anti-mouse IgE antibodies were allowed to capture IgE antibodies in mouse sera, 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 a diethanolamine buffer. Dilutions of a mouse serum pool containing defined amounts of IgE anti-OVA [0.05–3.2 arbitrary units (AU)/ml] were included on every plate for standard curve production. Optical density was measured at 405 nm in an MRX Microplate Reader (Dynatech Laboratories, Chantilly, Va., USA) connected to a PC using BioLinx software (Dynatech Laboratories) for instrument operation and calculation. Measurements of IgG2a anti-OVA were done in an analogous way to IgE anti-OVA, using plates coated with monoclonal rat anti-mouse IgG2a antibodies (LO-MG2a-7, LO-IMEX, University of Louvain, Belgium). The same mouse serum pool as mentioned above was used for the construction of a standard curve on each plate (0.002–1.28 AU/ml).

Total Mouse Serum IgE and IgG2a

The procedure for detection of total IgE was described in detail previously [8]. The following modifications were made: rat anti-mouse IgE (LO-ME-3, LO-IMEX) as capturing antibody, biotin-labelled rat anti-mouse IgE (Biotin-LO-ME-2, LO-IMEX) as secondary antibody and purified mouse IgE isotype standard (clone 15.3, PharMingen, San Diego, Calif., USA) were used to construct the standard curve (1–1,000 ng/ml). In short, microtitre plates coated with monoclonal rat anti-mouse IgE antibodies were allowed to capture IgE antibodies in mouse sera, followed by incubation with, in succession, biotin-labelled rat anti-mouse IgE, alkaline-phosphatase-labelled avidin and *p*-nitrophenyl phosphate in a diethanolamine buffer. Optical density was measured as described above. Determination of total mouse serum IgG2a was done as for total IgE. The following modifications were made: rat anti-mouse IgG2a (LO-MG2a-7, LO-IMEX) as capturing antibody, biotin-labelled rat anti-mouse (clone Biotin-LO-MG2a-3, LO-IMEX) as secondary antibody, purified mouse IgG2a isotype standard (03021D, PharMingen) were used to construct the standard curve (0.01–2 µg/ml), and pre-formed complexes of avidin and biotinylated phosphatase were used as the amplifier.

Study Design

In the first two experiments, we investigated the importance of the genetic background on the effect of PSP in the sensitisation process. Mice were given an intraperitoneal injection with PSP plus OVA on days 0 and 16, and then a challenge with OVA alone on day 42. The mice were exsanguinated on day 47. In the first experiment, we investigated the effect of OVA alone and OVA in combination with PSP in NIH/Ola, BALB/c and C3H/HeJ mice. In the second experiment, NIH/Ola and BALB/c mice were used to verify the findings in the first experiment. We used the same preparations as in the first experiment. To investigate the normal antibody levels (negative control), one group from each strain was left untreated. As a positive control, one group from each strain was given intraperitoneal injections with OVA in combination with Al(OH)₃. Al(OH)₃ is known to be an adjuvant enhancing IgE responses in mice [8, 18, 19].

In the third experiment, we further elucidated the importance of particles in the sensitisation process. One group of NIH/Ola mice was given an injection with OVA on days 0 and 16. Another group was given PSP plus OVA on day 0 and OVA alone on day 16, whereas the last group was given PSP plus OVA on both days. The mice were exsanguinated 7 days after the last injection.

In the last experiment, we investigated the importance of an induced allergen responder status (not genetically determined) on the effect of PSP. NIH/Ola mice were given OVA alone or in combination with Al(OH)₃ (on days 0 and 16) to pre-sensitise them weakly or strongly, respectively, in terms of IgE responsiveness. On day 42, the mice were challenged intraperitoneally with OVA alone, PSP plus OVA or PSP alone. They were exsanguinated on day 47. We used untreated NIH/Ola mice as a negative control group.

Statistics

Statistical analysis was performed with the SigmaStat® Statistical Analysis System for Windows Version 3.06 (Jandel Scientific, Erkrath, Germany). Student's *t* test was used for pairwise comparison when normality and equal variance tests 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. The limit for statistical significance was set at *p* = 0.050.

Results

PSP Had a Specific IgE- and IgG2a-Adjuvant Effect in NIH/Ola Mice but Not in C3H/HeJ and BALB/c Mice

Figures 1 and 2 show results and statistics from experiments 1 and 2, respectively. In NIH/Ola mice, injections with PSP plus OVA gave higher levels of allergen-specific IgE and IgG2a compared to injections with OVA alone (fig. 1a, b, 2a, b). Also, the levels of total IgE were higher in NIH/Ola mice given PSP plus OVA compared to mice given OVA alone (fig. 1c, 2c). In both groups, the levels of total IgG2a were low and, if anything, tended to be lower in mice given PSP plus OVA compared to mice given OVA alone (fig. 1d, 2d).

C3H/HeJ and BALB/c mice showed a response pattern different from NIH/Ola mice since there were no significant differences in the levels of allergen-specific IgE and IgG2a (fig. 1a, b, 2a, b). Also in these mouse strains, the levels of total IgE tended to be higher in mice given PSP plus OVA compared to mice given OVA alone (fig. 1c, 2c), whereas the levels of total IgG2a tended to be lower (fig. 1d, 2d).

NIH/Ola and BALB/c mice given Al(OH)₃ plus OVA showed an antibody response pattern similar to that of mice given PSP plus OVA, except for the levels of IgG2a anti-OVA in BALB/c mice; BALB/c mice given Al(OH)₃ plus OVA had significantly higher levels of IgG2a anti-OVA than mice given OVA alone (results not shown).

The Presence of PSP in Both OVA Injections Gave Higher Levels of Anti-OVA IgE Than the Presence of PSP in the First Injection Only

NIH/Ola mice given two intraperitoneal injections with OVA produced only small amounts of IgE anti-OVA. Mice given PSP plus OVA in the first injection only tended to have higher levels of IgE-anti-OVA than mice given OVA alone (*p* = 0.121). However, mice given two injections with PSP plus OVA had significantly higher levels of allergen-specific IgE compared to mice given two injections with OVA or mice given one injection with PSP plus OVA and then one injection with OVA alone (OVA: *p* ≤ 0.001; PSP + OVA: *p* = 0.010) (results not shown).

PSP Had an Adjuvant Effect in Mice That Were Weakly Pre-Sensitised to OVA, but Not in Strongly Pre-Sensitised Mice

When given a challenge with PSP plus OVA, weakly pre-sensitised NIH/Ola mice had significantly higher levels of allergen-specific IgE and IgG2a, and total IgE com-

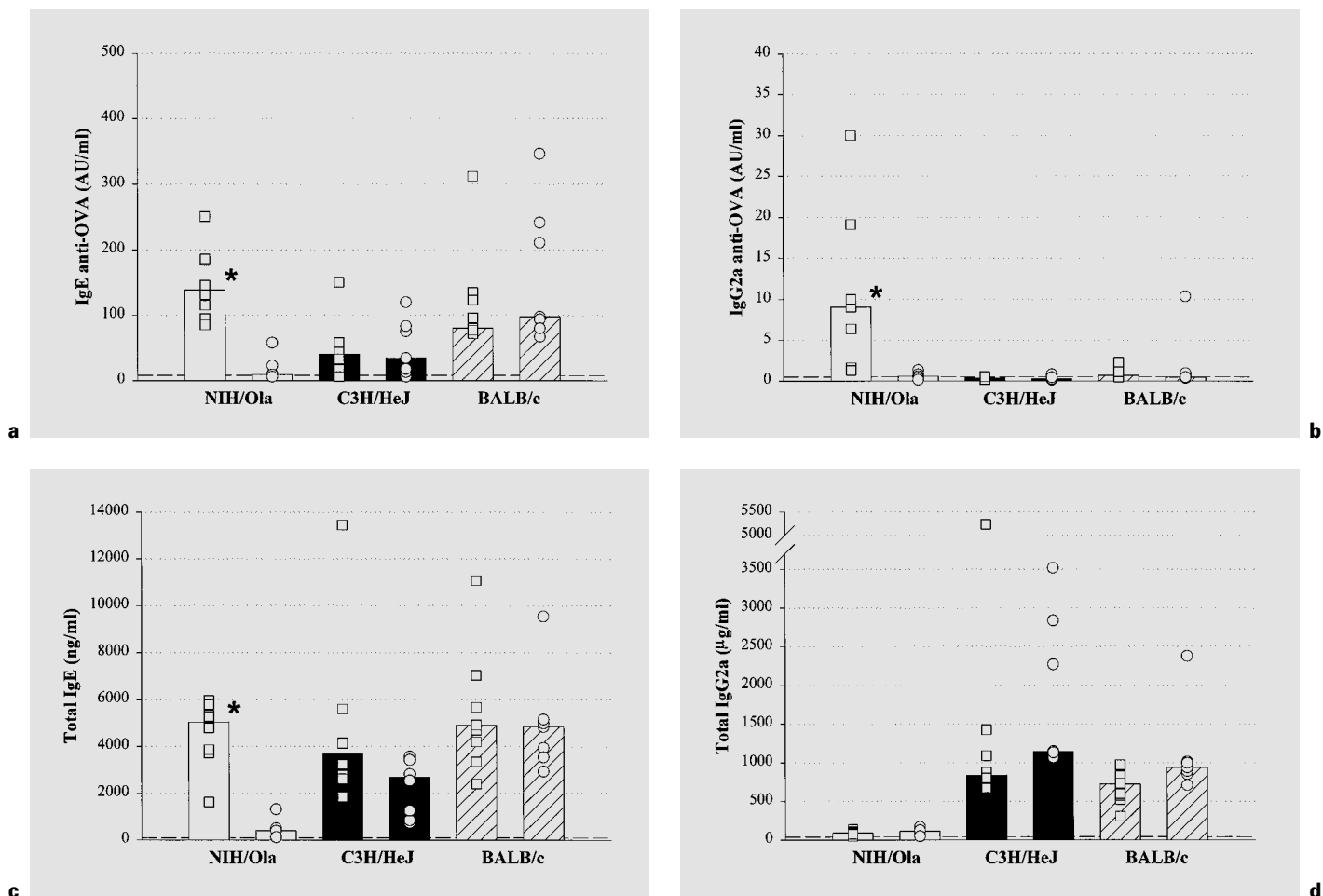


Fig. 1. The levels of IgE anti-OVA (**a**), IgG2a anti-OVA (**b**), total IgE (**c**) and total IgG2a (**d**) in NIH/Ola, C3H/HeJ and BALB/c mice given PSP+OVA (□) or OVA alone (○) (experiment 1). Individual (symbols) and median (columns) values for each group of 8–9 mice each are shown. A dotted black line denotes the detection limit of the different ELISA's. A statistically significant difference ($p \leq 0.050$) is

denoted by (*) (see details below). IgE anti-OVA – NIH/Ola: $p \leq 0.001$; C3H/HeJ: $p = 0.955$; BALB/c: $p = 0.333$. IgG2a anti-OVA – NIH/Ola: $p = 0.002$; C3H/HeJ: $p = 0.830$; BALB/c: $p = 0.295$. Total IgE – NIH/Ola: $p \leq 0.001$; C3H/HeJ: $p = 0.105$; BALB/c: $p = 0.832$. Total IgG2a – NIH/Ola: $p = 0.293$; C3H/HeJ: $p = 0.065$; BALB/c: $p = 0.059$.

pared to mice given OVA alone (IgE anti-OVA: $p = 0.001$; IgG2a anti-OVA: $p = 0.049$; total IgE: $p = 0.007$), whereas there were no differences in the levels of total IgG2a ($p = 0.127$). In contrast, the difference in the antibody levels in strongly pre-sensitised NIH/Ola mice given PSP plus OVA compared to mice given OVA alone was not statistically significant (IgE anti-OVA: $p = 0.272$; IgG2a anti-OVA: $p = 0.798$; total IgE: $p = 0.721$; total IgG2a: $p = 0.353$). Figure 3 shows the levels of IgE anti-OVA and total IgE.

Discussion

The genetic background seems to be important for the propensity of an individual to develop allergic immune responses [20–22]. In our model, NIH/Ola, C3H/HeJ and BALB/c mice appeared to be low, low-intermediate and high IgE responders, respectively, to OVA given without any adjuvant (fig. 1).

In NIH/Ola mice, PSP increased the levels of both allergen-specific IgE and IgG2a, and total IgE, whereas the levels of total IgG2a tended to be lower in mice given PSP plus OVA than in mice given OVA alone. The antibody-adjuvant effect of PSP was more pronounced when

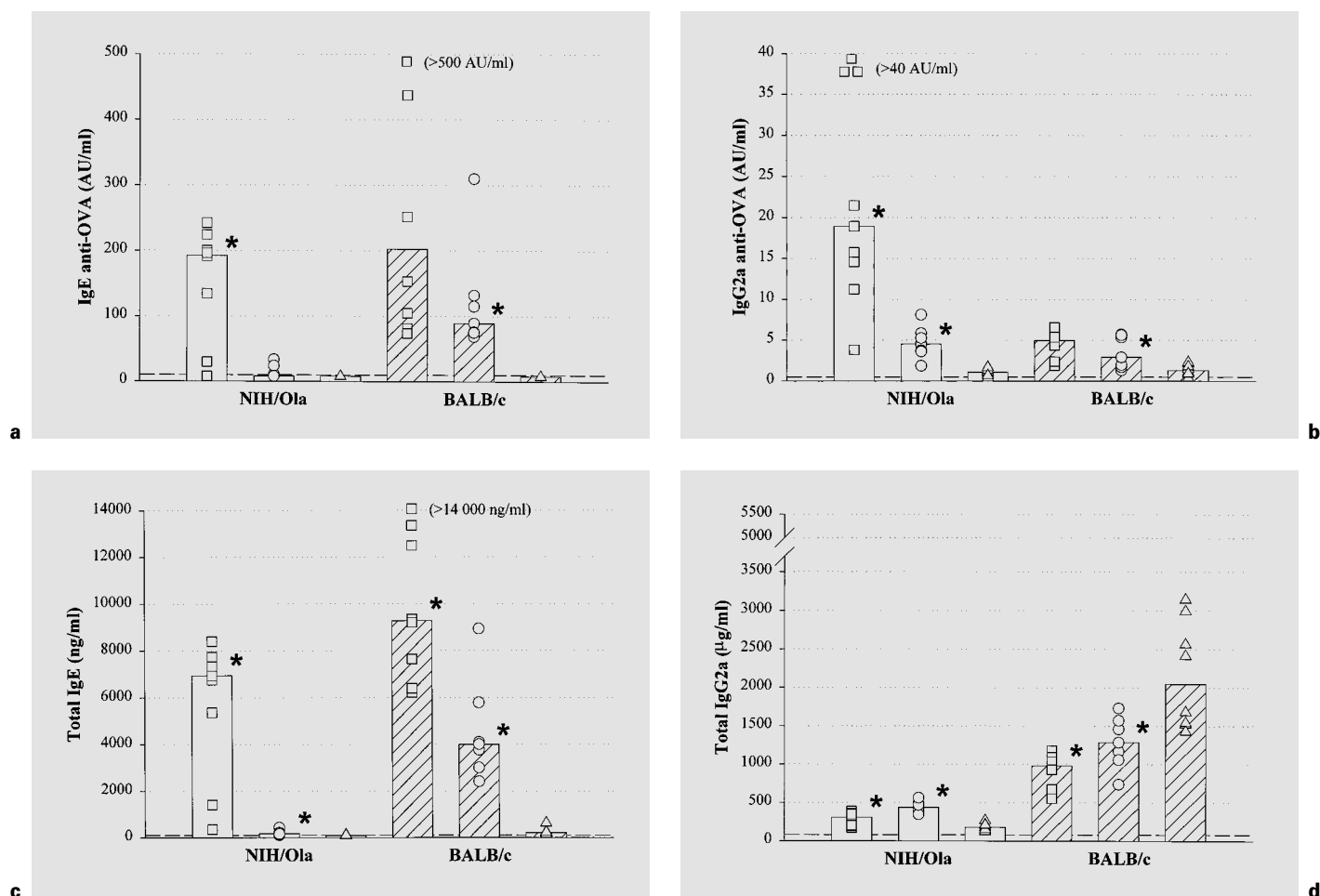


Fig. 2. The levels of IgE anti-OVA (**a**), IgG2a anti-OVA (**b**), total IgE (**c**) and total IgG2a (**d**) in NIH/Ola and BALB/c mice given PSP+OVA (□) or OVA (○) alone. One group of mice from each strain was left untreated (△). Individual (symbols) and median (columns) values for each group of 8–9 mice are shown. A dotted black line denotes the detection limit of the different ELISA's. A statistically significant difference ($p \leq 0.050$) is denoted by (*) (see details below). IgE anti-OVA – NIH/Ola: PSP + OVA vs. OVA $p = 0.005$, OVA vs. untreated $p = 0.234$; BALB/c: PSP + OVA vs. OVA $p =$

0.096, OVA vs. untreated $p \leq 0.001$. IgG2a anti-OVA – NIH/Ola: PSP + OVA vs. OVA $p = 0.003$, OVA vs. untreated $p \leq 0.001$; BALB/c: PSP + OVA vs. OVA $p = 0.290$, OVA vs. untreated $p = 0.029$. Total IgE – NIH/Ola: PSP + OVA vs. OVA $p = 0.001$, OVA vs. untreated $p = 0.003$; BALB/c: PSP + OVA vs. OVA $p = 0.003$, OVA vs. untreated $p \leq 0.001$. Total IgG2a – NIH/Ola: PSP + OVA vs. OVA $p \leq 0.001$, OVA vs. untreated $p \leq 0.001$; BALB/c: PSP + OVA vs. OVA $p = 0.019$, OVA vs. untreated $p = 0.010$.

PSP were present during allergen re-exposure, in that two injections of PSP plus OVA gave higher levels of IgE anti-OVA than one injection of PSP plus OVA followed by a second injection of OVA alone. In C3H/HeJ and BALB/c mice, PSP did not significantly alter the levels of allergen-specific IgE and IgG2a. However, in experiment 2, the levels of allergen-specific and total IgE tended to be higher in BALB/c mice given PSP plus OVA compared to mice given OVA alone. This had also been found in a previous study using BALB/c mice [unpubl. obs.]. These results

suggest that PSP can have a weak IgE-adjuvant effect in BALB/c mice. Our results indicate that particles may increase the probability of sensitisation to an allergen in individuals susceptible to the effect of particles.

It can be hypothesised that it is the level of systemic IgE antibodies, and not genetics, that determines the different magnitude of the antibody responses in NIH/Ola and BALB/c mice after exposure to particles. The experiment with strongly and weakly pre-sensitised NIH/Ola mice lends support to this view. Strongly pre-sensitised

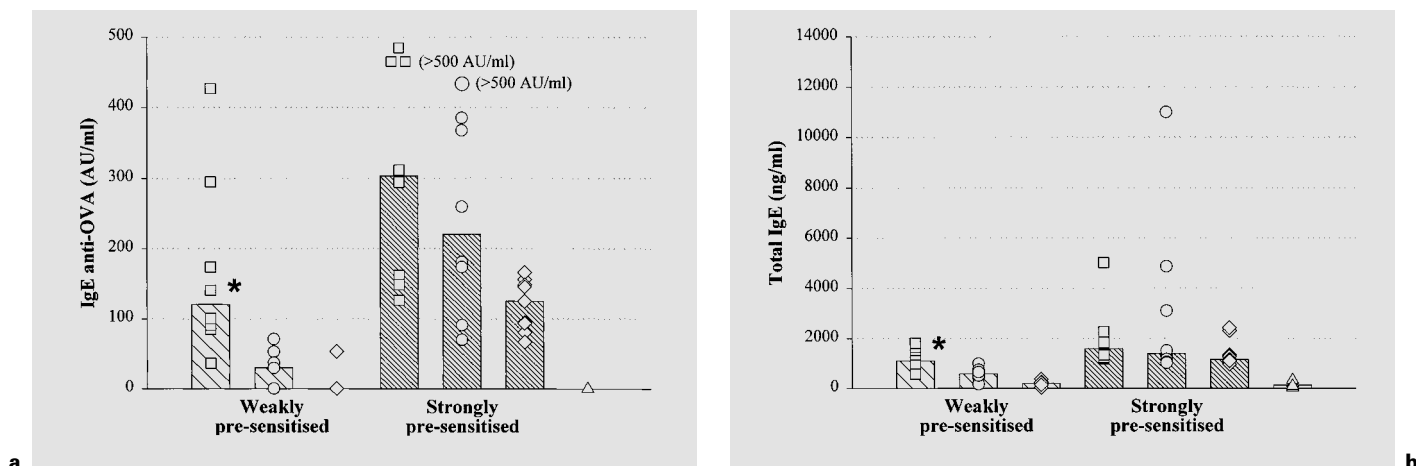


Fig. 3. Levels of allergen-specific (a) and total IgE (b), respectively, in weakly and strongly pre-sensitised NIH/Ola mice. Groups of weakly and strongly pre-sensitised mice were challenged with PSP + OVA (□), OVA alone (○) or PSP alone (◇) on day 42, whereas one group of mice was left untreated at all times (△). Individual (sym-

bols) and median (columns) values for each group of 7–8 mice are shown. A statistically significant difference ($p \leq 0.050$) is denoted by (*) (see details below). IgE anti-OVA – weak: $p = 0.001$; strong: $p = 0.272$. Total IgE – weak: $p = 0.007$; strong: $p = 0.721$.

NIH/Ola mice behaved as BALB/c mice when given a challenge with OVA plus PSP, in that there was no statistically significant increase in specific IgE levels compared to mice challenged with OVA alone. Weakly pre-sensitised NIH/Ola mice, in contrast, showed a statistically significant increase in the levels of allergen-specific IgE when given PSP plus OVA (fig. 3). These results suggest that particles may have less effect on the production of IgE in individuals with a high, genetic or induced, IgE responder status compared to individuals with a low IgE responder status.

C3H/HeJ mice showed a different pattern of response compared to naive and weakly pre-sensitised NIH/Ola mice. In spite of a low to intermediate response to OVA without PSP, C3H/HeJ mice did not show any adjuvant effect after exposure to PSP plus OVA. We cannot draw any conclusion from our study as to whether C3H/HeJ mice are 'resistant' IgE low-intermediate responders to OVA, or whether they may have a genetically determined lack of responsiveness to the adjuvant effect of particles. However, additional experiments using other particles than PSP and other allergens than OVA may reveal whether the C3H/HeJ mouse is unresponsive to adjuvant effects of particles.

In conclusion, our results indicate that the allergen responder status, either genetic or induced, may be important for the effect of environmental adjuvants such as particles. In addition, the adjuvant effect on the produc-

tion of allergen-specific antibodies may be dependent on putative 'adjuvant genes', that is particles may have a strong adjuvant activity only in certain individuals that are susceptible to the influence of particles. The genes in question may determine, for example, the cytokine response to irritative stimuli in general, or may act more specifically on the cellular response to particles. Regardless of the mechanisms involved, our results suggest that humans may differ in their susceptibility to the allergy-promoting effects of particles.

Acknowledgment

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 B.G. The authors acknowledge Else-Carin Groeng and Rita-Bente Leikvold for expert technical assistance in the laboratory. They also acknowledge Trude Olsen, Kari G. Løken, Mona Iren Skullerud and Victor Ong for technical assistance with the animals.

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