



Intranasal mite allergen induces allergic asthma-like responses in NC/Nga mice

Masafumi Shibamori ^{a,b}, Keiki Ogino ^{a,*}, Yasuhiro Kambayashi ^a, Hironobu Ishiyama ^b

^a Department of Environmental and Preventive Medicine, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8640, Japan

^b Third Institute of New Drug Discovery, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

Received 29 March 2005; accepted 5 June 2005

Abstract

Airway responses induced by intranasal administration of mite allergen without adjuvant were studied in NC/Nga mice. A crude extract of *Dermatophagoides farinae* (Df) was administered for 5 consecutive days and a single intranasal challenge booster dose was given 1 week after the last sensitization. 24 h after the single challenge, the airway hyperresponsiveness (AHR) was measured and the bronchoalveolar lavage fluid (BALF) was analyzed for numbers of eosinophils and neutrophils, and both cytokine and chemokine levels. There were marked increases in number of eosinophils in the BALF, AHR, Th2 cytokines (IL-5 and IL-13), and chemokine (eotaxin-1 and eotaxin-2) levels in the BALF following Df exposure. C57BL/6N, A/J, BALB/c, and CBA/JN mouse strains were also exposed to Df crude extract, but all of the measured responses were strongest in NC/Nga mice. Furthermore, Df-exposed NC/Nga mice showed the goblet cell hyperplasia, pulmonary eosinophilic inflammation, and increases in both total serum IgE and Df-specific IgG1. After intranasal exposure of NC/Nga mice to crude extract of *Dermatophagoides pteronyssinus*, the BALF eosinophilia and AHR were similar to responses induced by Df. None of the study parameters were increased in response to intranasal exposure to ovalbumin. These data demonstrated that NC/Nga mice developed allergic asthma-like responses after intranasal exposure to mite allergens.

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Keywords: Mite allergen; Intranasal administration; Airway inflammation; Airway hyperresponsiveness; NC/Nga mice

Introduction

The prevalence of asthma is increasing worldwide (Woolcock and Peat, 1997). The reasons for this increase are not known. However, both intrinsic and extrinsic factors may be involved. As extrinsic factors, proteinaceous allergens, such as mold, ragweed, pollens, cockroach, and dust mite, contribute to the etiology of asthma. House dust mites, including *Dermatophagoides farinae* (Df) and *Dermatophagoides pteronyssinus* (Dp), are known to be major extrinsic factors in the development of asthma (Huss et al., 2001; Peat et al., 1996; Platts-Mills et al., 1997; Sporik et al., 1990).

Pathophysiology of asthma is characterized by reversible airway obstruction, airway hyperresponsiveness (AHR), and airway inflammation. Several pathological changes, such as infiltration of activated eosinophils, goblet cell hyperplasia,

and mucus overproduction, are also involved (Busse and Lemanske, 2001). The eosinophil is thought to be a major effector cell in the pathogenesis of allergen-mediated AHR because it releases various types of cytotoxic granule proteins that induce airway injury. In animal asthma models, infiltration and activation of eosinophils are closely related to the development and resolution of AHR (Tomkinson et al., 2001; Underwood et al., 1995).

In general, most experimental allergic asthma models have used ovalbumin (OVA) as the allergen, although OVA is not considered to be a common human airway allergen. Furthermore, most experimental allergic asthma models have been developed by systemic sensitization that involves either intraperitoneal or subcutaneous injection with adjuvants, such as aluminum hydroxide. Some investigators have developed mouse models of allergic airway eosinophilia (Ichinose et al., 2003; Sadakane et al., 2002; Yu et al., 1999), allergen-induced AHR (Herz et al., 1996), or allergen-induced airway obstruction (Yasue et al., 1998) using only local exposure of mite

* Corresponding author. Tel.: +81 76 265 2218; fax: +81 76 234 4233.

E-mail address: ogino@pub.m.kanazawa-u.ac.jp (K. Ogino).

allergen without adjuvant. The intent of such models is to mimic the route of sensitization in humans as closely as possible. A novel model with characteristics of both airway eosinophilia and AHR induces the asthmatic response in BALB/c mice by intranasal exposure of mite allergen (Cates et al., 2004; Johnson et al., 2004).

NC mice were established as an inbred strain in 1955 (Kondo et al., 1969) and have been used as an animal model of human atopic dermatitis (AD) (Suto et al., 1999; Vestergaard et al., 2000). Repeated topical application of mite allergen to the skin induces the development of AD-like skin lesions in the NC strain (NC/Nga mice), but not in BALB/c mice (Matsuoka et al., 2003; Sasakawa et al., 2001). However, whether NC/Nga mice develop allergic asthma-like responses after intranasal exposure to mite allergens is unknown.

In the present study, we demonstrate that NC/Nga mice develop allergic asthma-like responses after intranasal exposure to mite allergens without using adjuvant. Furthermore, the cellular inflammation, cytokine and chemokine levels in the bronchoalveolar lavage fluid (BALF), and AHR in NC/Nga mice were compared with those of other mouse strains (C57BL/6N, A/J, BALB/c, and CBA/JN) after intranasal exposure to Df allergen.

Materials and methods

Animals

8-week-old, male NC/Nga, BALB/c, C57BL/6N, and CBA/JN mice (Charles River Japan, Osaka, Japan) and A/J mice (SLC Japan, Shizuoka, Japan) were used. Mice were maintained under specific pathogen-free conditions and a 12 h light–12 h dark cycle with free access to water and standard laboratory food. Mice were acclimated 1 week prior to use in the experiments. The care and handling of the animals were in accordance with the Guidelines for the Care and Use of Laboratory Animals at Takaramachi Campus of Kanazawa University.

Materials

Df crude extract, Dp crude extract and OVA were purchased from COSMOBIO (Tokyo, Japan). Acetylcholine chloride was purchased from Daiichi Pharmaceutical (Tokyo, Japan). Gallamine triethiodide was purchased from Sigma (St. Louis, MO). Sodium pentobarbital was purchased from Dainabot (Osaka, Japan).

Intranasal exposure of mite allergen to mice

To establish whether allergic asthma-like responses could be induced experimentally using mite allergens, the intranasal dose of mite allergens was similar to dose that was previously used (Yasue et al., 1998). Mice were anesthetized with an intraperitoneal (ip) injection of 40 mg/kg of sodium pentobarbital, followed by intranasal instillation of 100 µg of Df crude extract in 50 µl of physiological saline. The same dose of Df was given intranasally on 5 consecutive days (days 0–4, sensitization). On

day 11, a single challenge with 100 µg of Df crude extract in 50 µl of physiological saline was administered intranasally under anesthesia. The Dp and OVA allergens used in this study were administered using the same dose and schedule as for Df. Saline was used as control for comparison with allergens.

Measurement of AHR to acetylcholine

The degree of bronchoconstriction was measured according to the overflow method of Konzett and Rössler (1940). Dose–response curves to acetylcholine in anesthetized, ventilated mice were obtained 24 h after the single intranasal challenge. Mice were anesthetized with sodium pentobarbital (80 mg/kg, ip). The trachea was surgically exposed, cannulated, and connected to a rodent ventilator (Model 132; New England Medical Instrument Inc., Medway, Mass) and a bronchospasm transducer (Model 7020; Ugo Basile, Comerio-Varese, Italy). An animal was mechanically ventilated with air at 60 strokes/min with a stroke volume of 0.7 ml. After an incision in the abdomen, the abdominal vein was cannulated for administration of acetylcholine. A paralytic agent, gallamine triethiodide (350 µg/mouse), was administered intravenously (iv) to eliminate spontaneous respiration. After the airway pressure stabilized, acetylcholine was administered iv and dose was increased in a stepwise manner. The bronchoconstriction data were recorded on an Omnicore II data acquisition system (Model RA1300; NEC San-ei, Tokyo, Japan). Bronchoconstriction was expressed as the percentage of the respiratory overflow volume provoked by acetylcholine to the maximal overflow volume (100%) obtained by totally occluding the tracheal cannula (Foster et al., 1996; Nagai et al., 1993). The AHR to acetylcholine was evaluated by the concentration of acetylcholine that caused 25% level of the maximum constriction (PD₂₅; µg/kg) and the degree of increased AHR was evaluated by the ratio of PD₂₅ value in each strain of Df-exposed to that of the corresponding strain of naive mice.

Analysis of cells in the BALF

Immediately after the assessment of acetylcholine-induced AHR, the lungs were lavaged with 1.0 ml of physiological saline via the tracheal cannula while gently massaging the thorax. The number and differential cell types of leukocytes in the BALF were determined using a hematology analyzer (ADVIA 120; Bayer Medical, Tokyo, Japan) that was set in the mouse hematology analysis mode.

Levels of cytokines and chemokines in the BALF

The cell-free supernatant was recovered by centrifuging BALF samples at 150 × g at 4 °C for 10 min. The concentrations of IL-4, IL-5, IL-13, eotaxin-1 and IFN-γ were determined by ELISA kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Eotaxin-2 levels were determined in matched antibody pairs by ELISA (R&D Systems, Minneapolis, MN). Briefly, Immuno Maxisorp 96 well microtiter plates (Nunc, Roskilde, Denmark) were

coated with 0.2 $\mu\text{g}/100 \mu\text{l}$ /well of capture antibody against mouse eotaxin-2 (MAB528) and incubated overnight at 4 °C. After blocking with phosphate buffered saline (PBS) containing 5% fetal bovine serum (FBS) and washing with PBS containing 0.05% Tween 20 (PBST), BALF samples were added to the plate and incubated overnight at 4 °C. The mouse eotaxin-2 bound to the plates was quantitated using biotinylated anti-mouse eotaxin-2 (BAF528), horseradish peroxidase (HRP)–streptavidin conjugate (Zymed, San Francisco, CA), and 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (COSMOBIO, Tokyo, Japan). Standard curves were constructed using sequentially diluted mouse eotaxin-2 (R&D Systems, Minneapolis, MN). The detection limits of the assays for IL-4, IL-5, IL-13, eotaxin-1, IFN- γ , and eotaxin-2 were 31.3, 31.3, 31.3, 31.3, 18.8, and 313 pg/ml, respectively.

Total IgE and Df or OVA-specific IgG1 levels in the sera

In a separate experiment, mice were exposed to saline, Df or OVA, using the methods described for intranasal exposure. On day 12 and under anesthesia with diethyl ether, blood was

collected from the abdominal vein. Blood samples were centrifuged at $1300 \times g$ at 4 °C for 15 min to obtain sera. Both Df and OVA-specific serum IgG1 were measured by ELISA. Briefly, Immuno Microsorp 96 well microtiter plates (Nunc, Roskilde, Denmark) were coated with 2 $\mu\text{g}/100 \mu\text{l}$ /well of either Df or OVA and incubated overnight at 4 °C. After blocking with PBS containing 5% FBS and washing with PBST, serum samples diluted in PBS containing 5% FBS were added to the plate and incubated overnight at 4 °C. The bound IgG1 antibodies were quantitated colorimetrically using biotinylated anti-mouse IgG1 (Zymed, San Francisco, CA), HRP–streptavidin conjugate (Zymed, San Francisco, CA), and TMB substrate solution (COSMOBIO, Tokyo, Japan). Results were expressed as optical density (OD450 nm) after subtraction of background. For IgE, total levels were quantified by ELISA (Morinaga, Kanagawa, Japan).

Histopathological examination of the lungs

The lungs were fixed with 10% buffered formalin and embedded in paraffin. Sections (3 μm) were stained with

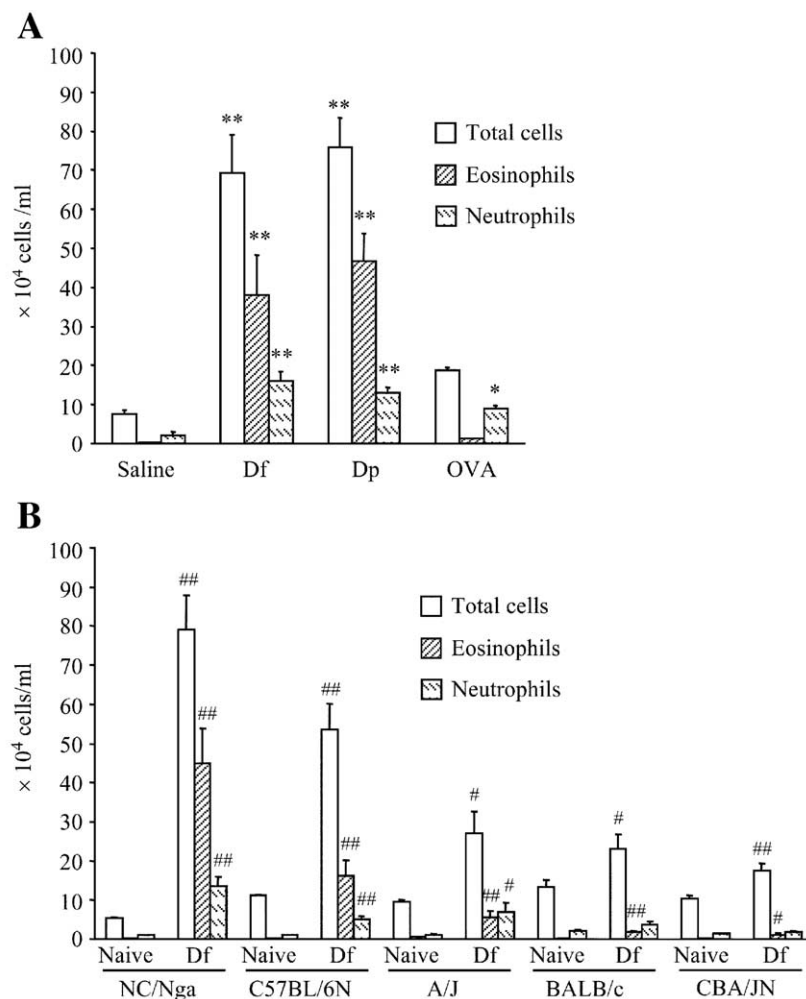


Fig. 1. The number of total cells, eosinophils and neutrophils in the BALF. (A) Effect of Df, Dp, or OVA induced airway eosinophilia in NC/Nga mice. Data were collected in 2 independent experiments and results obtained from 10 animals/group. (B) Strain differences in cellular inflammation following Df exposure to the 5 inbred strains of mice. Data were collected in 2 independent experiments and results from 7–13 animals/group are shown. The counts of total cells, eosinophils and neutrophils in the BALF are expressed as mean $\pm$ SE. * P < 0.05 and ** P < 0.01 vs. the saline-exposed group. # P < 0.05 and ## P < 0.01 vs. each naive group.

hematoxylin and eosin (H&E) for general morphologic analysis or with periodic acid-Schiff (PAS) for detecting the presence and distribution of goblet cells.

Statistical analyses

Unpaired *t*-test was used to evaluate the significance of differences between two groups. One-way analysis of variance

(ANOVA) followed by Dunnett's test was used to evaluate the significance of differences between more than two groups. Two-way ANOVA or two-way ANOVA followed by Dunnett's test was used to determine the significance of differences in the dose response studies. $P < 0.05$ was considered statistically significant. The PD25 and 95% confidence interval were calculated for each group with linear regression analysis. All measurements are expressed as mean $\pm$ SE. All statistical

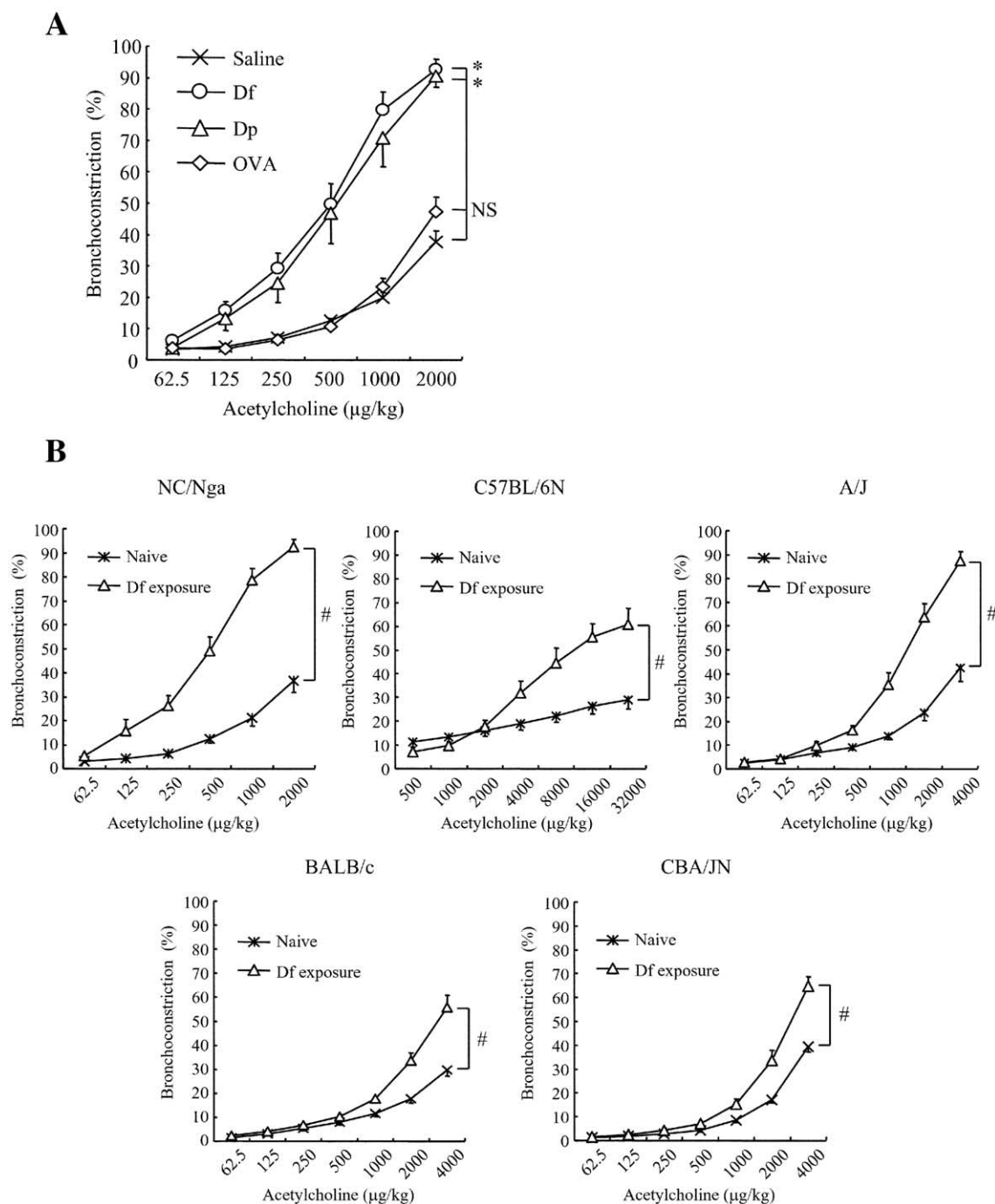


Fig. 2. The AHR to acetylcholine. (A) Effect of Df, Dp, or OVA induced increase in AHR in NC/Nga mice. Data were collected in 2 independent experiments and results obtained from 8–9 animals/group. (B) Strain differences in AHR following Df exposure to the 5 inbred strains of mice. Data were collected in 2 independent experiments and results from 7–12 animals/group are shown. The bronchoconstriction (%) is expressed as mean $\pm$ SE. * $P < 0.01$ vs. the dose response to acetylcholine in the saline-exposed group. # $P < 0.01$ vs. the dose response to acetylcholine in each naive group. NS, not significant.

Table 1
Impact of *Dermatophagoides farinae* (Df) exposure on PD25 values in 5 strains of mice

Mouse strain	PD25 ^a µg/kg (95% confidence interval)		Naive/Df-exposed ratio
	Naïve	Df exposed	
NC/Nga	1482 (1128–1947)	319 (275–371)	4.6
C57BL/6N	17,460 (9525–32,005)	4580 (3867–5426)	3.8
A/J	2202 (1709–2838)	854 (742–984)	2.6
BALB/c	3040 (2198–4206)	1517 (1356–1698)	2.0
CBA/JN	3190 (2443–4166)	1716 (1380–2133)	1.9

^a The concentration of acetylcholine that caused 25% level of the maximum constriction.

analyses were performed using SAS software (Release 8.1, SAS Institute Japan Ltd.).

Results

Distribution of cells in the BALF

The effect of mite allergen induced airway eosinophilia was investigated by instilling NC/Nga mice intranasally with saline, Df, Dp, or OVA (Fig. 1A). The eosinophils had the greatest increase of the cells in BALF after the exposure to Df and Dp in NC/Nga mice (Fig. 1A). OVA exposure resulted in no increase in eosinophils. The neutrophils in BALF of Df or Dp-exposed NC/Nga mice increased to a level that was less than half of that of eosinophils. The mononuclear cells; specifically lymphocytes and monocytes/macrophages, in Df, Dp and OVA-exposed mice also increased relative to the saline-exposed group (data not shown).

The strain differences in distribution of the BALF cells among 5 inbred strains of mice following intranasal administration of Df allergen were examined (Fig. 1B). The increases in total cell numbers, eosinophils and neutrophils in BALF were greatest in NC/Nga mice compared with C57BL/6N, A/J, BALB/c, and CBA/JN mice.

AHR to acetylcholine

The effect of mite allergen induced increase in AHR to acetylcholine was investigated by intranasal instillation of saline, Df, Dp, or OVA to NC/Nga mice (Fig. 2A). Significant increases in AHR to acetylcholine after intranasal administration of both Df and Dp, but not OVA, when NC/Nga mice were

compared to the saline-exposed group, were observed. The increased AHR levels between Df and Dp were not significantly different.

The differences in AHR to acetylcholine after intranasal administration of Df allergen to 5 inbred strains of mice are shown in Fig. 2B. All 5 strains of mice showed increases in AHR to acetylcholine compared to naive mice (Fig. 2B). Both PD25 and the ratio of PD25 values indicated that among the 5 strains tested, the highest AHR was in the NC/Nga mice (Table 1).

Cytokine and chemokine levels in the BALF

Cytokine and chemokine levels in the BALF of 5 strains of mice exposed with Df allergen are shown in Table 2. Df-exposed NC/Nga mice showed higher levels of IL-5, IL-13, eotaxin-1 and eotaxin-2 than C57BL/6N mice. The levels of IL-5, IL-13, eotaxin-1 and eotaxin-2 were undetectable in the BALF of Df-exposed BALB/c and CBA/JN mice. Only eotaxin-2 was elevated in the A/J mice. None of the 5 strains tested had detectable levels of IL-4 and IFN-γ.

Histopathology in the lungs of NC/Nga mice

Severe peribronchial and perivascular inflammation were observed in the lungs of Df-exposed mice (Fig. 3B) and a large number of eosinophils were observed in the inflammatory regions (Fig. 3C). The goblet cell hyperplasia in the lungs was also observed (Fig. 3E). In contrast, no inflammation (Fig. 3A) and only a few PAS-positive epithelial cells (Fig. 3D) were observed in the lungs of saline-exposed mice. The lungs of OVA-exposed mice had no evidence of either inflammation or goblet cell hyperplasia (data not shown).

Serum total IgE and Df or OVA-specific IgG1 levels

The serum total IgE levels were significantly increased in the Df-, but not in OVA-exposed, mice (Table 3). Both Df and OVA exposure significantly increased the antigen-specific IgG1 level in the sera.

Discussion

Our experiments showed that NC/Nga mice developed allergic asthma-like responses following intranasal exposure to mite allergens. Of the 5 mouse strains tested, the airway

Table 2
Cytokine and chemokine levels in the bronchoalveolar lavage fluid (BALF) of 5 strains of *Dermatophagoides farinae* (Df)-exposed mice

Cytokine/chemokine assayed	Levels of cytokine/chemokine quantified in mouse strain				
	NC/Nga	C57BL/6N	A/J	BALB/c	CBA/JN
IL-4 (pg/ml)	<31.3 ^a	<31.3	<31.3	<31.3	<31.3
IL-5 (pg/ml)	112.2±18.6	69.7±49.1	<31.3	<31.3	<31.3
IL-13 (pg/ml)	197.5±38.7	64.7±40.9	<31.3	<31.3	<31.3
IFN-γ (pg/ml)	<18.8	<18.8	<18.8	<18.8	<18.8
Eotaxin-1 (pg/ml)	67.3±9.4	<31.3	<31.3	<31.3	<31.3
Eotaxin-2 (pg/ml)	7087.1±1264.3	4605.1±2275.0	3102.8±1389.5	<313	<313

^a Results from 5 animals/group are shown and expressed as mean±SE.

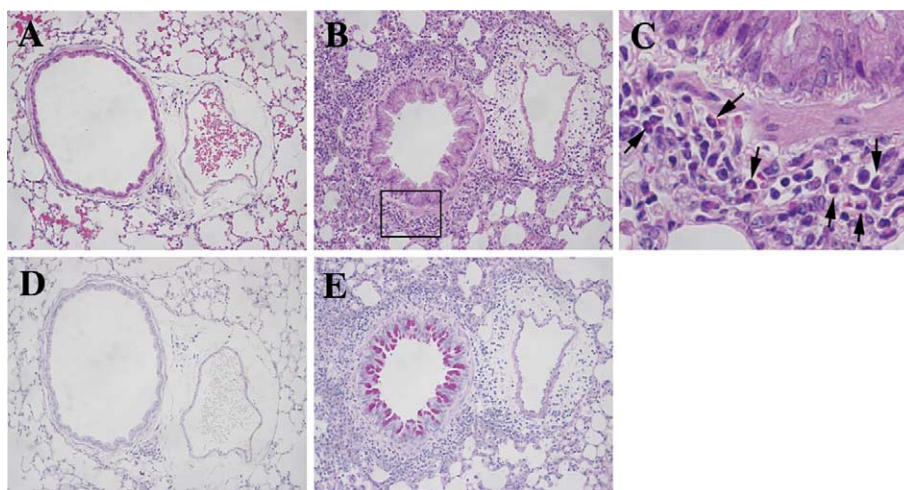


Fig. 3. Histopathology of lungs from saline or Df-exposed NC/Nga mice. H&E-stained lung sections (A) from the saline-exposed mice, (B) from the Df-exposed mice, and (C) from high magnification ($\times 600$) of (B) are shown. PAS-stained lung sections (D) from the saline-exposed mice, (E) from the Df-exposed mice are shown. Original magnification, $\times 200$. The arrows indicate eosinophils.

inflammation and severity of AHR were the strongest in Df-exposed NC/Nga mice. The presence of eosinophils is a characteristic feature of allergic asthma and a correlate of both disease severity and AHR. A number of studies have highlighted the eosinophil as a major effector cell in allergic AHR (Foster et al., 1996; Hamelmann et al., 1999; Hogan et al., 1997; Lee et al., 2004). We observed a selective increase in numbers of BALF eosinophils after Df-exposure in the NC/Nga strain. In addition, the number of eosinophils was higher than the number of neutrophils in the BALF of both Df-exposed NC/Nga and C57BL/6N mice. C57BL/6N mice are highly susceptible to the development of airway eosinophilia after intratracheal exposure to Df allergen compared with CBA/JN mice (Sadakane et al., 2002). Our results are consistent with this report. In the present study, an increased number of eosinophils in the BALF and an increase in AHR showed a tendency to correlate in the 5 mouse strains tested. Therefore, the eosinophils may contribute to the development of AHR. We have no direct evidence to show the association of AHR with activation of eosinophils. However, it has been reported that increased AHR correlates better with the degree of activation than the number of eosinophils (Pretolani et al., 1994; Tomkinson et al., 2001). The activated eosinophil releases cytotoxic granule proteins such as major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO), which may cause AHR (Gleich et al., 1993).

Table 3
Humoral responses in sera of NC/Nga mice following exposure to *Dermatophagoides farinae* (Df) and ovalbumin (OVA) allergens

Allergen	Total IgE (ng/ml)	Df or OVA-specific IgG1 (OD450 nm)	
		Df	OVA
Saline	10.3 $\pm$ 2.6 ^a	0.172 $\pm$ 0.055	0.007 $\pm$ 0.004
Df	32.8 $\pm$ 6.3 ^b	0.560 $\pm$ 0.099 ^b	ND ^c
OVA	12.9 $\pm$ 2.1	ND	1.088 $\pm$ 0.009 ^b

^a Results from 5 animals/group are shown as mean $\pm$ SE.

^b $P < 0.01$ vs. the saline-exposed group.

^c Not done.

Furthermore, we observed elevated Th2-associated immunoglobulins, such as total IgE and Df-specific IgG1 in the serum of Df-exposed NC/Nga mice. Allergen-specific IgG1 plays an important role in the activation and degranulation of eosinophils and manifestation of allergic disease (Kaneko et al., 1995; Oshiba et al., 1996). EPO activity in the supernatant of BALF of Df-exposed NC/Nga mice was observed (unpublished observation). It is not clear whether activation and degranulation of eosinophils occur or whether activated eosinophils contribute to AHR in Df-exposed NC/Nga mice.

The balance between CD4⁺ T-helper cell subsets (Th1 and Th2) plays an important role in the pathogenesis of various immune related disorders. Generally, an individual with allergic asthma shows the Th2-type immune state. Th2 cytokines, including IL-4, IL-5, and IL-13, play critical roles in allergic disorders (Barnes, 1999). In the present study, the various changes in the cytokines point to an imbalance in the Th1/Th2 states that accompany the allergic response. Specifically, Th2 cytokines (IL-5 and IL-13) in the BALF were detected in Df-exposed NC/Nga mice. The Th1 cytokine (IFN- γ) was not detected in Df-exposed NC/Nga mice. Notably, IL-13 can induce AHR (Grunig et al., 1998; Wills-Karp et al., 1998) and may also contribute to the development of AHR in Df-exposed NC/Nga and C57BL/6N mice. The IL-4 level was below the detection limit of the assay in the BALF of intranasal Df-exposed in all 5 strains of mice. Similarly, Yu et al. reported that IL-4 was undetectable by ELISA in the BALF of BALB/c mice that were given repeated intratracheal inoculations of mite allergen (Yu et al., 1999). Furthermore, Ward et al. compared the respiratory responses to intratracheal inoculation of *Metarhizium anisopliae* extract after intratracheal sensitization without adjuvant and intraperitoneal sensitization with adjuvant in BALB/c mice. By ELISA, IL-4 was detected in the BALF of intraperitoneally sensitized mice but not intratracheally sensitized mice (Ward et al., 2000). Following airway mucosal sensitization, the magnitude of IL-4 response may differ from that of general systemic sensitization with adjuvant.

Next, the levels of eotaxin-1 and eotaxin-2 were quantified in the BALF because they are specific chemokines for eosinophils and a large number of eosinophils in the airways were observed in Df-exposed NC/Nga mice. The levels of eotaxin were somewhat strain dependent since eotaxin-1 was detected only in BALF of Df-exposed NC/Nga mice and eotaxin-2 was detected in Df-exposed NC/Nga, C57BL/6N and A/J mice. The levels of eotaxin-2 and numbers of eosinophils in the BALF increased in tandem in these 3 mouse strains. Our data are consistent with the report that eotaxin-2 is a potent chemokine for eosinophils (Borchers et al., 2002). Eotaxin-2, as well as eotaxin-1, can induce eosinophil activation that results in actin polymerization and the release of reactive oxygen species (Elsner et al., 1998). Eotaxin-2 is now recognized to be a critical cofactor, along with IL-5, in regulating airway eosinophilia, IL-13 production, and the subsequent development of increased AHR (Yang et al., 2003). These findings led to the speculation that either or both eotaxin-1 and eotaxin-2 might be prerequisite for the development of airway inflammatory responses in Df-exposed NC/Nga mice.

Our results show that intranasal exposure to Df or Dp allergen, but not OVA, causes eosinophilia in the BALF and an AHR increase in NC/Nga mice. Moreover, Df allergen induced increases in numbers of BALF eosinophils and AHR in the 4 other strains tested. The studies of Johnson et al. also show continuous intranasal exposure of mite allergen, but not OVA, without adjuvant lead to the development of eosinophilia in the BALF and increase in AHR in BALB/c mice (Johnson et al., 2004). Collectively, these data suggest that intranasal exposure of mice to mite allergen has the potency to cause allergic airway responses without adjuvant.

Epidemiological studies show a high incidence of asthma and/or allergic rhinitis in the majority of infants and children with a history of AD (Spergel and Paller, 2003). Children with AD frequently have more severe asthma than asthmatic children without AD (Brinkman et al., 1997; Buffum and Settipane, 1966). The development of allergic disease including asthma and AD depends on both genetic and environmental factors (Cookson, 1999). NC/Nga mice are more sensitive than BALB/c mice to mite allergen applied intradermally or epicutaneously for the development of AD-like skin lesions (Sasakawa et al., 2001; Matsuoka et al., 2003). In our study, mite allergen exposed NC/Nga mice showed remarkable airway pathophysiological changes compared with other strains of mice. Therefore, NC/Nga mice may be useful in analyzing the mechanisms of development of asthma and atopic-like dermatitis induced by mite allergen exposure.

Acknowledgments

We thank Takahiro Asakuni, Yoko Sakamoto, and Shinya Kamitani for technical assistance.

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