

Effect of diesel exhaust particles and their components on the allergen-specific IgE and IgG1 response in mice

Yong Heo ^{a,1}, Andrew Saxon ^b, Oliver Hankinson ^{a,*}

^a *Department of Pathology and Laboratory Medicine and Jonsson Comprehensive Cancer Center, UCLA School of Medicine, University of California, Los Angeles, CA 90095-1680, USA*

^b *The Hart and Louise Lyon Laboratory Division of Clinical Immunology/Allergy, Department of Medicine, UCLA School of Medicine, University of California, Los Angeles, CA 90095-1680, USA*

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Abstract

Increased antigen-specific IgE expression is a hallmark of the allergic response in mice. IgG1 may also be involved. Co-injection of mice with diesel exhaust particles (DEP) and ovalbumin three times over a 2 week period lead to a rapid and marked elevation of ovalbumin-specific IgE, IgG1 and also IgG2a, compared with ovalbumin alone. When DEP were injected 1 day before or after ovalbumin on each occasion, their adjuvant effect was considerably muted, suggesting that the adjuvant effect of DEP is short-lived, or that a physical interaction between ovalbumin and DEP is required. DEP were extracted with methylene chloride. Both the resulting core carbon particles and the organic extract enhanced ovalbumin specific IgE and IgG1 levels. Thus the adjuvant effect of DEP in this model is due both to the physical and the chemical attributes of the particles. The tricyclic hydrocarbons phenanthrene (the most prevalent polycyclic aromatic hydrocarbon in DEP) and anthracene were both capable of enhancing antigen-specific IgE and IgG1 production. The phenolic antioxidant, butylated hydroxyanisole, which can affect gene expression via the antioxidant responsive element (ARE), had a lesser effect. Two agonists for the aryl hydrocarbon receptor, 3-methylchloranthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, either were without effect or suppressed the response, suggesting that DEP adjuvancy may not be mediated by this receptor. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

There has been a dramatic increase in the incidence of asthma over the last century, concurrent with the expansion of industrialization (Ishizaki et al., 1987; Corbo et al., 1993). A considerable amount of epidemiological evidence suggests that diesel exhaust particles have contributed towards

* Corresponding author. Tel.: +1-310-8252936; fax: +1-310-7949272.

E-mail addresses: yongheo@hotmail.com (Y. Heo), ohank@mednet.ucla.edu (O. Hankinson).

¹ Present address: Institute for Occupational and Environmental Health, 527-44 Inchang-Dong, Kuri-City, South Korea. Tel.: 82-31-5501065.

this increase (Nitta et al., 1993; Devalia et al., 1994; English et al., 1999). DEP have also been shown to exacerbate allergic airway disease in mice (reviewed in Takafuji et al., 1989; Nel et al., 1998). One of the hallmarks of allergic airway disease is the elevation of allergen-specific IgE. Previous studies have demonstrated DEP's adjuvant effect on IgE production utilizing inhalation (Ichinose et al., 1998; Takano et al., 1998), footpad injection (Løvik et al., 1997), intratracheal (Fujimaki et al., 1994; Ichinose et al., 1997) or intranasal instillation (Fujimaki et al., 1995; Maejima et al., 1997) or intraperitoneal injection (Muranaka et al., 1986; Suzuki et al., 1993) as a route of allergen or DEP administration. DEP consist of core carbon particles together with a complex mixture of organic compounds absorbed to the particles (Williams, 1982). Results of previous studies indicate that both the core particles and the absorbed chemicals contribute to the adjuvant effect of DEP on the allergic response in mice (Maejima et al., 1997; Granum et al., 1999). However, the mechanisms of DEP adjuvancy are unresolved, and in particular, the DEP-associated chemicals responsible for adjuvancy have not been identified (reviewed by Takafuji et al., 1989; Nel et al., 1998). The objectives of the current study were to develop a mouse model in which an enhancement of allergen-specific IgE (and IgG1) occurs rapidly after DEP treatment, and to use this model to begin to identify the component(s) of DEP responsible for their adjuvant effect on allergic responses.

2. Methods

2.1. Mice

Five- to six-week-old female BDF1 mice (F1 progeny from the cross between C57BL/6J and DBA/2 mice) weighing 16–18 g were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were housed in filter-topped cages under standard laboratory conditions ($23 \pm 2^\circ\text{C}$ room temperature, 60% relative humidity, and 12 h light/dark cycle), and maintained on autoclaved food and acidified water. The number of

mice used per treatment are given in the figure legends.

2.2. Reagents

Chicken egg ovalbumin (OVA, Sigma, St. Louis, MO) was prepared in physiological saline. Imject[®] Alum, an aqueous solution of aluminum hydroxide and magnesium hydroxide, was purchased from Pierce (Rockford, IL) and used according to the company's instructions. Mouse IgG was obtained from Zymed, South San Francisco, CA, IgE specific for trinitrophenol-keyhole limpet hemocyanin from Pharmingen (San Diego, CA), and amylase from Calbiochem, (San Diego, CA). The diesel exhaust particles were a generous gift from Dr. Masaru Sagai (National Institute for Environmental Studies, Japan). The DEP in this study were generated by a light-duty four-cylinder diesel engine (4JB1 type, Isuzu Automobile Co., Japan) using standard diesel fuel and obtained as previously described (Takenaka et al., 1995; Tsien et al., 1997). DEP were stored in a liquid nitrogen freezer. To expose animals to DEP, DEP were suspended in physiological saline containing 5% BDF1 mice serum on a magnetic stirrer at 4°C overnight, followed by sonication for 3 min on ice. Inclusion of mouse serum in the DEP suspension has been reported to greatly enhance uniform dispersion of the hydrophobic diesel exhaust particles (Løvik et al., 1997). To prepare extracts of extractable material from DEP (DEP-EX), 500 mg DEP were Soxhlet extracted with 200 ml methylene chloride (Sigma) for 18 h (Li et al., 1996), then the methylene chloride extract was concentrated under nitrogen gas. The resulting extract (108 mg in 1350 μl methylene chloride) was aliquoted and stored in a -20°C freezer. The percentage of methylene chloride extractable mass was determined by weighing the non-dissolved residue and was calculated to be 21.6%. The amounts of the major aromatic hydrocarbon constituents of the extract were determined by gas chromatography-mass spectroscopy. The non-extractable DEP mass was stored at 4°C . Phenanthrene, butylated hydroxyanisole (BHA), 3-methylcholanthrene (3MC), and anthracene were purchased from Sigma and dissolved in

methylene chloride at 500, 715, 26, and 87 mg/ml, respectively. Acenaphthylene was purchased from Aldrich Chemical Co. and dissolved in 333 mg/ml methylene chloride. The polycyclic aromatic hydrocarbons (PAH) stock solutions or DEP extract were further diluted with physiological saline containing 5% BDF1 mice serum for injection into mice. The diluted DEP-EX or PAH solutions were thoroughly mixed with mild heating for 1 h on a magnetic stirrer to evaporate trace amount of methylene chloride in the solution, then sonicated for 3 min on ice. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, >98% pure, from Wellington Laboratories, Ontario, Canada) was prepared at 10^{-4} M in acetone and this solution was added to corn oil and mixed as described above.

2.3. Immunization and DEP and related PAH exposure

The model of antigen immunization with DEP and its related PAH exposure was adopted from that described previously (Muranaka et al., 1986) with modifications. Mice were injected on three occasions at weekly intervals through the intraperitoneal (i.p.) route with 200 μ l physiological saline containing OVA with or without an adjuvant (45 μ l Imject[®] Alum containing 2 mg aluminum hydroxide). Some mice were exposed to DEP (2 mg), DEP-EX (5 μ l corresponding to 0.4 mg), or insoluble DEP (1.6 mg) in a total volume of 200 μ l of physiological saline containing 5% BDF1 mice serum in the presence or absence of 50 μ g OVA. The amount of DEP-EX and insoluble DEP was determined in accordance with the proportion of each component in the total DEP mass. Phenanthrene, BHA, 3MC, anthracene, or acenaphthylene mixed with or without 50 μ g OVA was injected i.p. into mice in a total volume of 200 μ l physiological saline containing 5% BDF1 mice serum, and the doses were 300, 15, 80, 300, and 300 mg/kg body weight, respectively. TCDD (20 μ g/kg body weight) mixed with or without 50 μ g OVA in 200 μ l total volume for an injection was given i.p. Mice were treated with the above compounds in the presence of OVA three times at weekly intervals. Blood was collected through periorbital bleeding at 2 days

before and 13 and 28 days after the first immunization. At day 56, mice were sacrificed and blood was collected by cardiac puncture. Sera were obtained by centrifugation and stored in a -80°C freezer until use.

2.4. Measurement of OVA-specific IgE level in mouse sera

To determine the serum level of OVA-specific IgE, we developed a sandwich ELISA method with high sensitivity and specificity using a commercial IgE capture antibody (Biosource, Camarillo, CA). Briefly, Immulon 2[®] microplates (Dynatech, Chantilly, VA) were coated with purified rat *anti*-mouse IgE capture Ab (100 μ l/well; 2 μ g/ml) by overnight incubation at 4°C . The plates were washed three times and, after a 2 h incubation with PBS containing 0.05% Tween 20 and 1% bovine serum albumin (Sigma), diluted serum samples or standard sera were added to each well. After overnight incubation at 4°C , the plates were washed and biotinylated OVA (100 μ g/well; 1.5 μ g/ml) was added and incubated at room temperature for 2 h. The plates were washed and further incubated with avidin-peroxidase (Sigma) for 1 h before detection using the substrate, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (Sigma). Absorbance was measured at 405 nm with an ELISA reader (Bio-Tek EL800, Winooski, VT). The standard serum from BDF1 mice was obtained from mice i.p. injected with OVA (50 μ g/injection) mixed with alum adjuvant weekly for 2 weeks. Blood was collected by cardiac puncture at Day 56 and serum was separated by centrifugation. The efficiency of the sandwich ELISA method for OVA-specific IgE was confirmed in various ways. A dose-response relationship ($R^2 = 0.997$) was obtained between optical density levels and the dilution titer of the OVA-IgE positive standard serum. No positive reaction was observed in the absence of capture Ab or biotinylated OVA. No cross-reactivity with IgG, the most predominant immunoglobulin isotype in serum, was found when mouse IgG serially diluted from 1.6 mg/ml to 6.25 ng/ml was applied to the system. Cross-reaction with unrelated IgE such as mouse IgE specific for trinitrophenol-key-

hole limpet hemocyanin or in serum from unimmunized BDF1 mice was also negligible. Serum from BDF1 mice immunized with α -amylase, which demonstrated an elevated level of IgE after 14 days of the immunization, did not give any positive reaction under the ELISA system using biotinylated OVA instead of biotinylated IgE as a detection reagent (data not shown). Amylase has been reported to induce production of antigen-specific IgE Ab (Hilton et al., 1994). Overall, the ELISA system used in this paper is a sensitive *in vitro* tool for determining serum levels of OVA-specific IgE. This is important since IgE levels should be extremely low in comparison with those of OVA-specific IgG after immunization of mice with OVA (Hessel et al., 1995). The amounts of OVA-IgE were expressed in arbitrary units and calculated from the linear portion of the standard curves. The standard serum diluted to 1/200 was defined to contain 100 units of OVA-IgE/ml.

2.5. Measurement of OVA-specific IgG1 and IgG2a level in sera

Measurement of IgG isotypes of anti-OVA (IgG1 and IgG2a) in serum was performed by ELISA (Heo et al., 1997). In brief, OVA (20 μ g/ml) was coated onto Immulon 2[®] microplates overnight at 4°C and then washed. After a 2 h incubation with PBS containing 0.05% Tween 20 and 1% bovine serum albumin, diluted serum samples or standard sera were added to each well and incubated overnight at 4°C. After washing, biotinylated detecting mAbs (100 μ l/well; 1 μ g/ml) were added and incubated at room temperature for 2 h. The plates were washed and further incubated with avidin-peroxidase for 1 h before detection using the 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) substrate. Absorbance was measured at 405 nm with the ELISA reader. The amounts of OVA-IgG1 or IgG2a were expressed in arbitrary units and calculated from the linear portion of the standard curves. The standard serum is the same serum that was used for OVA-IgE determination. The standard serum diluted to 1/20 000 and 1/200 was defined to contain 100 units of OVA-IgG1 and OVA-IgG2a/ml, respectively.

2.6. ELISA for total IgE, IgG1, and IgG2a quantification

Quantification of serum IgG isotypes was assayed by isotype specific ELISA using goat anti-mouse IgG1 or IgG2a and peroxidase-labeled goat anti-mouse IgG (Sigma), according to the manufacturer's instructions. The sensitivity of the assay was 5 ng/ml for IgG1 and IgG2a. Levels of serum IgE were determined as previously described (Heo et al., 1996) with the mAb pair of R35-72 and R35-92 supplied by PharMingen (San Diego, CA). The lower limit of detection was 5 ng/ml.

2.7. Statistical analyses

Data were initially evaluated for normal distribution. Statistical significances among groups were tested using Microsoft Excel[®] by single-factor ANOVA and Dunnett's *t*-test or Kruskal–Wallis ANOVA and Dunn's test, depending on normality of data. The significances were further confirmed by Student's *t*-test or the Mann–Whitney test. Differences were considered significant when *P* was less than 0.05.

3. Results

3.1. Adjuvant effect of DEP on antigen-specific IgE production

We first set out to establish a protocol in which DEP would elicit a robust and rapid adjuvant effect on antigen-specific IgE production. For these studies 6 week old female hybrids between the C57BL/6 and DBA/2 mouse strains (i.e. BDF1 mice) were injected with a mixture of OVA and DEP three times at one week intervals. We tested three doses of OVA. In general, the results obtained were similar at all OVA doses. Treatment with OVA and DEP did not affect body weight gain for 2 months following the initial injection (data not shown). The mice exhibited a strong adjuvant effect for DEP on ovalbumin-specific IgE within one month (Fig. 1). An adjuvant effect of DEP on OVA-IgE production became

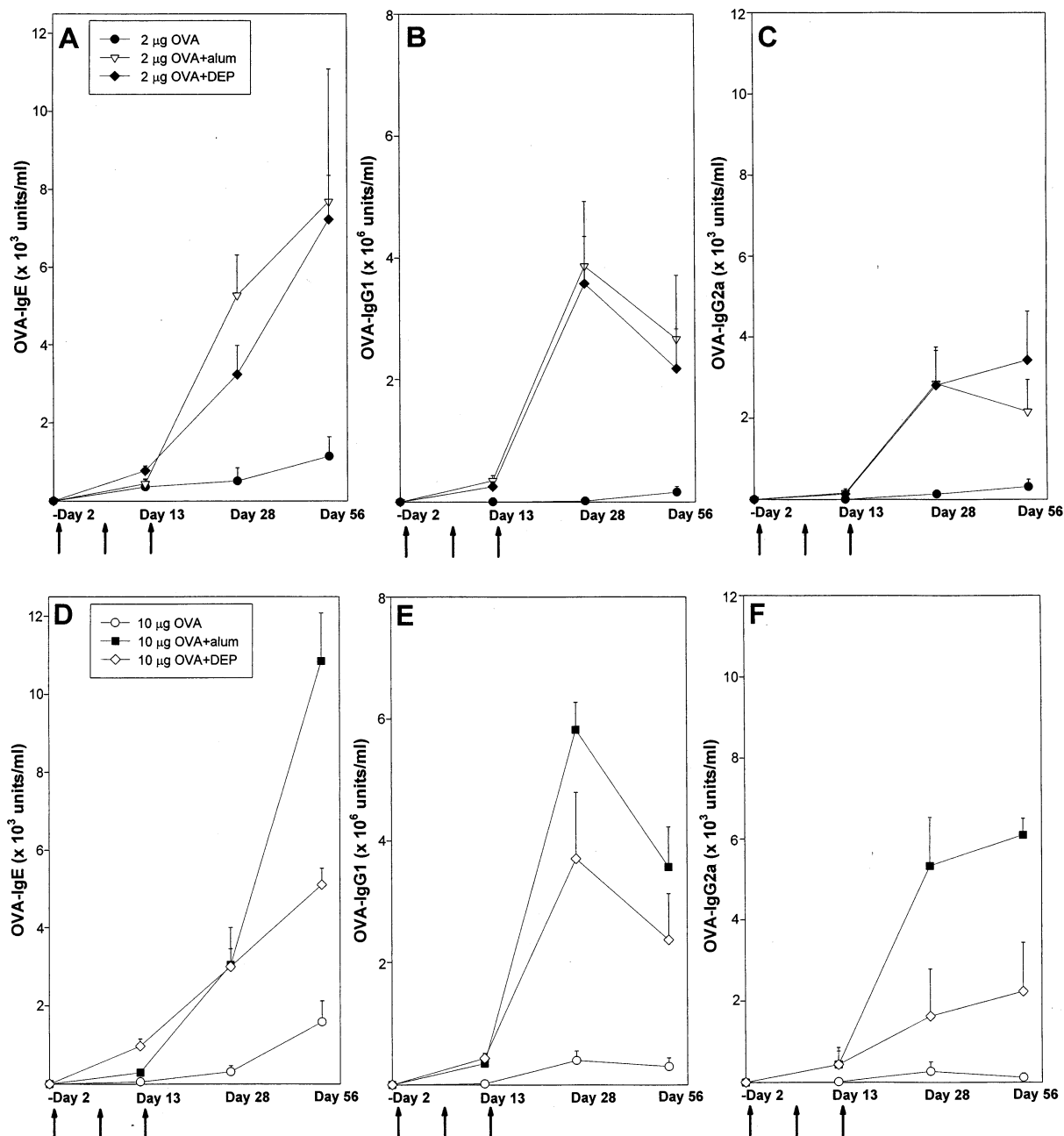


Fig. 1. Adjuvant effect of DEP on OVA-specific IgE, IgG1, and IgG2a production in BDF1 mice. Mice were i.p. immunized on days 0, 7 and 14 (pointed with arrows under the figure) with 2, 10 or 50 µg OVA with or without 2 mg DEP or 2 mg alum for 2 weeks. Sera were collected before (day 2) and at three time points (days 13, 28, and 56) after the first immunization. OVA-specific Ab levels in the sera were determined by ELISA. The results are expressed as the means $\pm$ SD of two separate experiments for 5 mice per treatment.

evident as earlier as day 13 and was dramatically demonstrated at day 28 and day 56, in that serum levels of OVA-IgE in the mice administered with OVA and DEP were significantly ($P < 0.05$) higher (six to eightfold higher on days 28 and 56) than for mice immunized with OVA alone (Fig. 1A, D, G). The degree of the DEP-mediated adjuvant effect on OVA-IgE production was similar to that of alum, a well recognized adjuvant. We also evaluated the effect of DEP exposure on OVA-specific IgG1 and IgG2a synthesis. Levels of OVA-IgG1 in mice administered the OVA/DEP or OVA/alum mixtures were significantly ($P < 0.05$) higher than the mice immunized with OVA alone throughout the experimental period, and reached peaks at day 28 (Fig. 1B, E, H). DEP also enhanced OVA-IgG2a production indicating that the immune response elicited by DEP in our model is not totally polarized to a helper T cell type 2-like phenotype (Fig. 1C, F, I). DEP-mediated alterations in serum levels of total IgE, IgG1, and IgG2a were also evaluated (Fig. 2. Only data for the 50 μ g OVA dose are presented). Total IgE and IgG1 concentrations in the sera collected at Day 28 and Day 56 were significantly ($P < 0.05$)

higher in the OVA/DEP-administered mice than the mice treated with OVA or saline alone. No significant increase in total IgG2a occurred in OVA/DEP-administered mice compared with OVA-treated mice. These results indicate that DEP mixed with an allergen induce a strong adjuvant effect, resulting in upregulated generation of allergen-specific IgE and IgG1. 50 μ g OVA was considered to be optimal in our animal model and was used for further experiments.

3.2. Effect of splitting the DEP and OVA treatment on the IgE response

We tested whether a 24 h interval between DEP and OVA treatments affected DEP's adjuvant effect. BDF1 mice were grouped as follows; Group I was immunized with OVA alone. Group II was treated with OVA 24 h after each DEP exposure. Group III was exposed to DEP 24 h after each OVA immunization. Group IV was administered OVA and DEP together. OVA-specific IgE, IgG1, and IgG2a responses universally followed the following order: Group IV > Group

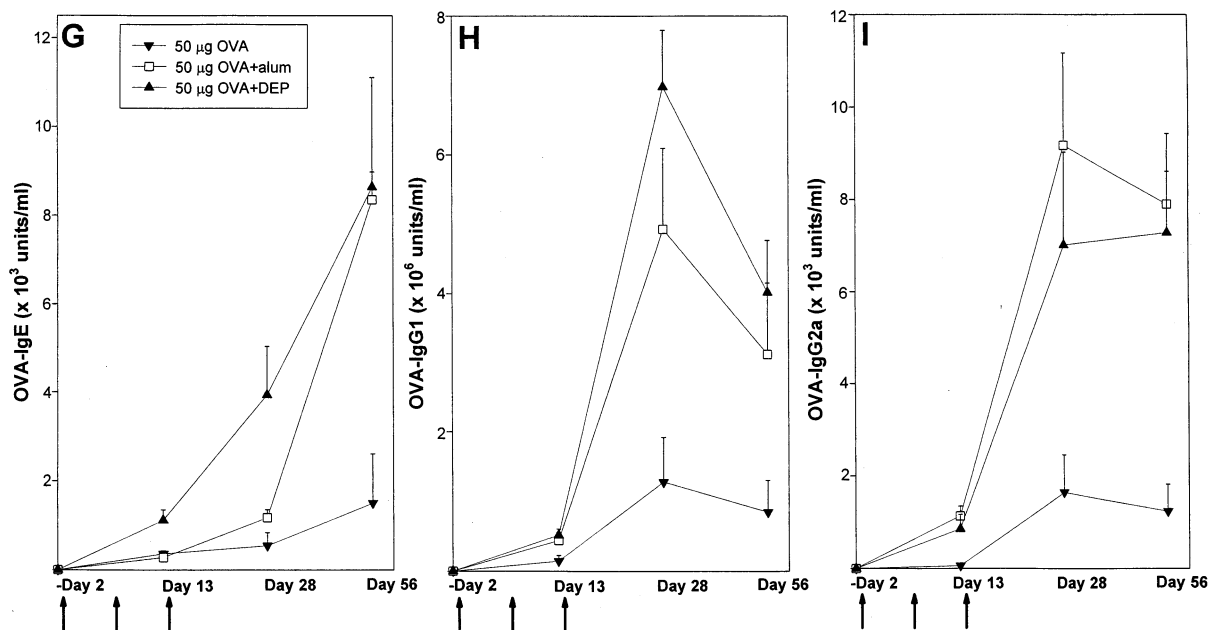


Fig. 1. (Continued)

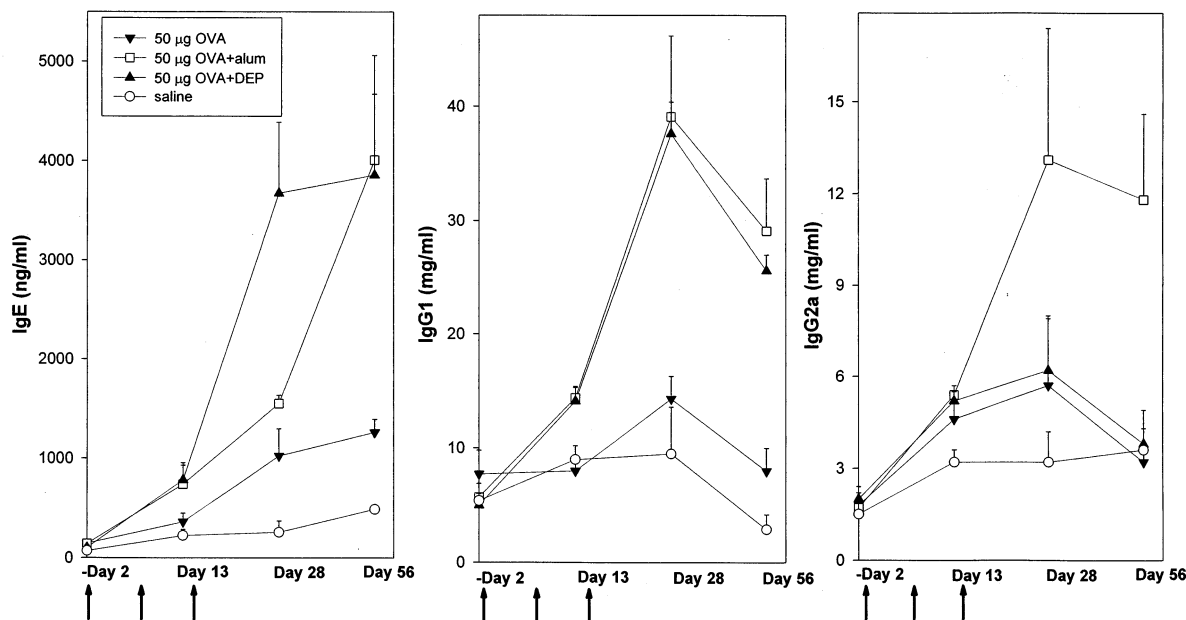


Fig. 2. Effect of DEP exposure on serum level of total IgE, IgG1, and IgG2a in BDF1 mice immunized with OVA. Serum levels of the immunoglobulins were determined for the mice treated with 50 µg OVA described in Fig. 1.

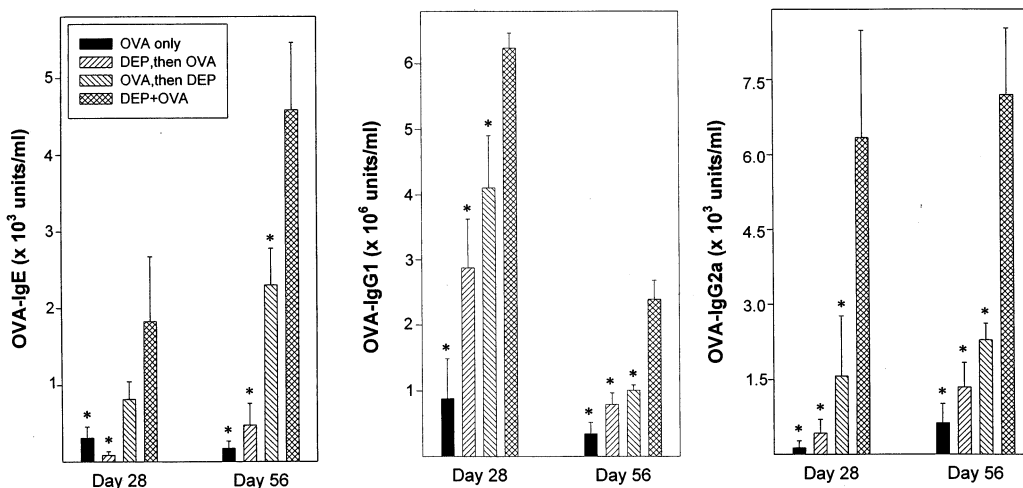


Fig. 3. Adjuvant effect of DEP on simultaneous administration of DEP and OVA compared with separate administration of DEP and OVA. Mice were injected i.p. on days 0, 7 and 14 with 50 µg OVA for 2 weeks. Two milligram DEP was administered 24 h before, simultaneously, or 24 h after OVA immunization on each occasion. The results are expressed as the means $\pm$ SD of two separate experiments for 5 mice per treatment. * ($P < 0.05$) Statistically significant difference from the simultaneous administration of OVA and DEP.

III > Group II > Group I (Fig. 3), indicating that co-administration of DEP and OVA is necessary

to obtain an optimal adjuvant effect by DEP. Furthermore, DEP was less efficacious when ap-

plied 24 h before OVA than when applied 24 h after.

3.3. Adjuvant effects of organic extract of DEP and the extracted core particles

We next investigated the relative contribution of the two major DEP components; namely the non-extractable ('inert') carbon particle core (insoluble DEP), and the methylene chloride extractable compounds absorbed to the core (DEP-EX), on the adjuvant effect of DEP. Phenanthrene (83 $\mu\text{g/g}$ DEP) was the the most dominant extractable component of the DEP-EX among 41 PAHs analyzed (data not shown), and its concentration was not much different from that of the standard reference DEP (SRM 1650; 79 μg phenanthrene/g DEP) from the United States National Institute of Standards and Technology. The doses of DEP-EX (0.4 mg) or insoluble DEP (1.6 mg) per injection were proportional to their amounts in 2 mg DEP. Treatment with DEP-EX or insoluble DEP did not affect body

weight gain for 2 months following the initial injection (data not shown). Both OVA-IgE and OVA-IgG1 levels in the sera obtained 13, 28, and 56 days after initiating the administration were significantly ($P < 0.01$) higher in the mice challenged with OVA plus insoluble DEP than the mice challenged with OVA alone. DEP-EX also increased the response to OVA, but to a lesser degree (Fig. 4). Total IgE and IgG1 levels were also significantly ($P < 0.01$) greater in mice treated with OVA plus insoluble DEP and to a lesser extent in mice treated with OVA plus DEP-EX ($P < 0.05$) in comparison to mice treated with OVA alone (Fig. 5). Interestingly, whereas DEP-EX produced a significantly ($P < 0.05$) elevated level of OVA-IgG2a compared to that in mice immunized with OVA alone, insoluble DEP had a much less marked adjuvant effect on OVA-IgG2a generation (Fig. 4). No significant change in total IgG2a level resulted from administration of insoluble DEP or DEP-EX in the presence of OVA (Fig. 5). Based on these results DEP-adjuvancy in our model of allergic antibody response is ascrib-

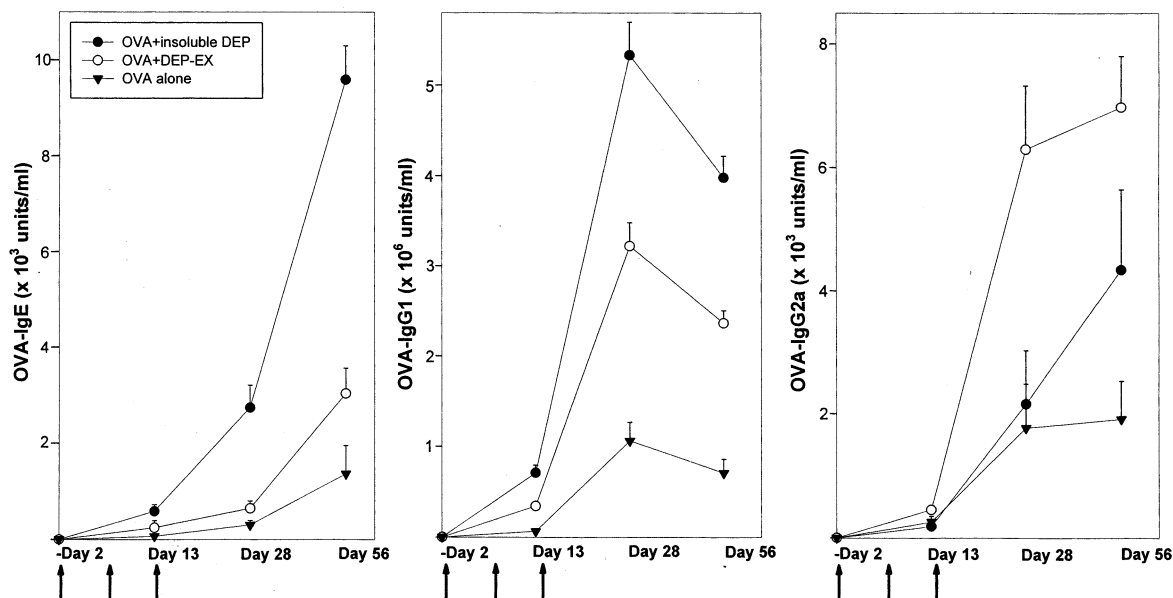


Fig. 4. Adjuvant effect of non-extractable DEP particles (insoluble DEP) and the extractable DEP fraction (DEP-EX) on OVA-specific IgE, IgG1 and IgG2a. Mice were i.p. exposed on days 0, 7 and 14 to 50 μg OVA or 0.4 mg DEP-EX or 1.6 mg insoluble DEP, together with 50 μg OVA. Sera were collected at the four different time points indicated before or after the first administration. The results are expressed as the means $\pm$ SD of three separate experiments for 8 mice per treatment.

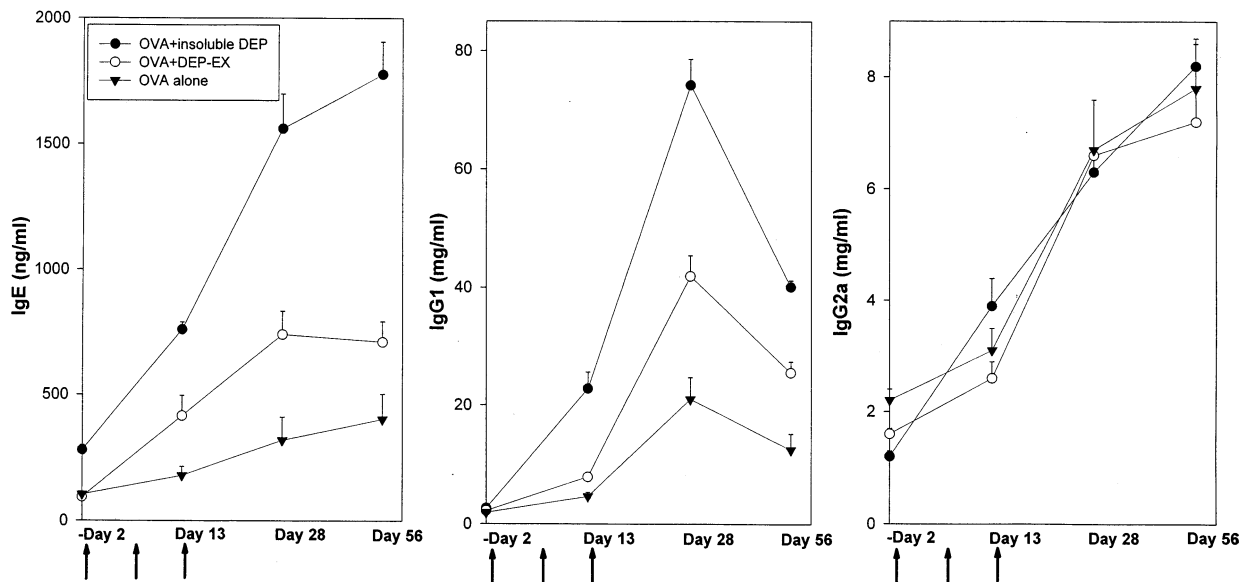


Fig. 5. Serum level of total IgE, IgG1, and IgG2a in BDF1 mice exposed to insoluble DEP and DEP-EX. Serum levels of the immunoglobulins were determined for the mice described in Fig. 4.

able to both the non-extractable DEP component, mostly composed of carbon particles, and the DEP extract, although the latter contributes to a lesser degree than the former to the DEP-induced adjuvant effect.

3.4. Adjuvant effect of certain polycyclic aromatic hydrocarbons and related compounds

We next investigated several model compounds for their potential adjuvant effects. We used these compounds at concentrations known to have a biological effect or at their maximal tolerated doses (see Section 4). Each compound was injected along with ovalbumin on days 0, 7 and 14. None of the treatments had an affect on total body weight gain for the duration of the experiment. Compared to OVA alone (vehicle control), phenanthrene and anthracene (see Section 2 and figure legends for doses used) both induced significantly more OVA-IgE and OVA-IgG1, and the response elicited was similar to that of DEP-EX (Fig. 6). These compounds also significantly ($P < 0.05$) increased total IgE and IgG1 levels (Fig. 7). BHA also significantly enhanced the OVA-specific

IgE and OVA-IgG1 responses at one time point. Neither 3-methylcholanthrene (3MC) nor acenaphthylene increased OVA-IgE or OVA-IgG1. Both compounds significantly suppressed OVA-IgG2a and total IgG2a production.

Finally we tested the effect of TCDD exposure on OVA-specific IgE, IgG1, and IgG2a production (Fig. 8), and total concentrations of IgE, IgG1, and IgG2a (Fig. 9). TCDD was dissolved and administered in corn oil. BDF1 mice were exposed i.p. to TCDD in the presence of 50 μ g OVA on three occasions at weekly intervals. This treatment did not affect total body weight gain for 2 months following the initial injection (data not shown). Mice treated with TCDD and OVA demonstrated significantly ($P < 0.05$) lower OVA-IgE and OVA-IgG1 serum levels in comparison to control mice immunized with OVA (dissolved in corn oil) or with OVA plus alum in the absence of TCDD (Fig. 8). When total IgE, IgG1, and IgG2a levels were compared between mice administered to TCDD plus OVA and mice immunized with OVA alone (Fig. 9), TCDD exposure resulted in downregulation of these immunoglobulin levels ($P < 0.05$).

4. Discussion

The present study was undertaken to gain insight into the component(s) of DEP which is (are) responsible for enhanced allergic inflammation. We first established a murine model in which DEP-induced adjuvant activities are appreciable within a relatively short period of observation. We chose BDF1 mice for these studies because these mice are known to exhibit a robust allergic response, to respond to DEP with an enhanced antigen-specific IgE response, and to possess a functional aryl hydrocarbon receptor system (Gonzalez et al., 1984; Muranaka et al., 1986). In addition to OVA-IgE production, we also measured OVA-IgG1 and OVA-IgG2a, since allergen-specific IgG1 has been shown to be important in induction of allergic airway hypersensitivity in mice (Oshiba et al., 1996), whereas IgG2a shows little relationship to the allergic response. Even though DEP have been shown to produce im-

munologic and pathologic changes linked to aggravation of respiratory allergic responses in animals (reviewed in Takafuji et al., 1989; Nel et al., 1998), it is not clear which constituent(s) of DEP contribute to the DEP-mediated immune alterations. Our further experiments were directed towards identifying which components/properties of DEP are responsible for their adjuvant effects.

We found that the insoluble DEP core particles induced a significantly greater adjuvant effect on allergen-specific IgE and IgG1 production compared with the soluble organic extract. Since an *in vivo* increase in allergen-specific IgE and IgG1 levels constitutes good serologic indicators for the development of the murine allergic response (Oshiba et al., 1996; Keane-Myers et al., 1998; Zuany-Amorim et al., 1998), our results suggest that the insoluble carbon core particles may make an important contribution to the DEP-mediated aggravation of allergic diseases in mice. This conclusion is consistent with the observation that

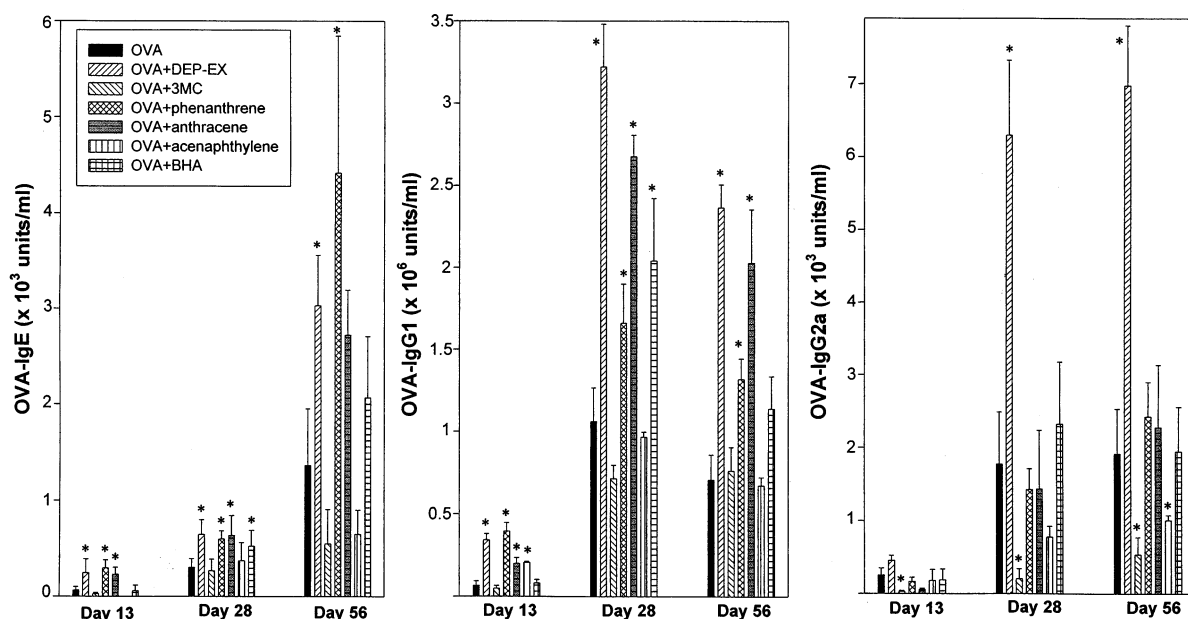


Fig. 6. Effects of various polycyclic aromatic hydrocarbons and related compounds on OVA-IgE, OVA-IgG1 and OVA-IgG2a levels. 3MC (80 mg/kg body weight), Phenanthrene (300 mg/kg body weight), BHA (15 mg/kg body weight), anthracene (300 mg/kg body weight), acenaphthylene (300 mg/kg body weight), or DEP-EX (5 μ l) mixed with 50 μ g OVA were injected three times on days 0, 7 and 14. The results are expressed as the means $\pm$ SD of two representative experiments for the pure compounds ($n = 6$) and three representative experiments for the DEP-EX and vehicle control ($n = 10$). * ($P < 0.05$) Statistically significant difference from the OVA alone (vehicle control).

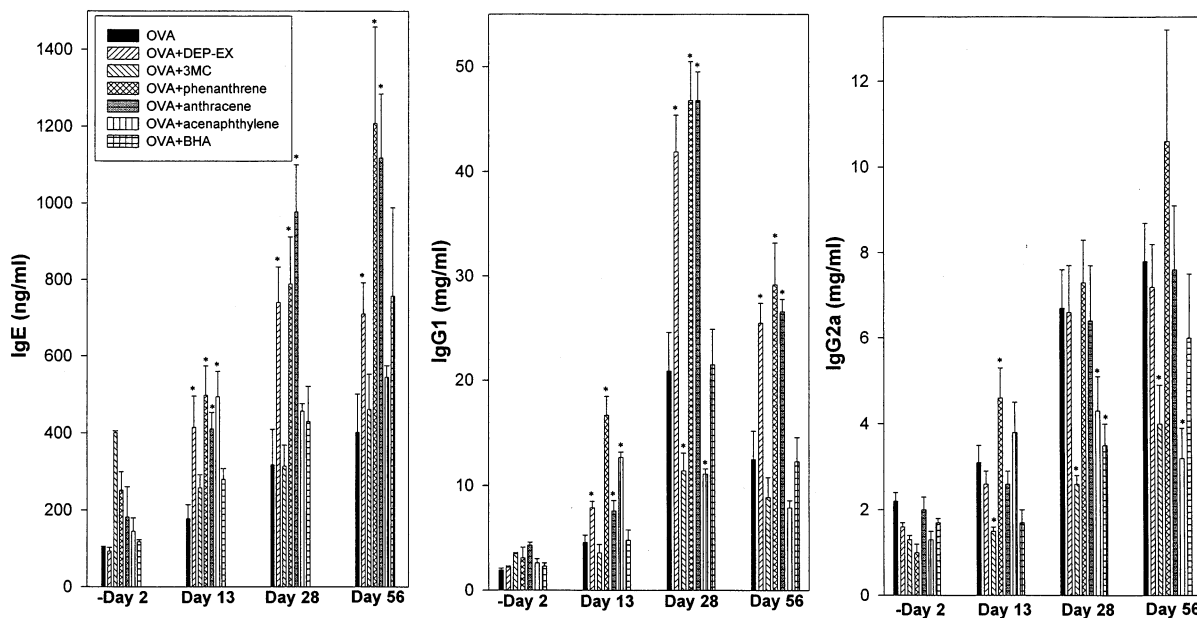


Fig. 7. Effects of various polycyclic aromatic hydrocarbons and related compounds on total Ig levels. Levels were measured in the same mice described in Fig. 6. * ($P < 0.05$) Statistically significant difference from the OVA alone (vehicle control).

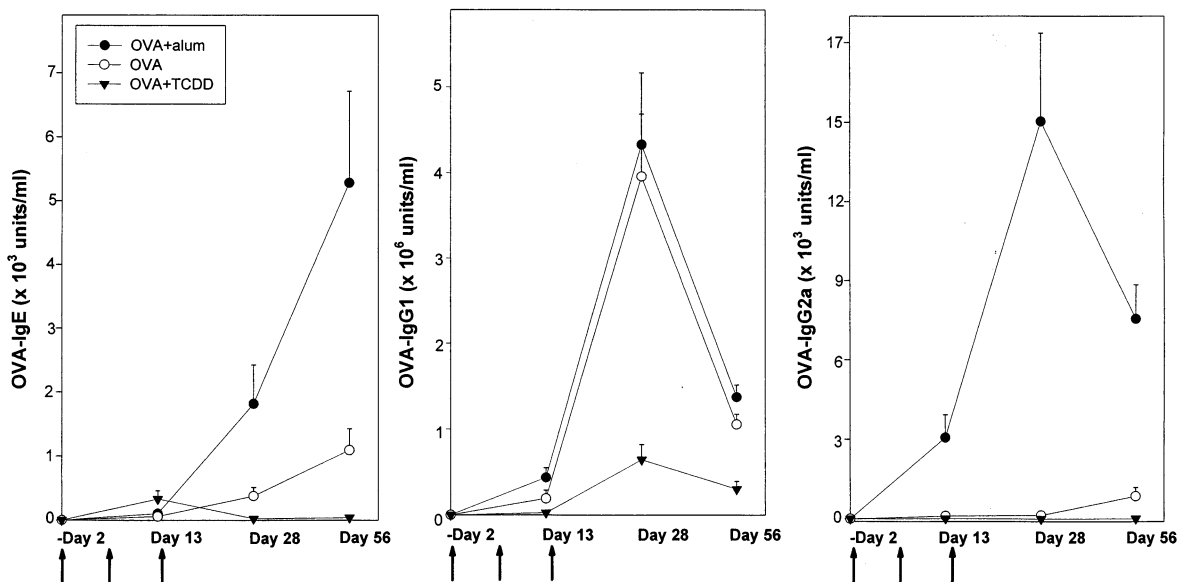


Fig. 8. Suppression of OVA-specific IgE and IgG1 by TCDD. OVA (50 μ g) dissolved in corn oil was injected i.p. on days 0, 7 and 14 in the presence or absence of TCDD (20 μ g/kg body weight). The total volume per injection was 200 μ l. Some mice were immunized with 50 μ g OVA plus 2 mg alum resuspended in 200 μ l physiological saline. The results are expressed as the means $\pm$ SD of two representative experiments for 6 mice per treatment.

artificial polystyrene particles with diameters smaller than 1 μm when injected intraperitoneally exhibit a strong adjuvant activity on OVA-specific IgE production in mice (Granum et al., 1999). In addition, carbon black, a surrogate for the non-extractable core of DEP, has been reported to have a significant adjuvant effect on OVA-specific IgE production after injection in BALB/c mice (Løvik et al., 1997). It is possible that allergen molecules adsorbed to carbon particle aggregates (Knox et al., 1997) within the DEP mass could be more efficiently carried into the phagocytic system, or may be much more slowly released into the surrounding microenvironment, resulting in a more persistent allergen exposure than allergen alone. Our observation that simultaneous administration of DEP and OVA elicited a stronger stimulatory effect on allergen-specific IgE and IgG1 compared with injecting DEP and OVA 24 h apart is consistent with the notion that a physical interaction between DEP and allergen contributes to the adjuvant effects of DEP.

The DEP organic extract (DEP-EX) demonstrated adjuvant activity in that it induced significantly elevated serum levels of OVA-IgE and

OVA-IgG1 compared to control mice immunized with OVA alone, although these effects were less than those elicited by the insoluble DEP core. This observation is in accordance with previous reports showing DEP-EX enhancement of IgE production from purified human B cells activated with IL-4 plus anti-CD40 mAb (Takenaka et al., 1995) or Epstein-Barr virus-transformed human B cells (Tsien et al., 1997). DEP-EX may exert its adjuvant effect through modulating the activity of several cell types participating in *in vivo* IgE and IgG1 production. The extract may upregulate IL-4 production from helper T cell type-2 or down-regulate IFN γ production from helper T cell-type 1, since IL-4 is known to induce switching to both IgE and IgG1 (Abbas et al., 1993) and IFN γ is regulatory for IL-4 activity (Snapper and Paul, 1987). This possibility is supported by previous observations of an increase in *in vitro* IL-4 production from cervical lymph node cells obtained from mice exposed to DEP and OVA through the intranasal route (Fujimaki et al., 1995). Furthermore, the DEP extract-induced enhancement of allergen-specific IgE and IgG1 production *in vivo* could promote activation of mast cells through

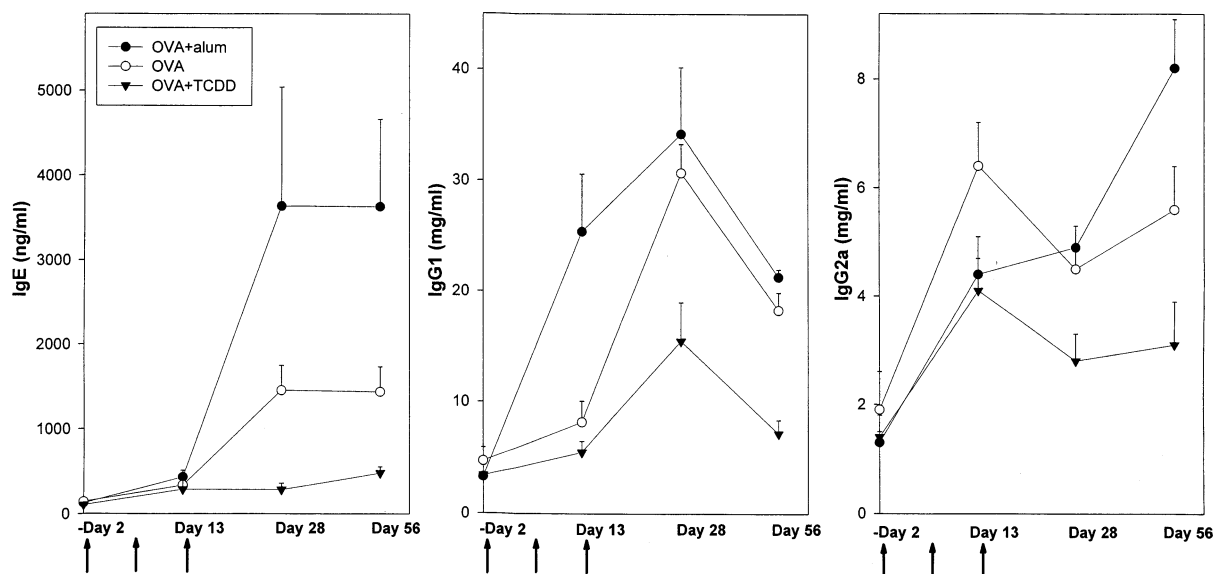


Fig. 9. Suppression of total immunoglobulin levels by TCDD. Serum levels of the immunoglobulins were determined for the mice described in Fig. 8.

their IgE and IgG1 receptors, respectively, followed by secretion of IL-4 (Daeron et al., 1980; Gordon et al., 1990). This mast cell IL-4 production will reciprocally trigger isotype-switching to IgE and IgG1 in B cells. Indeed we have shown in humans that mucosal challenge with DEP synergizes with allergen to drive basophils/mast cells' early IL-4 production and release. Furthermore, this early IL-4 release results in an increased polarization of the immune response toward an allergic phenotype (Diaz-Sanchez et al., 1997; Wang et al., 1999; Diaz-Sanchez et al., 2000).

We also investigated the effects of certain model compounds to gain insight into the mechanism(s) involved in the adjuvant effect of the chemicals present in DEP-EX. The tricyclic hydrocarbons, phenanthrene, anthracene, and acenaphthylene were each applied at a dose of 300 mg/kg body weight i.p. to mice. These compounds all induce murine hepatic CYP1A2 at this dose (Chaloupka et al., 1994). Phenanthrene was the predominant PAH present in the DEP-EX used in our experiments. Phenanthrene was previously reported to significantly increase IgE mRNA and protein production from IgE-secreting transformed human B cells in vitro (Tsien et al., 1997). Furthermore, we have found that nasal challenge of humans with phenanthrene can drive enhanced allergic responses in vivo, although less well than DEP (Diaz-Sanchez, Penichet-Garcia and Saxon, unpublished observations). Phenanthrene and anthracene were found to have a certain degree of adjuvant effect on OVA-IgE, OVA-IgG1, total IgE, and IgG1 production in our study. Both phenanthrene and anthracene induce cytochrome P4501A2 gene expression by an unknown mechanism that apparently does not involve the aryl hydrocarbon receptor, (Chaloupka et al., 1994). However, since acenaphthylene induces CYP1A2 by the same mechanism, but does not exhibit an adjuvant effect in our model, it seems unlikely that the responsible component(s) of DEP-EX elicit their adjuvant effect via this mechanism. Butylated hydroxyanisole (BHA) is not present in DEP, but was used as a compound that activates transcription from the antioxidant response element (ARE), like many PAH quinones that are present in DEP or are produced by metabolism of polycyclic aromatic

hydrocarbons. The dose of BHA (15 mg/kg body weight) was chosen based on the LD₅₀ of BHA dissolved in dimethyl sulfoxide and given i.p. to mice (29 mg/kg body weight, Corte and Sgaragli, 1984). BHA also exhibited a slight adjuvant effect in our model, suggesting that compounds in DEP that act directly or indirectly on the ARE may be partly responsible for its adjuvancy.

The organic extract of DEP acts as an agonist for the aryl hydrocarbon receptor (Soontjens et al., 1997). 3MC, was used as a representative of polycyclic aromatic hydrocarbons present in PAH that can elicit several biological responses via binding to this receptor. 3MC was used at a dose of 300 mg/kg body weight. This concentration of 3MC is known to maximally induce cytochrome P4501A1 in BDF1 mice (Boobis et al., 1977; Gonzalez et al., 1984). 3MC has been reported to suppress the antibody response to the T-dependent antigen, sheep red blood cells, in vitro and in vivo (Lubet et al., 1984; White et al., 1985; Ladics et al., 1991), and to suppress human T cell proliferation induced by phytohemagglutinin (Davila et al., 1996). 3MC moderately suppressed OVA-IgG2a production, but not OVA-IgE nor OVA-IgG1, in our studies. TCDD is present in DEP (Clunies-Ross et al., 1996). The compound has been reported to modulate expression of certain cytokine genes (Kerkvliet et al., 1996; Fan et al., 1997; Vogel et al., 1997), to induce thymic atrophy (Poland and Glover, 1979; Fernandez-Salguero et al., 1996; Staples et al., 1998), suppress B cell differentiation into antibody-secreting cells (Tucker et al., 1986), inhibit the antibody plaque-forming cell response to T cell-dependent and -independent antigen (Smialowicz et al., 1996), downregulate cytotoxic T cell activity (Kerkvliet et al., 1996), and interfere with helper T cell activation through evoking impairment of B7-2 expression on antigen presenting cells (Prell and Kerkvliet, 1997). We chose 20 µg/kg body weight TCDD for injection, at which concentration the immune alterations mentioned above have been reported. Our observation that TCDD negatively affects in vivo generation of antigen-specific IgE, IgG1, and total IgE, IgG1, and IgG2a adds to the list of inhibitory effects of TCDD on the immune system. Since 3MC had no positive effect and TCDD suppressed the IgE and IgG1 response in our model, the aryl hydrocarbon receptor may

not be involved in the DEP-mediated adjuvant effect on allergen-specific antibody production. However, since the concentration of TCDD we employed has potent general immunologic inhibitory effects, the possibility cannot be fully excluded that, at lower doses where such immunosuppression is limited, TCDD or other agonists of the aryl hydrocarbon receptor may act as adjuvants that enhance the allergic response.

In conclusion, we find that the core carbon particles contribute more than the organic extractable components to the adjuvant effect observed following co-exposure to allergen and DEP, although the methylene chloride extractable organic components do enhance production of allergen-specific IgE and IgG1. Certain tricyclic PAHs contained in DEP, such as phenanthrene and anthracene, may be components responsible for the DEP extract-mediated immunostimulatory effect. In addition, our protocol for toxicant exposure and allergen immunization should be useful in further defining environmental toxicants causing allergic responses.

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