

Murine Strain Differences in Airway Inflammation Induced by Diesel Exhaust Particles and House Dust Mite Allergen

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Key Words

Allergic asthma · Cytokines · Diesel exhaust particles · Eosinophils · Mite allergen · Strain differences

Abstract

Background: Differences in allergic airway inflammation induced by ovalbumin (OVA) + diesel exhaust particles (DEP) in various murine strains have already been reported. However, there is no report that different murine strains respond differently towards house dust mites or DEP, which are known to aggravate allergic asthma.

Methods: The *Dermatophagoides farinae* allergens Der f (1 µg) or Der f (1 µg) + DEP (50 µg) were administered intratracheally to two different mouse strains (CBA/JN and C57BL/6N). Histological changes in the lung tissues, asthma-relevant cytokines in the lungs, and allergen-specific immunoglobulins in plasma were investigated.

Results: Der f treatment led to the proliferation of goblet cells, production of mucus plugs, and the recruitment of eosinophils and lymphocytes to the airways of the mice. The manifestation of the airway inflammation in the C57BL/6N mouse was much greater than in the CBA/JN

mouse. The protein levels of interleukin (IL)-4 and IL-5, regulated on activation, normal T cell expressed, and presumably secreted (RANTES), and eotaxin in the lung tissue of C57BL/6N mice were higher than those in CBA/JN mice by a factor of 1.26 (IL-4), 5.26 (IL-5), 2.07 (RANTES) and 3.27 (eotaxin). DEP aggravated the manifestations of the eosinophilic inflammation in CBA/JN mice through goblet cell proliferation. However, the exact effect of DEP could not be evaluated in C57BL/6N because of its severe enhancement of the inflammation. DEP enhanced the local expression of IL-5, RANTES, and eotaxin in the CBA/JN mouse, and consequently triggered an increased IgG1 production in both strains. Allergen-specific IgE antibodies were lower than 1 titer in both mice. **Conclusion:** The murine strain differences in the pathogenesis of allergic airway disease caused by mite allergen might be related to the local expression of the cytokines we screened. The aggravating effect of DEP may be mediated by an increase in the local expression of IL-5, RANTES, eotaxin, and the production of an antigen specific to IgG1.

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Introduction

Recently, the number of allergic asthma patients among schoolchildren has been increasing steadily in many countries, including Japan [1–3]. House dust mites and mold are increasing in airtight buildings with high humidity [4–5], conditions that tend to cause allergic asthma and atopic dermatitis [5–7]. In particular, house dust mites are known as the predominant cause of the current increase in asthma prevalence [6].

Epidemiological studies show that ambient particulate matter (PM₁₀; PM_{2.5}) is associated with an increase in hospital admission for asthma [8] and the development of respiratory symptoms in childhood asthma [9]. Some experimental studies have reported that diesel exhaust particles (DEP) or diesel exhaust, together with some other allergens, may modulate the bronchial or nasal allergic reactions [10–15].

Allergic asthma is clinically characterized by chronic inflammatory eosinophilic bronchitis and airway hyper-responsiveness. A model study involving murine asthma, DEP, and ovalbumin (OVA) showed that DEP enhances airway hyperresponsiveness and manifestations of eosinophilic inflammation [16–18]. This inflammation was not induced by the dose of DEP alone. The airway inflammation aggravated by DEP was shown to be associated with an increase in local cytokine expression, such as interleukin (IL)-2 and IL-5 [12, 19]. The production of OVA-specific IgG1 and the manifestations of eosinophilic airway inflammation were also found to differ between mouse strains. For example, CBA/JN and BALB/C mice were low responders regarding IgG1 production and the development of allergic airway inflammation following exposure to OVA. On the other hand, C57BL/6N and C3H/He mice are high responders to the allergens present in OVA [20]. Mice strain differences in IgE production and airway response to house dust mite allergen have also been reported. The A/J mouse is a highly responsive antigen-specific IgE producer and shows immediate airway constriction if exposed to native *Dermatophagoides farinae* (Der f) and recombinant Der f. On the other hand, C57BL/6 and C3H/He mice are low responders to Der f [21]. DEP reportedly triggers adjuvant activity against Der f-specific IgE production in A/J mice [22]. However, there have been no reports on murine strain differences in allergic airway inflammation caused by house dust mites or DEP.

In the present study, differences between mouse strains in the manifestation of allergic airway inflammation induced by a mite allergen or DEP were investigated. After

injecting CBA/JN and C57BL/6N mice with DEP or a mite allergen intratracheally, the following reactions were measured: production of antigen-specific IgG1 and IgE in plasma, airway inflammation (characterized by the infiltration of eosinophils and lymphocytes, as well as goblet cell proliferation), and asthma related to the lung cytokines IL-2, IL-4, IL-5, granulocyte macrophage-colony stimulating factor (GM-CSF), RANTES and eotaxin.

Materials and Methods

Animals

C57BL/6N mice (5 weeks old) and CBA/JN mice (5 weeks old) were purchased from Charles River (Kanagawa, Japan). Their body weight was recorded and they were examined for possible infections. The mice were then used 1 week later. Commercial animal food (CE-2; CLEA Japan, Tokyo, Japan) and water were accessible ad libitum. The mice were housed in plastic cages with plywood chips. The cages were placed in a conventional room air-conditioned at 23°C and 55–70% humidity, and a light/dark cycle of 12 h/12 h. The study adhered to the US National Institutes of Health guidelines for the use of experimental animals. The animal care method was approved by the Animal Care and Use Committee at Oita University of Nursing and Health Science in Oita, Japan.

Study Protocol

Mice of each strain were divided into two groups. Each group was injected with Der f alone or Der f and DEP. DEP were prepared from a light-duty (2,740 cm³), four-cylinder diesel engine according to a method reported previously [12, 19, 20]. The diameters of the particles were measured using the low-pressure type Andersen air sampler (Tokyo Dylec, Tokyo, Japan). The DEP had a mass median aerodynamic diameter of 0.4 µm. Crude extract of Der f was purchased from LSL (Tokyo, Japan). One microgram of Der f was dissolved in a pH 7.4 phosphate-buffered saline (PBS) solution (Nissui Pharmaceutical, Tokyo, Japan) containing 0.05% Tween 80 (Nacalai Tesque, Kyoto, Japan), and 0 or 50 µg of DEP. The samples were injected intratracheally into 32 mice four times at 2-week intervals. Three days after the last injection, the mice were sacrificed by exsanguination under deep ethyl ether anesthesia. Plasma prepared from sacrificed mice was stored at –80°C until assayed for Der f-specific IgG1 and IgE. The lungs were removed, snap-frozen in liquid nitrogen, and stored at –80°C until analyzed for cytokines.

Histologic Analysis

Ten out of 16 mice from each group were used for histologic examination. The lungs were fixed by intratracheal instillation of 10% neutral phosphate-buffered formalin at a pressure of 20 cm H₂O. After separation of the lobe, 2-mm-thick blocks were taken for paraffin embedding. Embedded blocks were sectioned at a width of 3 µm and then stained with hematoxylin and eosin to evaluate the degree of infiltration of eosinophils or lymphocytes in the airways from proximal to distal. The sections were stained with periodic acid-Schiff (PAS) to evaluate the degree of proliferation of goblet cells in the bronchial epithelium. Histologic analyses of inflammatory cells and epithelial cells in the airway were performed using an Olympus BH-2 light microscope (Olympus, Tokyo, Japan). The degree of

eosinophil and lymphocyte infiltration in the airways and the proliferation of goblet cells in the bronchial epithelium were graded in a blind fashion: – = not present; ± = very slight; + = slight; ++ = moderate; +++ = moderate to marked; ++++ = marked. ‘Very slight’ was defined as an eosinophilic inflammatory reaction affecting less than 10% of the airways (less than 10% goblet cells stained with PAS); ‘slight’: 20–30% of the airways are affected; ‘moderate’: 40–50%; ‘moderate to marked’: 60–70%, and ‘marked’: more than 80% of the airways are affected [10, 11, 20].

Immunohistochemistry for RANTES and Eotaxin

After the slides had been deparaffinized with an auto dewaxer (Res Gen, Huntsville, Ala., USA), the endogenous peroxidase activity was blocked by treatment with a 2% hydrogen peroxide in a 60% methanol solution for 2 h at room temperature. The slides were subsequently soaked in a 0.2% azide solution for 20 min. After hydration and a rinse in universal buffer (Res Gen), the nonspecific antigens were blocked by exposing them to 2% normal rabbit serum for 30 min at room temperature. Endogenous biotin and avidin binding were sequentially blocked using a Vector Blocking Kit (Vector Lab, Burlingame, Calif., USA). After thorough washing in a universal buffer, the sections were incubated with 2.5 µg/ml of rat monoclonal anti-eotaxin mouse IgG2a (R & D Systems, Minneapolis, Minn., USA) in a PBS solution containing 0.1% bovine serum albumin (BSA) for 1 h at room temperature. After the sections had been rinsed in universal buffer, they were incubated in 0.5% biotinylated anti-rat IgG (Vectastain Elite ABC kit, Vector Lab) for 30 min at room temperature. After rinsing in universal buffer, the sections were incubated in an avidin-biotin complex for 30 min at room temperature. The sections were rinsed in PBS solution and then stained with a 1% 3,3'-diaminobenzidine chromogen solution (DAKO, Carpinteria, Calif., USA) containing an imidazole HCl-buffered substrate (pH 7.5). The sections were then washed with water, lightly counterstained with Myer's hematoxylin (Wako, Tokyo, Japan), dehydrated, and mounted with Pristin Mount (Res Gen).

Controls were prepared using the conditions described above, except that the nonimmune rat IgG2a (Zymed Laboratories, San Francisco, Calif., USA) was used instead of a primary antibody. The remaining slides were deparaffined and then immunostained for RANTES using the protocol described above. The sections were incubated with 5 µg/ml of rat monoclonal anti-RANTES mouse IgG2a (R & D Systems) or nonimmune rat IgG2a (Zymed Laboratories) for 1 h at room temperature and then treated in the same manner as above.

Quantitation of Cytokine Protein Levels in Lung Supernatants and Plasma

The cytokine protein levels of IL-2, IL-4, IL-5, GM-CSF, RANTES, and eotaxin were examined in lung tissue supernatants obtained from 6 animals in each group. IL-4, IL-5 and RANTES were also examined in the plasma. The lungs were homogenized according to a method reported previously [10–12, 16, 19]. The homogenate was then centrifuged at 105,000 *g* for 1 h at 4°C. The lung supernatants were stored at –80°C until analysis.

IL-2, IL-4 (Amersham, Little Chalfont, UK), IL-5, RANTES (R & D Systems), eotaxin (R & D Systems) and GM-CSF were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Endogen, Cambridge, Mass., USA). Subtractive readings at 550 nm from the readings at 450 nm were converted to pg/ml using values obtained from the standard curves. The standard curves were

prepared using varying concentrations of recombinant IL-2, IL-4, IL-5, GM-CSF, RANTES and eotaxin. The detection limits of these assays were 3 pg/ml (IL-2), 5 pg/ml (IL-4), 5 pg/ml (IL-5), 5 pg/ml (GM-CSF), 7.8 pg/ml (RANTES), and 3 pg/ml (eotaxin).

Determination of Der f-specific IgG1 Titer

Der f-specific IgG1 in the plasma of the 10 animals from each group used for histologic analysis was subsequently measured using ELISA with solid-phase antigen. Briefly, microtiter plate was coated with Der f at a concentration of 2 µg/ml overnight at 4°C according to a method reported previously [10–12, 16, 18–20]. The plate was incubated with 1% BSA-PBS solution (pH 7.4) containing 0.01% thimerosal for 1 h at room temperature. After washing, 100 µl of each diluted sample (1:5, 1:50, 1:500 and 1:5,000) was placed on the microplate well and incubated for 1 h at room temperature. After additional washing with a PBS solution containing Tween 20, the plate was incubated with biotinylated rabbit anti-mouse IgG1 at a concentration of 1.67 µg/ml (Zymed Laboratories) for 1 h at room temperature. After a second washing, the wells were incubated with horseradish peroxidase-conjugated streptavidin (Sigma, St. Louis, Mo., USA) for 1 h at room temperature. These wells were subsequently incubated with *o*-phenylenediamine and hydrogen peroxide for 30 min in the dark at room temperature. The enzyme reaction was stopped by adding 4 *N* H₂SO₄, and the absorbance at 492 nm was measured using a microplate reader (SPECTRAFLUOR; Tecan, Salzburg, Austria). Each plate contained previously screened standard plasma with a high titer of anti-Der f antibodies. The results were expressed in titers, which were calculated based on the titers of the standard plasma. Cut-off values for antibody-positive plasma were set at twice the mean absorbance of preimmune plasma.

Determination of Der f-Specific IgE

The assay protocol for Der f-specific IgE was a slight modification of the one for Der f-specific IgG1. The samples, double diluted, were incubated overnight at 4°C in microtiter plates coated with Der f at a concentration of 2 µg/ml. The samples were reacted with biotinylated rabbit anti-mouse IgE at a concentration of 1 µg/ml (Biosource International, Camarillo, Calif., USA). The results were expressed as absorbance (blank value was subtracted).

Statistical Analysis

Statistical analysis of the histologic evaluation of inflammation and epithelial cells in the airway was performed using the ANOVA test. The values of cytokine levels were expressed as mean ± SE. Statistical analysis of cytokine proteins and Der f-specific IgG1 or IgE was conducted using Student's *t* test. Differences between groups were considered statistically significant at a level of *p* < 0.05.

Results

Histologic Changes in Murine Airways

Figure 1 shows the histologic changes in the lungs from mice treated with Der f alone or Der f + DEP. In the case of the CBA/JN mice, Der f administration caused a slight recruitment of eosinophils to the submucosal layer of the bronchi and bronchioles. Der f + DEP administration caused a moderate but significant recruitment of

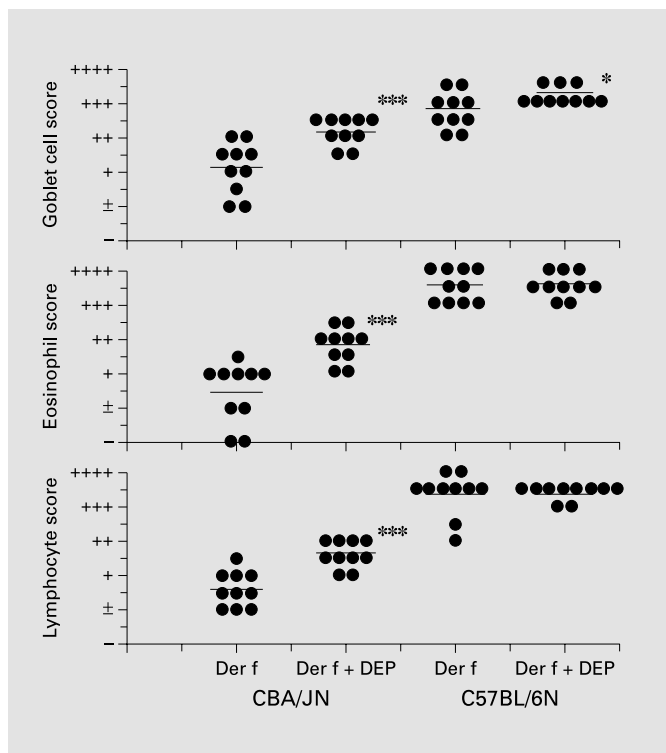


Fig. 1. Histologic changes in goblet cell proliferation, recruitment of eosinophils and lymphocytes in the airways; $n = 10$ in each group. Results are shown as mean and individual values. Individual values are the mean of two experiments. Significant differences were evaluated with the ANOVA test; * $p < 0.05$ and *** $p < 0.001$ between the groups treated with Der f alone and Der f + DEP.

eosinophils. In the case of the C57BL/6N mice, Der f alone or Der f + DEP led to a moderate to marked recruitment of eosinophils. However, the exact effect of DEP could not be evaluated well because of the severe symptom enhancement it causes. Similar changes in lymphocytes were observed between the two strains treated with Der f alone and Der f + DEP.

Der f alone induced a marked proliferation of goblet cells in the airway epithelium of the C57BL/6N mice. The degree of proliferation induced in these mice was much greater than that in the CBA/JN mice. Der f + DEP induced a far greater proliferation of goblet cells both in the CBA/JN mice ($p < 0.001$) and the C57BL/6N ($p < 0.05$) than Der f alone. The degree of goblet cell proliferation in a group treated with Der f or Der f + DEP was consistent with the degree of recruitment of eosinophils or lymphocytes to the submucosal layer of the airways.

Figure 2 shows the immunohistochemical localization of RANTES. The immunoreactivity of RANTES was often observed in alveolar macrophages and eosinophils in the CBA/JN (fig. 2a) and C57BL/6N mice treated with Der f alone. A positive protein was found in the ciliated and the nonciliated epithelial cells of the bronchus (fig. 2b). There were less RANTES-positive cells in the airway epithelium of the CBA/JN mice treated with Der f + DEP than in those treated with Der f alone.

The immunohistochemical localization of eotaxin is seen in figure 2c, d. Immunohistochemical staining of eotaxin was frequently observed in alveolar macrophages, alveolar cells, endothelial cells in the alveolar walls, eosinophils recruited into the submucosal layer, and in the interstitium around the peribronchial vessels. Immunohistochemical staining of eotaxin was observed particularly in mice treated with Der f + DEP. A positive protein was found in ciliated and nonciliated airway epithelial cells (which contain Clara cells, the fibroblasts in the lamina propria of airways) and in the smooth muscle of vessels. The immunoreactivity of eotaxin-positive cells in CBA/JN and C57BL/6N mice treated with Der f + DEP was more intense than that of eotaxin-positive cells in CBA/JN and C57BL/6N mice treated with Der f alone.

Local Cytokine Expression in Lung Tissue Supernatant

Table 1 shows the cytokine protein levels of IL-2, IL-4, IL-5, eotaxin, RANTES and GM-CSF in the lung tissue supernatants obtained from mice treated with Der f or Der f + DEP. In CBA/JN mice, Der f + DEP increased the protein levels of IL-4, IL-5, RANTES and eotaxin markedly more than Der f alone. This phenomenon was particularly striking in the case of IL-5 and eotaxin. The increased protein levels of IL-5 ($p < 0.05$), RANTES ($p < 0.05$) and eotaxin ($p < 0.001$) were statistically significant. When C57BL/6N and CBA/JN mice were treated with Der f alone, the protein levels of IL-4, IL-5, RANTES and eotaxin in the lung tissue of C57BL/6N mice were higher than in CBA/JN mice by a factor of 1.26 (IL-4), 5.26 (IL-5), 2.07 (RANTES) and 3.27 (eotaxin). DEP significantly increased the eotaxin in the lung tissue of C57BL/6N mice. IL-2 and GM-CSF protein levels in the lung tissues of CBA/JN and C57BL/6N mice treated with Der f or Der f and DEP were at or below the detection limits. However, the GM-CSF protein level in the lung tissue from Der f + DEP-treated C57BL/6N mice increased significantly compared with GM-CSF levels in the lung tissue of mice treated with Der f alone.

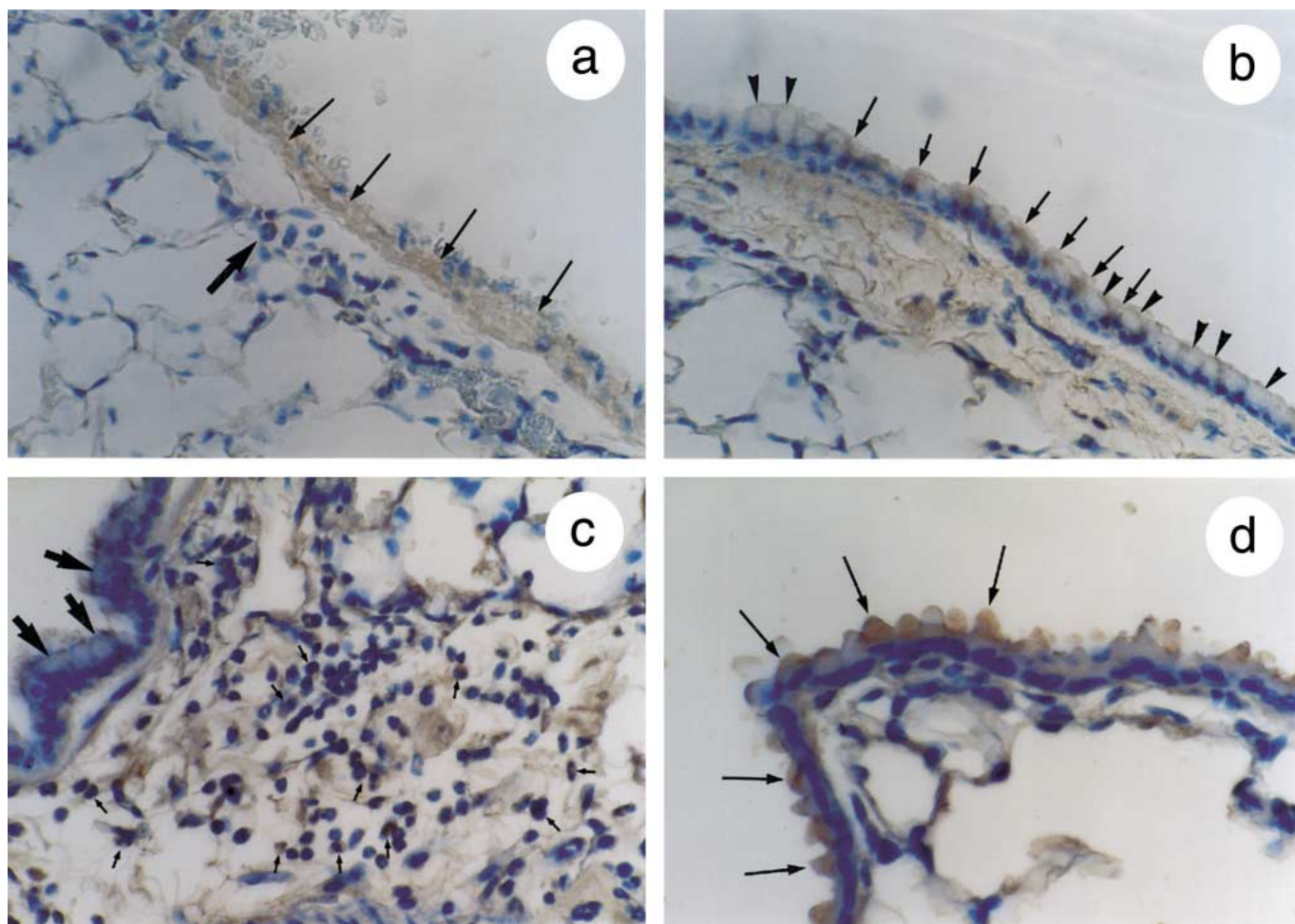


Fig. 2. Immunohistochemical localization of RANTES and eotaxin. a Light micrograph of the vessel of a CBA/JN mouse treated with Der f alone. The thinner arrows show the RANTES, localized in the smooth muscles of a vessel. The thicker arrow shows the RANTES-positive eosinophil in the interstitium around a vessel. $\times 132$. b Light micrograph of the airway of a CBA/JN mouse treated with Der f alone. Arrows show the RANTES with positive epithelial cells. Arrowheads indicate the absence of RANTES-positive goblet cells in

the bronchial epithelium. $\times 132$. c Light micrograph of the airway of a CBA/JN mouse treated with Der f and DEP. Small arrows show that the eotaxin is localized in eosinophils in the submucosal layer of the airway. The large arrows indicate the absence of eotaxin-positive goblet cells in the bronchial epithelium. $\times 132$. d Light micrograph of the airway of a CBA/JN mouse treated with Der f alone. Arrows show the eotaxin localized in Clara cells of the bronchial epithelium. $\times 200$.

Table 1. Protein levels of cytokines in murine lung tissue supernatants (pg/total lung tissue supernatants)

Mouse strain	Group	IL-2	IL-4	IL-5	Eotaxin	RANTES	GM-CSF
CBA/JN	Der f	ND	233.6 $\pm$ 25.2	37.7 $\pm$ 16.5	315.0 $\pm$ 72.9	108.4 $\pm$ 19.4	ND
	Der f + DEP	ND	268.4 $\pm$ 19.5	198.4 $\pm$ 75.2*	1,998.2 $\pm$ 65.0***	183.0 $\pm$ 15.6*	ND
C57BL/6N	Der f	ND	293.6 $\pm$ 12.8	112.6 $\pm$ 22.2	1,030.0 $\pm$ 108.3	224.5 $\pm$ 21.9	3.7 $\pm$ 1.1
	Derf + DEP	9.11 $\pm$ 1.9	294.4 $\pm$ 32.4	142.5 $\pm$ 34.3	1,772.6 $\pm$ 288.6*	225.3 $\pm$ 23.9	14.0 $\pm$ 3.6*

ND = Not detected; * $p < 0.05$, *** $p < 0.001$ compared with the group treated with Der f alone; $n = 6$ in each group. Results are shown as mean $\pm$ SE.

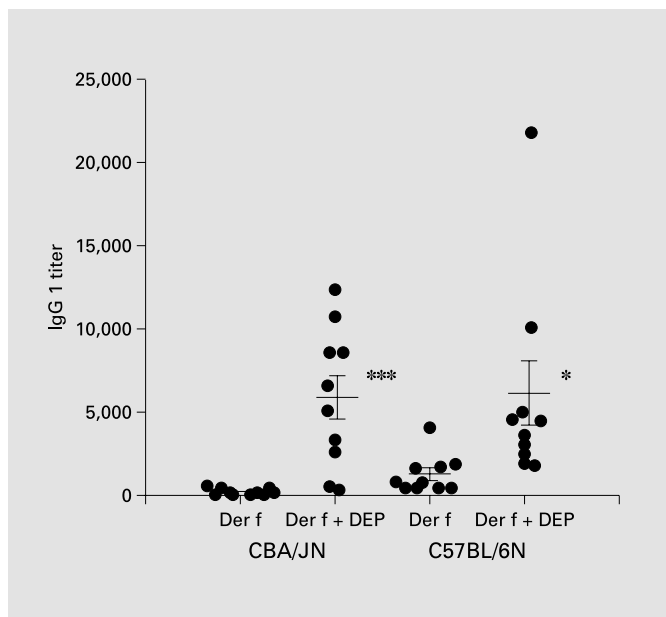


Fig. 3. Antigen-specific IgG1 responses in plasma of CBA/JN and C57BL/6N mice treated 4 times with Der f or Der f + DEP at 2-week intervals; n = 10 in each group. Results are shown as mean $\pm$ SE and individual values. Significant differences were examined using Student's t test; * $p < 0.05$ and *** $p < 0.001$ compared with a group treated with Der f alone.

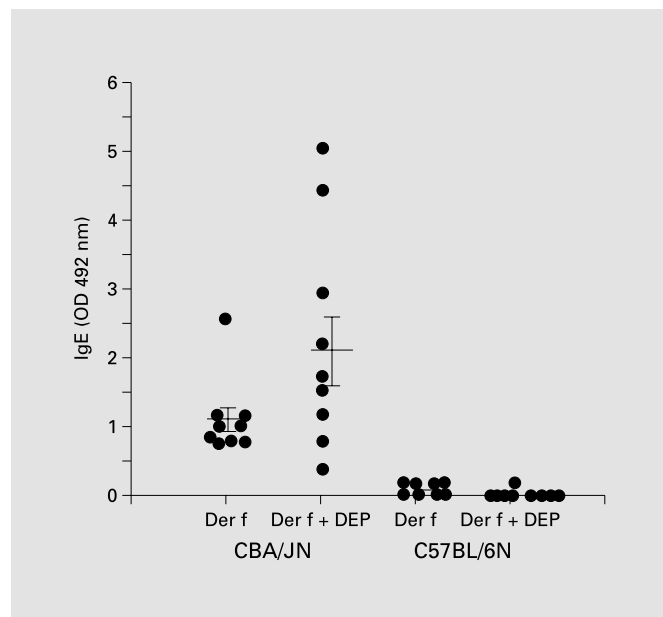


Fig. 4. Antigen-specific IgE responses in plasma of CBA/JN and C57BL/6N mice treated 4 times with Der f or Der f + DEP at 2-week intervals; n = 10 in each group. Results are shown as mean $\pm$ SE and individual values. The samples with an absorbance above 3 (upper limit of detection) were double diluted 2 and then measured.

Table 2. Protein levels of cytokines in murine plasma (pg/ml)

Mouse strain	Group	IL-5	RANTES	IL-4
CBA/JN	Der f	ND	8.7 ± 1.0	ND
	Der f + DEP	ND	3.0 ± 0.5	ND
C57BL/6N	Der f	10.4 ± 1.7	9.2 ± 0.7	6.0 ± 0.9
	Derf + DEP	10.0 ± 2.2	9.6 ± 0.8	4.5 ± 1.5

ND = Not detected; n = 6 in each group. Results are shown as mean $\pm$ SE.

Cytokine Expression in Plasma

Table 2 shows the cytokine protein levels in murine plasma. The protein levels of IL-5, and IL-4 in the plasma from CBA/JN mice treated with Der f alone or Der f + DEP were below the detection limits. The protein levels of IL-5, IL-4 and RANTES in the plasma from C57BL/6N mice treated with Der f alone or Der f + DEP were slightly higher than the detection limits. However, these protein levels were unaffected by DEP.

Der f-Specific Immunoglobulin Production in Plasma

Figure 3 shows the titers of Der f-specific IgG1 in the plasma of CBA/JN and C57BL/6N mice. The mean titers of antigen-specific IgG1 in CBA/JN and C57BL/6N mice treated with Der f alone were 137 ± 50 and $1,005 \pm 328$, respectively. The mean titers of antigen-specific IgG1 in CBA/JN and C57BL/6N mice treated with Der f + DEP were $5,831 \pm 1,318$ and $6,073 \pm 1,920$, respectively. The adjuvant effect of DEP on IgG1 production was observed in both strains treated with Der f + DEP. The adjuvant activities in the groups treated with Der f + DEP were statistically significant (CBA/JN: $p < 0.001$ and C57BL/6N: $p < 0.05$). The titers of antigen-specific IgG1 increased more in C57BL/6N mice than in CBA/JN mice when they were treated either with Der f alone or Der f + DEP. The production levels of the antigen-specific IgG1 did not always correspond to the degree of the histologic manifestations in the airways.

Figure 4 shows the absorbance of Der f-specific IgE in the plasma of the two strains. The results are shown in absorbance units as allergen-specific IgE antibodies, which were lower than 1 titer in both mouse strains. Mean values of the antigen-specific IgE in CBA/JN and C57BL/

6N mice treated with Der f alone were 1.12 ± 0.17 and 0.10 ± 0.02 , respectively. Mean values of the antigen-specific IgE in CBA/JN and C57BL/6N mice treated with Der f + DEP were 2.10 ± 0.5 and 0.04 ± 0.03 , respectively. The adjuvant effect of DEP on IgE production was observed in CBA/JN mice when they were treated with Der f + DEP. The allergen or DEP did not participate in the increase in Der f-specific IgE in C57BL/6N mice. The levels of antigen-specific IgE in the two strains did not correspond to the degree of the histologic manifestations in the asthma model used in the present study.

Discussion

The murine asthma models with an intratracheal injection of mite allergen exhibited symptoms also seen in human asthma, including proliferation of goblet cells, production of mucus plugs and recruitment of eosinophils and lymphocytes to the airways. A previous study reported that the manifestation of allergic airway inflammation induced by OVA was much greater in C57BL/6N mice than in CBA/JN mice [20]. Similar results were observed in the present study. The airways of C57BL/6N mice were more susceptible to allergic reactions induced by mite allergens than those of CBA/JN mice. The manifestation of eosinophilic inflammation and goblet cell proliferation in CBA/JN mice was increased by DEP. However, the exact effect of DEP on eosinophilic inflammation could not be estimated in C57BL/6N mice because of the severe enhancement it causes. The transformation of airway epithelial cells into goblet cells by mite allergens in two strains correlates with the amount of eosinophils introduced into the submucosa of the airways. Eosinophils cause epithelial injury and hyperresponsiveness of the airways after releasing mediators, including platelet-activating factors, reactive oxygen radicals, leukotrienes, and major basic proteins [23]. Therefore, the injury of airway epithelial cells along with goblet cell proliferation may be induced by activated eosinophils.

It has been suggested that eosinophil chemotactic cytokines – such as IL-5, GM-CSF, eotaxin and RANTES – are implicated in the pathogenesis of allergic diseases by recruiting and/or activating eosinophils [24–29]. In the present study, protein levels of IL-5, RANTES and eotaxin in the lung tissues from C57BL/6N mice (which exhibited a high recruitment of eosinophils after Der f treatment) were higher than those in CBA/JN mice (which exhibited a low recruitment of eosinophils). The results of the present study suggest that these eosinophil chemotac-

tic cytokines are associated with the pathogenesis of allergic airway diseases caused by mite allergens. The differences observed in the two mouse strains may be due to the differences in the local expression of these cytokines.

DEP increased the production of RANTES, particularly IL-5 and eotaxin, in CBA/JN mice. Therefore the modulation of eosinophilic airway inflammation caused by DEP may be associated with the inflammatory mediators increased by DEP. Immunostaining of RANTES or eotaxin was observed in alveolar macrophages, ciliated epithelial cells, Clara cells and lamina propria in the airways, and also in the smooth muscles of vessels and bronchi. On the other hand, no RANTES- or eotaxin-positive goblet cells were observed in the bronchial epithelium. Hence these positive cells, including recruited eosinophils and macrophages, might be the source of these chemokines. Recruited Th2 lymphocytes might be the source of IL-5. Although the airways of C57BL/6N mice seem to be more susceptible to DEP than those of CBA/JN mice, the DEP did not increase eosinophilia in the airways of C57BL/6N mice. The airway inflammation caused by Der f alone in C57BL/6N mice might have reached the maximum eosinophil recruitment before the end of the experimental period. The role of DEP in the airway inflammation may be observed in the early stage of the experiment.

IL-2 is a Th1-type cytokine. No induction of IL-2 by a mite allergen was observed in the asthma model used in the present study. IL-4, which is secreted from Th2 lymphocytes, plays an important role in the formation of immunoglobulin and the development of Th2 lymphocytes in B cells [30]. When the two strains were treated with Der f, the IL-4 protein level was higher in C57BL/6N mice than in CBA/JN mice. In addition, DEP modulated IL-4 production in CBA/JN mice. These results suggest that the airway inflammation in C57BL/6N mice is more dominated by Th2 lymphocytes than that in CBA/JN mice. The IL-4 levels in CBA/JN mice suggest that DEP accelerates the differentiation from Th0 lymphocytes to Th2 lymphocytes.

Upon exposure to allergens, eosinophilic inflammation and bronchial hyperreactivity occur even in mice lacking IgE, mast cells, IL-4 or B cells [31–34]. In the present study, the level of allergen-specific IgE antibody was lower than 1 titer in the CBA/JN mice. No IgE antibody induction was observed in C57BL/6N mice. These results suggest that the airway inflammation induced by mite allergens – with or without DEP – is not associated with the antibody.

It has been suggested that the antigen-specific IgG1 antibody plays an important role in the degranulation of eosinophils. This antibody achieves the degranulation of antigen-specific eosinophils via an Fcγ RII receptor on the surface of eosinophils [35]. In the present study, the adjuvant effect of DEP on immunoglobulin production induced by mite allergens was detected in both strains used, suggesting that the degranulation of antigen-specific eosinophils was induced by an increased antigen-specific antibody.

The present study demonstrates that mite allergens from house dust could induce manifestations of allergic airway inflammation. This inflammation was different in

the two mouse strains used. DEP enhanced the allergic reaction in our asthma model. The differences in the pathogenesis of allergic airway inflammation caused by mite allergens in the two strains may be associated with the local expression of IL-4, IL-5, RANTES and eotaxin. The aggravating effect of DEP may be due to an increase in the local expression of these eosinophil chemotactic cytokines and the production of antigen-specific IgG1. The results of the present study suggest that the cause of individual differences in humans at the onset of allergic asthma could be associated with differences in the eosinophil expression relevant to cytokines and antigen-induced immune responses such as IgG1.

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