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Murine strain differences in airway inflammation caused by diesel exhaust particles

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Murine strain differences in airway inflammation caused by diesel exhaust particles. Y. Miyabara, R. Yanagisawa, N. Shimojo, H. Takano, H.B. Lim, T. Ichinose, M. Sagai. ©ERS Journals Ltd 1998.

ABSTRACT: To elucidate whether immunoglobulin (Ig) E or IgG are involved in the murine asthma model, we compared the pathogenic features of mice that were high IgG responders (C3H/He) with mice that were high IgE responders (BALB/c) after intratracheal instillation of diesel exhaust particles (DEP) and ovalbumin sensitization.

Both mouse strains received DEP intratracheally once a week for 5 weeks. After the second injection of DEP, ovalbumin and aluminium hydroxide were injected intraperitoneally. After the last DEP administration, the mice were challenged by exposure to an aerosol of ovalbumin.

DEP caused increased IgG1 production and airway hyperresponsiveness after ovalbumin sensitization in C3H/He mice, although IgE production did not change in either strain. Furthermore, in C3H/He mice, the number of eosinophils and goblet cells in the bronchial epithelium, and the expression of interleukin-5 and interleukin-2 were increased by DEP and ovalbumin treatments. In contrast, the pathogenic changes in BALB/c mice were weak, even though the same protocol was used.

In conclusion, murine strain differences in response to air pollutants and allergens seem to be related to antigen-specific immunoglobulin G1 production and cytokine expression in the lungs.

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Urban areas have been polluted heavily by nitrogen dioxide (NO_2) and suspended particulate matters (SPM) [1]. The primary source of NO_2 and SPM in urban areas is vehicle emissions. The ratio of vehicle emission to total oxides of nitrogen (NO_x) exhausts in Tokyo and Osaka in 1990 were 70% and 56%, respectively [2]. The number of diesel-powered cars has been increasing, and diesel vehicles emit more NO_2 and particulates than petrol-engined cars. Therefore, the ratio of diesel exhaust particles (DEP) to SPM is very high in urban air; in Tokyo in 1989 it was at least 40% [2]. DEP contains elemental carbon nuclei that adsorb a variety of organic compounds and a trace amount of heavy metals. Some of these organic compounds are strong mutagens and carcinogens [3–5]. It has been established that DEP causes lung tumours in a dose-dependent manner [6].

It has been shown that the prevalence of allergic rhinitis among schoolchildren is significantly higher in districts polluted by automobile exhaust than in nonpolluted districts [7]. Although, there have been numerous attempts to demonstrate the relationship between NO_2 and bronchial asthma, no direct link has been demonstrated experimentally. Furthermore, no experimental study has addressed the relationship between DEP and asthma. A previous report from our laboratory showed that repeated intratracheal instillations of DEP in mice induced chronic airway inflammation with infiltration of eosinophils and lymphocytes, and airway hyperresponsiveness with hypersecretion of mucus [8].

It is clear in allergic diseases such as asthma that allergen-specific immunoglobulin (Ig) E plays a central role in hypersensitivity reactions, and that the IgE-mediated reactions are followed by chronic inflammation leading to increased airway responsiveness. It has also been shown that DEP has adjuvant effects on IgE production in mice in cases of allergic rhinitis [9]. Intranasal instillation of DEP and antigen induced an increase of antigen-specific IgE antibody in mouse sera [10]. However, some observations suggest the existence of alternative and/or additional pathways of hypersensitivity reactions. Firstly, allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in IgE and mast cell-deficient mice [11, 12]. Secondly, immediate hypersensitivity and airway hyperresponsiveness were induced by the administration of ovalbumin (OVA)-specific IgE or IgG1, but not IgG2a or IgG3 [13]. We developed a murine asthma model by intratracheal instillation of DEP and OVA, and found that DEP enhanced the production of allergen specific IgG1, airway inflammation and airway hyperresponsiveness, before it enhanced IgE production [14]. Thus, both IgG and IgE antibodies are involved in allergic airway inflammation and airway hyperresponsiveness in that murine model of asthma.

To determine which immunoglobulin is more important in allergic asthma, we compared the production of antigen-specific IgE and IgG1, airway inflammation, airway hyperresponsiveness, and local cytokine expression between high IgG responders (C3H/He) [15] and high IgE responders (BALB/c) [16] after intratracheal instillation of DEP and OVA sensitization.

Materials and methods

Animals

Male C3H/He and BALB/c mice, aged 8–10 weeks, weighing 21–25 g, were obtained from Japan Clea Co. (Tokyo, Japan). They were fed a commercial stock diet (Japan Clea Co.) and water *ad libitium*. The mice were housed in an animal facility maintained at 24–26°C with 55–75% humidity and a light/dark cycle of 14 h/10 h. The study adhered to the National Institute of Health guidelines for the use of experimental animals.

Study design

Both strains of mice were divided into five groups: normal controls (nontreatment group); vehicle-saline; DEP-saline; vehicle-OVA and DEP-OVA. Vehicle or DEP were instilled by the intratracheal route through a polyethylene tube under anaesthesia with 4% halothane. The vehicle was 50 mM phosphate buffered saline (PBS; pH 7.4) containing 0.05% Tween 80. DEP was suspended in the vehicle at a concentration of 250 $\mu g \cdot mL^{-1}$ and the suspension was sonicated for 5 min using an ultrasonic disrupter (UD-201; Tomy Seiko, Tokyo, Japan) at 50% power with ice cooling.

The vehicle and DEP treated groups received 0.1 mL of the vehicle or DEP suspension once a week for 5 weeks. At 24 h after the second intratracheal injection of DEP or vehicle, 1 mg of OVA dissolved in 0.5 mL of a 3 $mg \cdot mL^{-1}$ suspension of aluminium hydroxide in saline solution was injected intraperitoneally as a sensitization step. At 24 h after the last DEP administration, these mice were challenged by exposure to an aerosol of 1% OVA in saline administered through an ultrasonic nebulizer (NE-U07; Omron Co., Tokyo, Japan) in a 2 L chamber for 15 min. Unsensitized mice were injected with saline instead of OVA-aluminium hydroxide and challenged with aerosol-saline as described above. The animals were killed 24 h after the challenge with saline or OVA.

Drugs and chemicals

Acetylcholine (ACh), diethyl ether, thimerosal, phenylmethane sulphonyl fluoride, Tween 20, and Tween 80 were purchased from Nacarai Tesque (Kyoto, Japan). Anti- mouse IgE rat horseradish peroxidase-conjugated streptavidin, bovine serum albumin (BSA), ethylenediamine tetraacetic acid (EDTA), 4-methyl-umbelliferyl-β-galacto-side and OVA (grade V) were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Biotinylated rabbit anti-mouse IgG1 and β-D-galactosidase-conjugated streptavidin were obtained from Zymed Laboratories (San Francisco, CA, USA). Leupeptin and pepstatin were supplied by Peptide Institute (Osaka, Japan). Halothane was purchased from Hoechst Japan (Tokyo, Japan). PBS (pH 7.4) was obtained from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Diff-Quik was supplied by International Reagents Co., Ltd. (Kobe, Japan). Anti-mouse IgE rat mo-noclonal antibody was purchased from Yamasa Shoyu Co., Ltd. (Chiba, Japan). Shiff's reagent was obtained from Merck (Darmstadt, Germany). All chemicals were of the highest grade available.

Collection of DEP

A4JB-1-type, light-duty (2,740 mL), four-cylinder diesel engine (Isuzu Automobile Co., Tokyo, Japan) was connected to an EDYC dynamometer (Meiden-sha, Tokyo, Japan) to regulate driving conditions. The engine was operated using standard diesel fuel at a speed of 1,500 revolutions per minute (rpm) under a load of 10 $kg \cdot m^{-1}$. DEP were collected as described previously [17]. Most of the particles were globular in shape. The mean diameter of the particles was 0.4 μm.

Blood retrieval and analysis

Mice were anaesthetized with diethyl ether. The chest and abdominal walls were opened, and blood was retrieved by cardiac puncture. Plasma was prepared and frozen at -80°C until assay for OVA-specific IgE and IgG1.

OVA-specific IgE determination

The titre of OVA-specific IgE antibody was measured by enzyme-linked immunosorbent assay (ELISA) [18]. In brief, microplate wells (Dynatech, Chantilly, VA, USA) were coated with an anti-mouse IgE rat monoclonal antibody at 37°C for 3 h, and incubated at 37°C for 1 h with PBS containing 1% BSA and 0.01% thimerosal. After washing with PBS containing 0.05% Tween 20 (PBST), diluted serum samples were added to the wells and incubated overnight at 4°C. After washing with PBST, biotinylated OVA was added to each well and incubated for 1 h at room temperature. After washing, wells were incubated for 1 h at room temperature with β-D-galactosidase-conjugated streptavidin. After the final washing, wells were incubated with 4-methylumbelliferyl-β-galactoside as the enzyme substrate at 37°C for 2 h. The enzyme reaction was stopped with 0.1 M glycine-NaOH buffer solution (pH 10.3). The fluorescence intensity was read by a microplate reader (Fluoroskan Flow Laboratories, Costa Mesa, CA, USA). Each plate included a previously screened standard plasma that contained a high titre of anti-OVA antibodies. For standardization, a titre of one was defined as twice the fluorescence of pre-immune plasma (blank). The OVA-specific IgE was calculated using a standard curve made by serial dilution of the standard plasma and dilution factor. Samples with a titre lower than one were considered negative. ELISA antibody titre was expressed as the highest plasma dilution giving a positive reaction.

OVA-specific IgG1 determination

OVA-specific IgG1 was measured by ELISA with solid phase antigen. In brief, microplate wells were coated with OVA overnight at 4°C. Then the plate was incubated at room temperature for 1 h with 1% BSA-PBS containing 0.01% thimerosal. After washing with PBST, diluted serum samples were added to the wells and incubated at room temperature for 1 h. After one more washing with PBST, the plate was incubated at room temperature for 1 h with biotinylated rabbit anti-mouse IgG1. After another washing, wells were incubated with horseradish peroxidase-conjugated streptavidin at room temperature for 1 h. Then, wells were washed and incubated with *o*-phenylenediamine and hydrogen peroxide (H_2O_2) in the dark at room temperature for 30 min. The enzyme reaction was stopped with 4N sulphuric acid. Absorbance at 490 nm was read by a microplate reader (Model 3550; Bio-Rad Laboratories, Hercules, CA, USA). Each plate included standard serum that contained a high titre of anti-OVA IgG1 antibodies. OVA-specific IgG1 titres were calculated as above for IgE titres.

Measurement of airway responsiveness

Pulmonary function was measured by the method of SORKNESS *et al*. [19], with a minor modification. In brief, each mouse was anaesthetized with pentobarbital sodium (50 $mg \cdot kg^{-1}$, *i.p.*), and a tracheostomy was performed with an 18-gauge cannula. Each mouse was mechanically ventilated with a rodent respirator (Model 683; Harvard Apparatus, South Natick, MA, USA) in a plethysmograph box with a pneumotachometer (BUXCO Electronics, Inc., Sharon, CT, USA) at a constant tidal volume (0.3 mL) and at a respiratory frequency of 120 $breaths \cdot min^{-1}$. Spontaneous respiration was inhibited by pancuronium bromide (1 $mg \cdot kg^{-1}$, *i.m.*). Continuous measurements of endotracheal pressure, flow, respiratory resistance (R_{rs}), and dynamic lung compliance (C_{dyn}) were recorded on a six-chan- nel recorder (BUXCO Electronics, Inc.). A 4 s average of R_{rs} and C_{dyn} was also recorded. An ACh challenge was performed with ACh solution (0.313–10 $mg \cdot mL^{-1}$) inhaled for 2 min. The solution was aerosolized by an ultrasonic nebulizer (NE-U07; Omron Co.), which generates aerosol particles of approximately 5 μm in diameter.

Histological evaluation of eosinophils, lymphocytes, neutrophils and goblet cells in the lung

After exsanguination, the lungs were fixed by intratracheal instillation with 10% neutral phosphate-buffered formalin at a pressure of 20 cmH_2O for at least 72 h. Slices (2–3 mm thick) of all pulmonary lobes were embedded in paraffin. Sections (3 μm thick) were prepared and stained with Diff-Quik to quantify the number of infiltrated eosinophils, lymphocytes and neutrophils. The length of the basement membrane of all airways in each specimen was measured by video micrometer (VM-30; Olympus, Tokyo, Japan). The numbers of eosinophils, lymphocytes and neutrophils under bronchial epithelium in each sample were counted with a micrometer (AX80; Olympus) under oil immersion. Results were expressed as the number of inflammatory cells per millimetre of basement membrane. To quantify goblet cells, the sections were stained with periodic acid Schiff (PAS). The number of goblet cells that stained red in the bronchial epithelium were counted by micrometer. Results were expressed as the number of goblet cells per millimetre of basement membrane.

Quantification of cytokine protein levels in lung tissue supernatants

Murine lungs were removed after exsanguination, frozen in liquid nitrogen and stored at -80°C until assay. Each lung was homogenized with 10 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA, 0.1 mM phenylmethane sulphonyl fluoride, 1 μM pepstatin, and 2 μM leupeptin. The homogenate was then centrifuged at 105,000×g for 1 h at 4°C. The supernatant was stored at -80°C.

ELISAs for interleukin-5 (IL-5), granulocyte-macrophage colony stimulating factor (GM-CSF), and interleukin-2 (IL-2) were conducted using matching antibody pairs (Endogen, Cambridge, MA). The antibody pairs for detection of IL-5, GM-CSF and IL-2 were TRFK5 and TRFK4, MP1-22E9 and MP1-31G6 and S4B6 and H5 4.1.1, respectively. The ELISA for interleukin-4 (IL-4) was conducted using matching antibody pairs (Amersham, Buckinghamshire, UK) according to the manufacturer's instructions. The second antibodies were conjugated to horseradish peroxidase. Subtractive readings of 550 nm from the readings at 450 nm were converted to picograms per millilitre using values obtained from standard curves generated with varying concentrations of recombinant IL-5, GM-CSF, IL-2 and IL-4. The detection limits of each assay were 5, 5, 3 and 5 $pg \cdot mL^{-1}$, respectively.

Statistical analysis

Data are reported as mean±SEM Differences in the numbers of infiltrated inflammatory cells and goblet cells, airway hyperresponsiveness, cytokine protein levels and immunoglobulin titres among groups were determined using analysis of variance (Statview; Abacus Concepts, Inc., Berkeley, CA, USA). If differences among groups were significant ($p<0.05$), Fisher's protected least significant difference test or Scheffe's F-test were used to distinguish between pairs of groups.

Results

Airway inflammation

The inflammatory cellular profiles of five groups in C3H/He and BALB/c mice are shown in table 1.

In C3H/He mice, OVA sensitization (vehicle-OVA) caused eosinophilic infiltration, and the combined DEP and OVA treatment (DEP-OVA) led to a marked and significant infiltration under bronchial epithelia. The number of eosinophils in the DEP-OVA group were 13 and seven times higher than that in the vehicle-saline ($p<0.01$)

Table 1. – Effects of diesel exhaust particles (DEP) and/or ovalbumin (OVA) administration on inflammatory cell counts of respiratory tract in C3H/He and BALB/c mice

Group	Eosinophil counts·mm^{-1}		Neutrophils counts·mm^{-1}		Lymphocytes counts·mm^{-1}	
	C3H/He	BALB/c	C3H/He	BALB/c	C3H/He	BALB/c
Normal control	0.003±0.003	0.005±0.005	0.015±0.007	0.028±0.007	0.477±0.063	0.467±0.081
Vehicle-saline	0.114±0.046	0.003±0.003	0.030±0.009	0.032±0.022	0.592±0.129	0.418±0.127
DEP-saline	0.033±0.011	0.013±0.003	0.040±0.007	0.026±0.023	0.510±0.096	0.365±0.106
Vehicle-OVA	0.215±0.066	0.110±0.028*	0.069±0.014*	0.052±0.016	0.560±0.184	0.621±0.135
DEP-OVA	1.450±0.211**	0.314±0.063**	0.066±0.034*	0.058±0.009	1.078±0.123*	0.959±0.102*

Animals (n=5) received intratracheal instillation of vehicle or DEP for 5 weeks with or without OVA sensitization. Lungs were removed and fixed 24 h after the OVA challenge. Sections were stained with Diff-Quik for measurement of inflammatory cells around the airways. Results are expressed as number of cells per length of basement membrane of airways. Data are presented as mean±SEM *: p<0.05; **: p<0.01, compared to vehicle-saline group (Student's t-test or Cochran-Cox test).

and the vehicle-OVA (p<0.01) groups, respectively. There was a small increase in eosinophils in the nonsensitized groups (vehicle-saline and DEP-saline) above the normal control group (p<0.05). The numbers of infiltrated neutrophils in the DEP-OVA group were only 1.0–1.6 times higher than that in the vehicle-OVA and DEP-saline groups. A twofold increase in infiltrated lymphocytes was observed in the DEP-OVA group as compared to the vehicle-saline or vehicle-OVA groups (p<0.05). Thus, the inflammation induced in C3H/He mice by DEP and OVA was characterized by a marked infiltration of eosinophils.

In BALB/c mice, OVA sensitization (vehicle-OVA and DEP-OVA) caused eosinophilic infiltration, but it was not as marked as that of the C3H/He mice. The number of eosinophils in the DEP-OVA group was three times higher than that of the vehicle-OVA group (p<0.05). The combined DEP and OVA instillation induced a less than 2.2 times elevation of infiltrated neutrophils as compared with either the vehicle-OVA or DEP-saline groups. A 1.5 fold increment in infiltrated lymphocytes in the DEP-OVA group was observed compared with the vehicle-OVA group (p<0.05).

Infiltration by inflammatory cells in the DEP-OVA groups of both strains were characterized by an increase of eosinophils. The inflammatory cells of the normal control groups were approximately equal for both strains. However, the number of eosinophils in C3H/He mice compared with BALB/c mice for vehicle-saline, DEP-saline, vehicle-OVA, and DEP-OVA groups were 38, two, three and five times higher, respectively.

Mucus hypersecretion

The lung specimen was stained with PAS to estimate the hypersecretion of mucus in the airway. The numbers of goblet cells per length of basement membrane of the airways in C3H/He and BALB/c mice are shown in figure 1.

In the C3H/He mice groups, a marked number of goblet cells were observed in the DEP-OVA group. The number of goblet cells in the DEP-OVA group was 27 times higher than that of the vehicle-saline group (p<0.05) and four times higher than that of the vehicle-OVA (p<0.05) groups. The numbers of goblet cells in the nonsensitized groups (vehicle-saline and DEP-saline) were small.

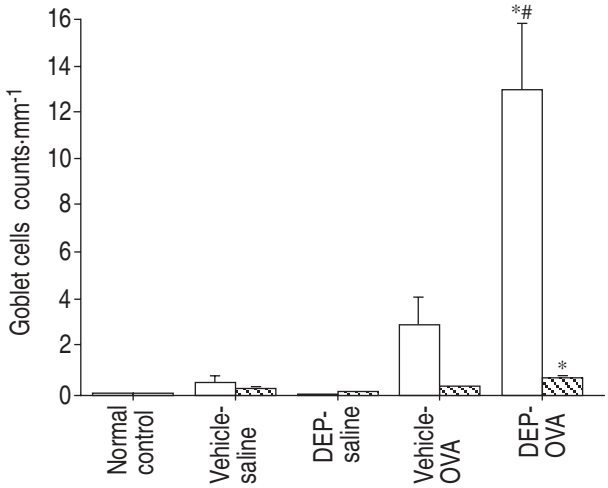

Fig. 1. – Effects of diesel exhaust particles (DEP) and/or ovalbumin (OVA) administration on increase of goblet cells in C3H/He (□) and BALB/c mice (▨). Two strains of mice were intratracheally instilled with vehicle or DEP once a week for 5 weeks with or without OVA sensitization. Lungs were removed and fixed 24 h after saline or OVA challenge. Sections were stained with peroxide acid Schiff for measurement of goblet cells under bronchial epithelia. Results are expressed as mean±SEM (n=5). *: p<0.05 *versus* vehicle saline group; #: p<0.05 *versus* vehicle OVA group (Fisher's protected least significance difference test or Scheffe's F-test).

In BALB/c mice, a small number of goblet cells were observed in the DEP-OVA group. The number of goblet cells in the DEP-OVA group was 3.3 times higher than that in the vehicle-saline group (p<0.05) and 1.6 times higher than that in the vehicle-OVA group (p=NS). The numbers of goblet cells were small in the nonsensitized groups.

The combined instillation of DEP and OVA increased the number of goblet cells in both strains, but the increment in BALB/c mice was small compared with that in the C3H/He mice. The number of goblet cells in the DEP-OVA group of the C3H/He mice was 17.5 times higher than that of the BALB/c mice.

Airway hyperresponsiveness

We measured the R_{rs} of C3H/He and BALB/c mice upon inhalation of ACh to compare the effects of DEP and/or OVA on airway hyperresponsiveness. The increase of R_{rs} in both mice groups is shown in figure 2. In C3H/

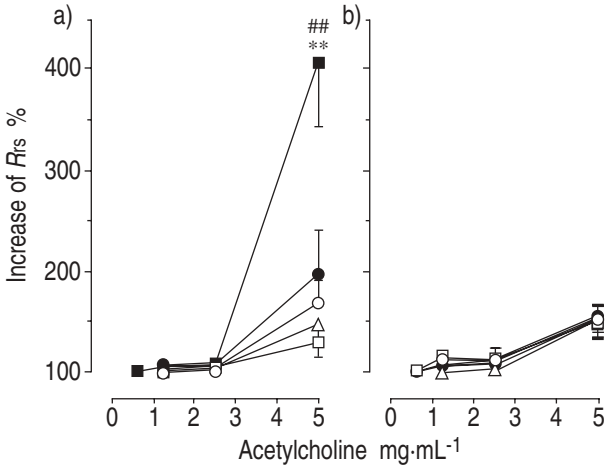

Fig. 2. – Effects of DEP and/or OVA administration on airway responsiveness (airway resistance (R_{rs})) to acetylcholine in a) C3H/He and b) BALB/c mice. Mice were intratracheally instilled with vehicle or DEP once a week for 5 weeks with or without OVA sensitization. R_{rs} to acetylcholine was measured 24 h after saline or OVA challenge. Results are expressed as mean±SEM (n=7–8). **: p<0.01, compared to vehicle-saline group; ##: p<0.01, *versus* vehicle-OVA group (Fisher's least significant difference test or Scheffe's F-test). —○—: normal control; –△–: vehicle-saline; —●—: DEP-saline; —□—: vehicle-OVA; —■—: DEP-OVA. For definitions see legend to figure 1.

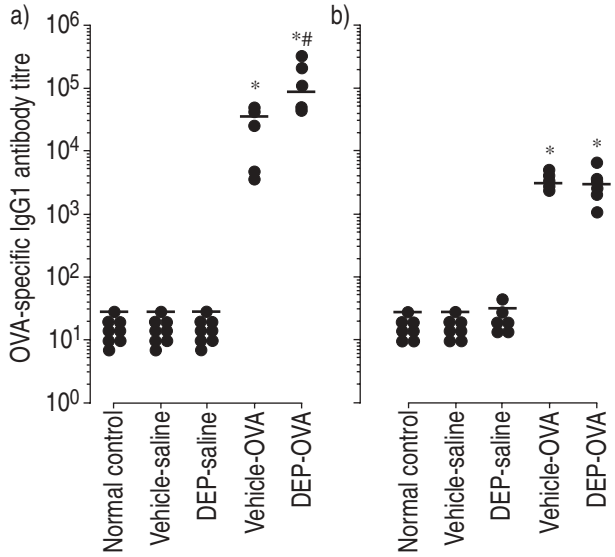

Fig. 3. – Effects of DEP and/or OVA administration on OVA-specific immunoglobulin G1 (IgG1) antibody productions in a) C3H/He and b) BALB/c mice. The mice were intratracheally instilled with vehicle or DEP once a week for 5 weeks with or without OVA sensitization. Plasma samples were retrieved 24 h after saline or OVA challenge. OVA-specific IgG1 was analyzed using enzyme-linked immunosorbent assay (ELISA) (see text). Results are expressed as individual data. The horizontal bars are the means (n=7–8). *: p<0.05, compared to vehicle-saline group; #: p<0.05, compared to vehicle-OVA group (Fisher's protected least significant difference test or Scheffe's F-test). For definitions see legend to figure 1.

He mice, R_{rs} was significantly higher only in the DEP-OVA group compared with the other four groups (p<0.05 *versus* each other group). The provocative concentration of ACh causing a 50% increase in R_{rs} (PC_{150}) in the DEP-OVA group was significantly lower than in the other four groups (PC_{150} 3.1 mg·mL^{-1} *versus* 5.9–8.7 mg·mL^{-1}, p<0.05). However, there were no significant differences in the increase of R_{rs} among the BALB/c mice groups. The averages of PC_{150} in BALB/c mice groups were 4.5–6.9 mg·mL^{-1} of ACh. There were no significant differences between the normal control C3H/He and BALB/c mice (PC_{150} 5.9–6.5 mg·mL^{-1}).

Antigen-specific IgE and IgG1 production

OVA-specific IgE and IgG1 in both C3H/He and BALB/c mice were measured to examine the involvement of these immunoglobulins in allergic asthma, and whether DEP had adjuvant activity on antigen-specific immunoglobulin production after intratracheal instillation of OVA. The titres of OVA-specific IgE were not significantly increased by antigen challenge with or without DEP instillation in either strain, despite manifestations of airway inflammation, mucus hypersecretion and airway hyperresponsiveness. Most values were below the detection limits (<10 titre) in both strains of mice.

The titres of OVA-specific IgG1 in the serum of both C3H/He and BALB/c mice are shown in figure 3.

OVA-specific IgG1 was significantly elevated by OVA sensitization in both groups of mice. In C3H/He mice, the average concentration of IgG1 in the DEP-OVA group was 2.6 times higher than that in the vehicle-OVA group (p<0.05). However, there was no significant difference between the vehicle-OVA and DEP-OVA groups in BALB/c mice. When C3H/He and BALB/c mice were compared, the titres of IgG1 in the vehicle-OVA and DEP-OVA groups of C3H/He mice were 11 and 28 times higher than those of BALB/c mice, respectively. The titres of IgG1 in both vehicle-saline and DEP-saline groups were very low (<27 titres).

Local cytokine expression

To explore what kinds of cytokines are involved in the enhancing effects of DEP on antigen-induced airway inflammation, mucus hypersecretion and airway hyperresponsiveness, we quantified protein levels of IL-5, GM-CSF, IL-2, and IL-4 in the lung supernatants (fig. 4).

The amount of IL-5 increased dramatically in the DEP-OVA and vehicle-OVA groups of C3H/He mice, and these increases were 34 and 26 fold greater than the vehicle-saline group, respectively (p<0.0001). The amount of IL-5 in the normal control group of C3H/He mice was three times lower than that of BALB/c mice. However, the IL-5 levels in BALB/c mice did not significantly increase after antigen challenge in the presence or absence of DEP.

GM-CSF in the DEP-OVA and vehicle-OVA groups were significantly greater in C3H/He and BALB/c mice than in the vehicle-saline group (p<0.01), but the increases were only 1.3 to 1.7 fold, respectively. There was no significant difference between the vehicle-OVA and DEP-OVA groups in either strain. The amount of GM-CSF in C3H/He mice was 3.9 to 5.2 fold higher than in BALB/c mice.

IL-2 in the DEP-OVA and vehicle-OVA groups of C3H/He mice showed 17 and 29 fold increases over the vehicle-saline group, respectively (p<0.0001). However, antigen challenge did not affect IL-2 levels in BALB/c mice with or without DEP treatment. No meaningful differences in IL-4 were observed among mouse strains.

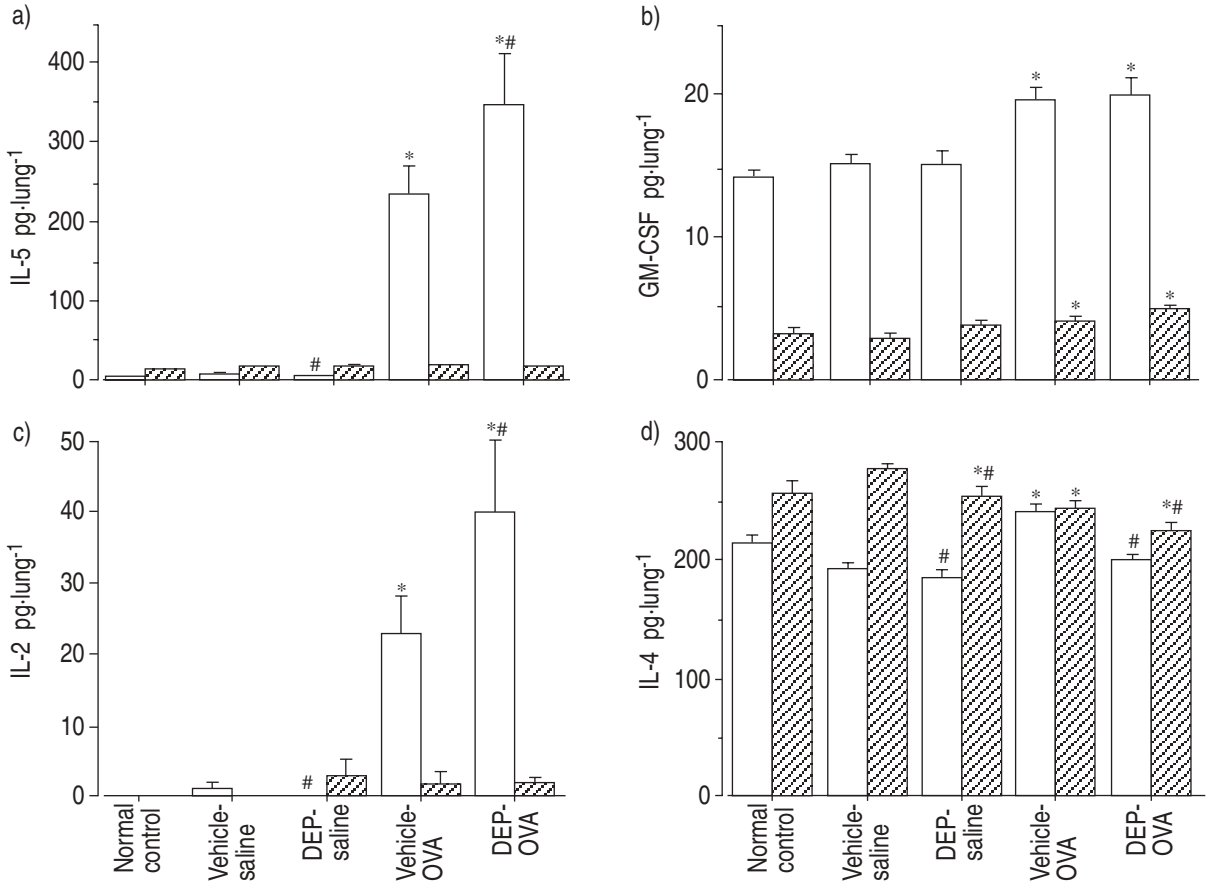

Fig. 4. – Effects of DEP and/or OVA administration on local cytokine expressions in C3H/He (▭) and BALB/c (▨) mice. The mice were intratracheally instilled with vehicle or DEP once a week for 5 weeks with or without OVA sensitization. Lungs were removed 24 h after saline or OVA challenge and frozen until use. Protein levels of four cytokines in lung tissue supernatants were analyzed using enzyme-linked immunosorbent assay (ELISA) (see text). Results are expressed as mean±SEM (n=7–8). *: $p<0.05$, compared to vehicle-saline group; #: $p<0.05$, compared to vehicle-OVA group (Fisher's protected least significant difference test or Scheffe's F-test). For definitions, see legend to figure 1.

Discussion

In the present study, we have demonstrated that airway inflammation with a marked infiltration of eosinophils was induced by the DEP administration with antigen challenge in both C3H/He and BALB/c mice. The recruitment of eosinophils was accompanied by a hyperplasia of goblet cells in bronchial epithelium as a maker of mucus hypersecretion. Eosinophils and goblet cells were more induced in C3H/He mice than in BALB/c mice after the DEP and OVA treatment. Airway hyperresponsiveness, IL-5 and IL-2 expressions were enhanced by the DEP administration with OVA challenge in C3H/He mice, but not in BALB/c mice. The combination of intratracheal administration of DEP and single antigen challenge by aerosol inhalation induced a significant increase of antigen-specific IgG1 in C3H/He mice, but not of antigen-specific IgE in either mouse strain. Thus, the production of IgG1 varied along with the infiltration of eosinophils, airway hyperresponsiveness (PC_{150}) and hyperplasia of goblet cells, but not IgE. However, after the intratracheal administration of DEP and three times of OVA challenge every other day, the antigen-specific antibodies of both IgE and IgG1 isotypes were remarkably increased even in BALB/c mice, and airway hyperresponsiveness, marked infiltration of eosinophils and lymphocytes, hyperplasia of goblet cells were also observed (data not shown). These results indicate that DEP had an adjuvant effect on OVA-specific IgG production in not only high IgG responders (C3H/He) but also high IgE responders (BALB/c) and that the former is more sensitive to the administration of DEP with OVA than the latter.

Inbred strains of mice have been shown to vary in their ability to produce immunoglobulins. These inbred mice are classified into six H-2 haplotypes, $H\text{-}2^a$, $H2^b$, $H\text{-}2^d$, $H\text{-}2^k$, $H\text{-}2^q$, and $H\text{-}2^s$. It has been reported that the $H\text{-}2^k$ haplotype strain shows higher IgG responsiveness to ovalbumin than $H\text{-}2^b$ and $H\text{-}2^d$ strains [15]. In the present study, C3H/He mice ($H\text{-}2^k$) were more sensitive to OVA than BALB/c ($H\text{-}2^d$) mice. The titre of IgG1 in the serum of C3H/He mice was 28 times higher than that of BALB/c mice after the same DEP and OVA treatments. The production of IgG1 was accompanied by recruitment of eosinophils and an increase of goblet cells in bronchial epithelium. This may indicate that genetic factors, such as the H-2 haplotype, are important to the expression of asthmatic features in mice as well as humans.

It has been reported that intranasal DEP administration with antigen demonstrated adjuvant activity for IgE production in mice [10]. Although IgE is an important component of many allergic reactions, there are some reports which suggest the existence of alternative and/or

additional pathways of hypersensitivity reactions with IgG prduction in mice [11–14]. Judging from these reports and the present results, DEP with antigen may be able to induce allergic airway inflammation and hyperresponsiveness not with IgE antibody production but with IgG antibody production. It seems that IgG antibody is more important than IgE in murine asthma model, and antigen-specific IgE and IgG production is altered by the administration route of allergen. In humans, late asthmatic reactions to an inhaled allergen challenge are associated with IgG1 rather than IgE antibody [20]. Significantly elevated levels of IgE and IgG antibodies to inhaled allergens have been detected in children with asthma, and in adults with allergic asthma [21]. IgG antibody as well as IgE antibody may be involved in allergic bronchial asthma.

Allergic asthma is often associated with activation of IL-4, IL-5, and the GM-CSF gene cluster. Significant associations have been observed between the numbers of cells expressing mRNA for IL-4, IL-5, and GM-CSF and air flow restriction, bronchial hyperresponsiveness, and asthma score [22]. A recent report concerning IL-5-deficient mice supports the concept that IL-5 and eosinophils are central mediators in the pathogenesis of allergic lung diseases [23]. Another study with IL-4-deficient mice implied a significant role for IL-4 in antigen-induced eosinophilic airway inflammation and bronchial responsiveness [12, 24]. GAVETT *et al*. [25] have recently provided the first direct evidence that antigen-induced airway in-flammation and hyperresponsiveness are associated with increases in protein levels of IL-4 and IL-5 in murine BAL supernatants.

In the present study, airway inflammation (eosinophils), airway hyperresponsiveness (PC_{150}), hypersecretion of mucus (goblet cell), IgG1, IL-5, and IL-2 in lung tissue were increased after DEP administration and antigen challenge. These results show that IL-5 and IL-2 are important cytokines in distinguishing strain differences and mechanisms of airway inflammation and hyperresponsiveness. IL-5 acts in the lung to recruit, activate, and prolong the life of eosinophils. Our results agree with previous reports showing that the combined administration of DEP and OVA induced eosinophilic infiltration and IL-5 production in ICR mice [14]. IL-2 causes lymphocyte activation and proliferation and affects the regulation of immunoglobulin production and lymphokine expression related to the activation of other cells. It has been reported that IL-2 administration induced OVA-specific IgG production and airway inflammation [26]. In addition, T-cells from eosinophilic patients produce IL-5 after IL-2 stimulation [27]. It seems that the elevated levels of IL-2 in the present experiment play a role in lymphocyte activation and induce the OVA-specific IgG production and cytokine expression. Furthermore, the relatively high level dose of GM-CSF in C3H/He mice may enhance the infiltration and activation of inflammatory cells. Although the protein level of IL-4 did not increase in our model, it may be related to the production of IgG1 and IgE. The IL-4 IgG1 dose-response curve was bimodal, and IgG1 can be induced by normal levels of IL-4; the response of IgE is directly dependent on a high concentration of IL-4. The amount of IL-4 needed for optimal IgE secretion is 100 fold higher than that needed for the first peak of IgG1 production [28]. In our study, the concentration of IL-4 seems to correspond to the first peak, resulting in high levels of IgG1 but not IgE. Furthermore, it was reported that allergen-specific IgG1 antibodies in patient sera contributed to antigen-specific eosinophilic degranulation *via* FcγRII on the eosinophil surface. IgG1 with antigen is a strong agonist for eosinophilic degranulation *in vitro* [29, 30]. The resulting cytokine expression induces IgG1 production and eosinophil activation, inducing airway inflammation with epithelial damage and airway hyperresponsiveness by degranulation of eosinophils.

Thus, manifestations of asthma-like features in our murine model were well explained with the cytokine expression in the lungs. These strain differences may be related to IL-5 and IL-2 gene expression. In particular, IL-5 positive lymphocytes have been observed in the bronchial mucosa of C3H/He mouse by immunohistochemistry (unpublished data). Therefore, the cytokine synthesis and/or release, and its mRNA expression patterns of individual cells *in vivo* should be investigated in future. It will clarify the mechanism of allergic asthma and human individual variations.

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