

ENHANCEMENT OF MITE ALLERGEN-INDUCED EOSINOPHIL INFILTRATION IN THE MURINE AIRWAY AND LOCAL CYTOKINE/CHEMOKINE EXPRESSION BY ASIAN SAND DUST

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Data on the effects of sand dust toward allergic asthma produced by indoor allergens, such as house dust mites, are not currently available. This study was undertaken to clarify the role of Asian sand dust on mite allergen, Dermatophagoides farinae (D. farinae)-induced eosinophilic inflammation in the murine lung, using sand dusts from the Maowusu Desert (Inner Mongolia) (SD-1) and the Tengger Desert (China) (SD-2). ICR mice were intratracheally administered saline; SD-1 alone; SD-2 alone; D. farinae alone; D. farinae + SD-1; and D. farinae + SD-2, 4 times at 2-wk intervals. The two sand dusts enhanced infiltration of eosinophil in the airway, along with goblet-cell proliferation related to D. farinae. The degree of eosinophil infiltration induced with SD-2 was greater than with SD-1. The SD-1, which contained higher amounts of β -glucan, increased the expression of interferon (IFN)- γ in bronchoalveolar lavage fluids (BALF) with or without D. farinae, but SD-2 did not. Synergistically or cumulatively elevated levels of interleukin (IL)-5, eotaxin, and monocyte chemotactic protein in BALF related to D. farinae were higher with D. farinae + SD-2 than with D. farinae + SD-1. These results suggest that increased cytokine and chemokines in BALF play an important role in the enhancement of eosinophil infiltration in the airway induced by D. farinae + sand dusts. The reduced eosinophil infiltration in the SD-1-treated mice could be due to suppression of Th-2 cytokine and eotaxin via interferon- γ induced by microbial materials, such as β -glucan.

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Recently the number of allergic asthma patients has been steadily increasing globally, including in Japan (Bjorksten et al., 1998; JMESC, 2000; Shamssain & Shamssain, 2001). In particular, house-dust mites were shown to be the predominant source of the current increase in asthma prevalence (Huss et al., 2001).

Epidemiologic studies showed that ambient particulates PM_{2.5} (particle sizes less than 2.5 µm) and PM₁₀ (particle sizes of 10 µm–2.5 µm) are associated with an increase in hospital admissions for asthma (Schwartz et al., 1993) as well as development of respiratory symptoms in childhood asthma (Romieu et al., 1996; Milligan et al., 1998). PM_{2.5} and PM₁₀ contain a variety of particles, such as industrial dust, diesel soot, sea salt, and sand dust (mineral particles) of soil origin (Penner et al., 2001). The increases in atmospheric concentration of sand dust are associated with asthma severity (Rutherford et al., 1999) and adverse respiratory health effects. For example, Asian sand dust events, which spread over large areas, including East China, the Korean Peninsula, and Japan (Husar et al., 2001), are associated with an increase in daily mortality in Seoul, Korea (Kwon et al., 2002), and Taipei, Taiwan (Chen et al., 2004), and with pregnancy outcome (Yang et al., 2003). The dust also produced cardiovascular and respiratory problems in Seoul, Korea (Kwon et al., 2002), and neutrophilic airway inflammation in mice (Ichinose et al., 2005). Although it was found that diesel soot modulated the onset of bronchial asthma in several experimental studies (Ichinose et al., 1998, 2003; Takano et al., 1997), there are no investigations on the relationship between sand dust (mineral dust) and allergic airway diseases.

This study was undertaken to clarify the effects of Asian sand dusts of two different desert origins on allergic airway inflammation induced by mite allergen, *Dermatophagoides farinae* (*D. farinae*). In particular, the relationship between cytokines/chemokines and eosinophilic inflammation in the airway was examined.

MATERIALS AND METHODS

Experimental Animals

In total, 96 male ICR mice (5 wk of age) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). ICR male mice (5 wk of age) that were reported to be responsive to airway inflammation induced by *D. farinae* were used (Ichinose et al., 2003). Six-week-old mice were used after body weight and absence of infection were observed for 1 wk. They were fed a commercial diet of CE-2 (CLEA Japan, Inc., Tokyo) and were given water ad libitum. Mice were housed in plastic cages lined with softwood chips. The cages were placed in a conventional room, which was air-conditioned at 23°C and 55–70% humidity with a light/dark (12-h/12-h) cycle. The study adhered to the U.S. 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 Sciences in Oita, Japan.

Preparation of Particle Samples

Sand dust (SD) was collected from desert surface soils at two sites where dust storms occur frequently. Two samples were prepared: (1) SD-1, a sample from Yanchi Shabianzi on the southern fringe of the Maowusu Desert in the Inner Mongolia region; and (2) SD-2, a sample from Shapotou on the southern fringe of the Tengger Desert in north central China. After collection from both sites, the sands were sieved with a 250-mesh screen and then the particles were further sieved to smaller size by a separation system through a wind tunnel (16 m length) under controlled conditions (2 m/s wind speed). The collected particles were sieved to a diameter less than 10 μm using an Andersen air sampler. Approximately 1 g raw sand material was placed in a 2-L glass bottle and then purged with a clean air stream at 28.3 L/min. The headspace containing sand particulates was subsequently drawn into the Andersen air sampler, which had eight impact plates. Sand samples were recovered from the impact plates, which contained sand particulates ranging from 0.4 to 11 μm in diameter. Approximately 20 mg sand sample was obtained from 1 g raw sand material. Kaolin particles (Engelhard, Iselin, NJ) were used to examine possible contamination by lipopolysaccharide (LPS) and β -glucan from air or experimental apparatus. The kaolin particles were sieved to a diameter less than 10 μm using an Andersen air sampler as well. The particle diameter of the samples (a total of 600 particles) was measured under a scanning electron microscope (JSM-5800 JEOL Ltd., Tokyo). This procedure was repeated three times. The mean distribution peak of particle diameter in each sample was observed at 5.5 μm .

Analysis of Elements, LPS, and β -Glucan in Particles

The concentration of each element was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; 61E Trace and ICP-750, Thermo Jarrell-Ash, Grand Junction, CO). Lipopolysaccharide (LPS) and β -glucan in particles were measured according to the Bacterial Endotoxins Test in *The Japanese Pharmacopoeia* (Japan Ministry of Health and Labor, 2001).

Study Protocol

ICR mice were divided into six groups for treatment with the two different particles: saline; *D. farinae* alone; SD-1 alone; SD-2 alone; *D. farinae* + SD-1, and *D. farinae* + SD-2. These particles were suspended in normal saline for instillation (Otsuka Co., Kyoto, Japan). *Dermatophagoides farinae* was dissolved in the same saline. The instillation dose of particles was 0.1 mg per mouse (weekly doses of 0.1 mg). The amount of samples deposited in the lungs of a single mouse was 3.3-fold higher than the amount (approximately 30 μg in the case of 100% deposition) of the Japanese national air quality standard for suspended particulate matter (0.1 mg/m^3) accumulated in the lung of one mouse per week in the deposition efficiencies calculated by the tidal volume (0.15 ml) and breathing rate (200 breaths/min). However, in the case of the human respiratory tract model for radiological protection (James et al.,

1994), the deposition rate into alveoli was approximately 3% for particulate matter with 5.5 μm diameters. The amount of particulates deposited directly into alveoli was 100 $\mu\text{g}/\text{mouse}$ in the present study. Therefore, the instillation dose of particulates (100 μg) into alveoli in the present study was 111-fold higher than that going through a respiratory system (0.9 μg in the case of 3% deposition).

The instillation dose of *D. farinae* was 1 μg per mouse. Mice were intratracheally instilled with these particles through a polyethylene tube under anesthesia with 4% halothane (Takeda Chemical, Osaka, Japan). The control mice were instilled intratracheally with 0.1 ml normal saline (Otsuka Co., Kyoto, Japan) per mouse. The instillation was repeated 4 times at 2-wk intervals for the murine asthma model (Ichinose et al., 2003).

Pathological Evaluation and Bronchoalveolar Lavage

The lungs were fixed by 10% neutral phosphate-buffered formalin. After separation of the lobe, 2-mm-thick blocks were taken for paraffin embedding. Embedded blocks were sectioned at a thickness of 3 μm , and then were stained with hematoxylin and eosin (HE) to evaluate the degree of infiltration of eosinophils or lymphocytes in the airway 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. Pathological analysis of inflammatory cells and epithelial cells in the airway was performed using an Olympus BH-2 light microscope (Olympus Co., Tokyo). The degree of infiltration of eosinophils and lymphocytes in the airway or proliferation of goblet cells in the bronchial epithelium was graded on the following scale: 0, not present; 1, very slight; 2, slight; 3, moderate; 4, moderate to marked; 5, marked. “Very slight” was defined as less than 10% of the airway with eosinophilic inflammatory reaction or with goblet cells stained with PAS, “slight” as 20% to 30% of the airway, “moderate” as 40% to 50%, “moderate to marked” as 60% to 70%, and “marked” as more than 80% of the airway (Ichinose et al., 1998, 2003). Bronchoalveolar lavage fluids (BALF) and cell counts were examined by a previously reported method (Takano et al., 1997). The BALF was stored at -80°C until analysis for cytokines and chemokines.

Quantitation of Cytokines and Chemokines in BALF

The protein levels of cytokine and chemokines in BALF were determined using enzyme-linked immunosorbent assays (ELISAs). Interleukin (IL)-5 and granulocyte–macrophage colony-stimulating factor (GM-CSF) were measured using an ELISA kit from Endogen, Inc. (Cambridge, MA). Monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1 α , regulated on activation, normal T-cell expressed and presumably secreted (RANTES), and eotaxin were measured using an ELISA kit from R&D Systems, Inc. (Minneapolis, MN). Interferon (IFN)- γ and tumor necrosis factor (TNF)- α in the BALF were measured using an ELISA kit (Endogen, Inc., Cambridge, MA).

Measurement of IgG1 and IgE Antibodies

Dermatophagoides farinae-specific immunoglobulin (Ig) G1 antibody and IgE antibody were measured by a previously reported method (Takano et al., 1997). The absorption at 492 nm for *D. farinae*-specific IgG1 antibody was measured using a microplate reader (SPECTRAFluor; TECAN, Salzburg, Austria). The fluorescence intensity for *D. farinae*-specific IgE antibody was measured using a microplate reader (SPECTRAFluor; TECAN, Salzburg, Austria).

Statistical Analysis

Statistical analyses on the pathologic evaluation in the airway and on cytokine and chemokine proteins in BALF were conducted using Fisher's protected least significant differences (P/SD) test in analysis of variance (ANOVA) (Statview; Abacus Concepts, Inc., Berkeley, CA). Differences among groups were determined as statistically significant at a level of $p < .05$.

RESULTS

Concentration of Elements, LPS, and β -Glucan in the Sand Dust Samples

The element contents in SD-1 and SD-2 were 0.28 and 0.28 g/g for Si; 0.059 and 0.058 g/g for Al; 0.064 and 0.054 g/g for Ca; 0.029 and 0.029 g/g for Fe; and 0.018 and 0.017 g/g for K. The content of LPS in SD-1 and SD-2 was 2 EU/mg and 1.9 EU/mg, respectively. The content of β -glucan in SD-1 and SD-2 was 26.4 pg/mg and 12 pg/mg, respectively. LPS and β -glucan were not detected in kaolin particle sieved by an Andersen air sampler.

Enhancement of Pathologic Changes by *D. Farinae* and Sand Dusts

The immune system changes in the airway of the mice treated with *D. farinae* and/or sand dusts are shown in Table 1. *Dermatophagoides farinae* with SD-1 or SD-2 significantly increased infiltration of eosinophils compared with *D. farinae* or sand dust alone. However, the degree of infiltration of eosinophils induced by SD-2 was greater than SD-1. *Dermatophagoides farinae* + SD-1 or SD-2 also increased accumulation of lymphocytes in the airway compared with *D. farinae* or sand dust alone. The accumulation of lymphocytes induced by SD-1 was greater than by SD-2. There was also enhanced proliferation of goblet cells in the airway compared with *D. farinae* or sand dust alone. The increased levels were similar in the cases of *D. farinae* + SD-1 group and *D. farinae* + SD-2 group. Light micrographs of the pathologic changes are shown in Figure 1.

Enhancement of *D. farinae*-Induced Eosinophil Numbers in BALF by Sand Particles

Table 2 shows the cellular profile in BALF. SD-1 significantly increased neutrophil count compared with control, but SD-2 did not. *Dermatophagoides farinae* with SD-1 or SD-2 further elevated the cell number compared with

TABLE 1. Immune Parameter Changes in the Murine Airway

Group ^a	Animals (n)	Pathologic changes		
		Lymphocytes	Eosinophils	Proliferation of goblet cells
Control	8	0	0.13 ± 0.35	0
SD-1	8	1.61 ± 0.35 ^b	0.38 ± 0.16	0.44 ± 0.33
SD-2	8	1.50 ± 0.27 ^b	0.44 ± 0.15	0.38 ± 0.18
<i>D. farinae</i>	8	0.88 ± 0.30 ^b	1.13 ± 0.39 ^b	1.31 ± 0.33 ^b
<i>D. farinae</i> + SD-1	8	3.25 ± 0.25 ^{b, c}	2.75 ± 0.23 ^{b, c}	2.50 ± 0.30 ^{b, c, d}
<i>D. farinae</i> + SD-2	8	3.00 ± 0.42 ^{b, d}	3.31 ± 0.95 ^{b, d, e}	2.65 ± 0.29 ^{b, d, e}

^a Six groups of mice were treated intratracheally with normal saline (control), SD-1 (sample from Maowusu desert), SD-2 (sample from Shapotou desert), *Dermatophagoides farinae* (*D. farinae*), *D. farinae* + SD-1, and *D. farinae* + SD-2, 4 times at 2-wk intervals. The values are mean ± SD (n = 8).

^b Significant at $p < .05$ vs. control.

^c Significant at $p < .05$ vs. SD-1.

^d Significant at $p < .05$ vs. *D. farinae*.

^e Significant at $p < .05$ vs. SD-2.

SD-1 or SD-2 alone ($p < .001$). SD-1, SD-2, or *D. farinae* alone produced no change in eosinophil numbers. *Dermatophagoides farinae* with SD-1 or SD-2 significantly increased the white blood cell numbers compared with the *D. farinae* alone, or with SD-1 or SD-2. The eosinophil numbers in the *D. farinae* + SD-2 group were somewhat higher than for the *D. farinae* + SD-1 group. *Dermatophagoides farinae* with SD-1 or SD-2 increased lymphocyte count compared with SD-1 or SD-2 alone or *D. farinae* alone. A synergistic phenomenon was observed in the rise of these inflammatory cells in the combined groups.

Induction of Cytokines and Chemokines in BALF by the Sand Dust Alone

SD-1 alone significantly increased protein levels of IFN- γ and TNF- α (Table 3) compared with the control. SD-2 alone also elevated the protein level of TNF- α , but not of IFN- γ . The increased levels of IFN- γ were higher in SD-1 group than in SD-2 group. SD-1 or SD-2 alone significantly enhanced GM-CSF compared with control (Table 3). SD-1 or SD-2 alone also significantly increased RANTES and MIP-1 α compared with the control (Table 4).

Enhancement of Cytokines and Chemokines in BALF Related to *D. farinae* by Sand Dusts

Dermatophagoides farinae + SD-1 significantly increased IL-5, eotaxin, and MCP-1 (Tables 3 and 4) compared with SD-1 alone. *Dermatophagoides farinae* + SD-2 significantly enhanced IL-5, eotaxin, and MCP-1 (Tables 3 and 4) compared with SD-2 alone. *Dermatophagoides farinae* with SD-1 or SD-2 significantly increased levels of eotaxin, RANTES, MCP-1, and MIP-1 α

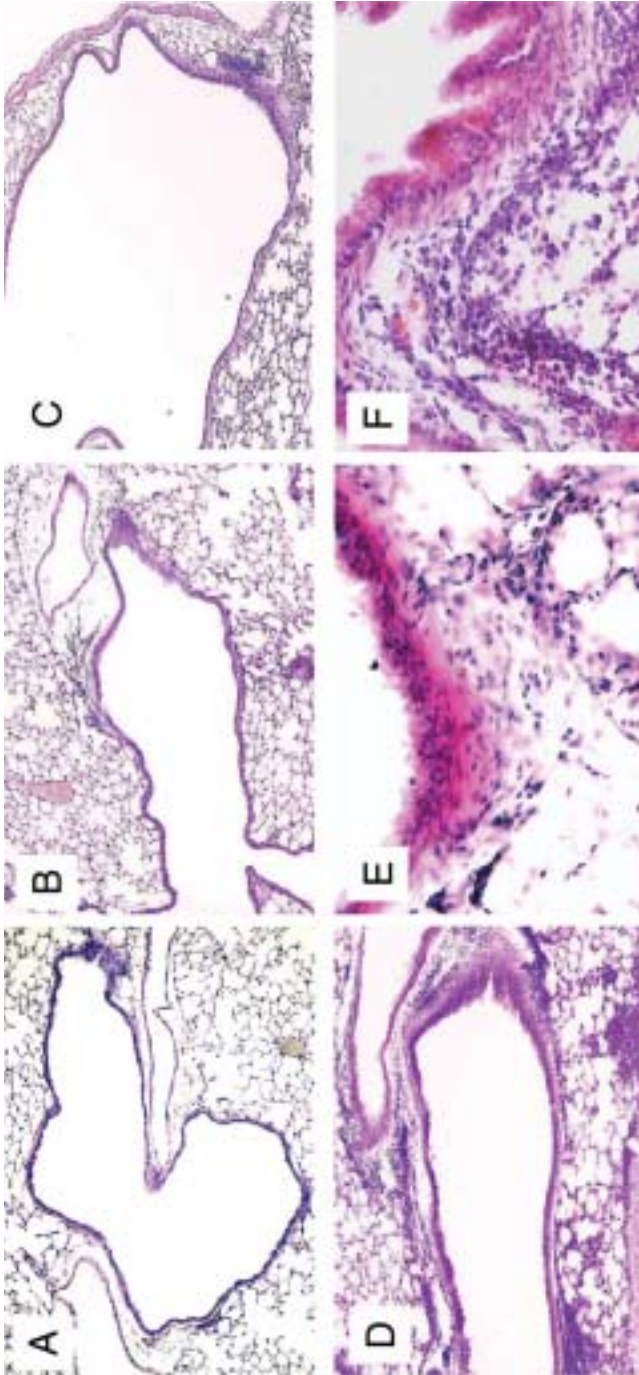


FIGURE 1. Effects of sand dust on pathological changes in the lungs. (A) No pathological changes in the lungs treated with saline. (B) The proliferation and hypertrophy of epithelial cells in the airway, and a slight infiltration of inflammatory cells in the connective tissue around the airway treated with sand dust (SD-1). (C) Slight proliferation of goblet cells that have mucus stained pink with PAS solution in the airway (treated with *D. farinae* alone). (D) Marked proliferation of goblet cells in the airway epithelium and the infiltration of inflammatory cells into connective tissue around the airway (treated with *D. farinae* + SD-2). (A)–(D) Original magnification $\times 25$ (PAS stain). (E) Slight infiltration of eosinophils into connective tissue around the airway (treated with *D. farinae* alone). (F) Numerous eosinophils and lymphocytes in the lamina propria of the airway (treated with *D. farinae* + SD-2). (E) and (F) Original magnification $\times 100$ (H&E stain).

TABLE 2. Cellular Profile in Bronchoalveolar Lavage Fluid

Group ^a	Animals (n)	Cell number ($\times 10^3$ /total BALF)				
		Total cells	Macrophages	Eosinophils	Neutrophils	Lymphocytes
Control	8	15.5 $\pm$ 1.40	15.4 $\pm$ 1.36	0	0.21 $\pm$ 0.05	0.01 $\pm$ 0.01
SD-1	8	53.1 $\pm$ 6.08	17.6 $\pm$ 1.44	0.43 $\pm$ 0.13	34.0 $\pm$ 4.96 ^b	1.00 $\pm$ 0.25
SD-2	8	30.4 $\pm$ 8.73	12.2 $\pm$ 1.93	0.48 $\pm$ 0.14	17.4 $\pm$ 1.00	0.63 $\pm$ 0.17
<i>D. farinae</i>	8	69.2 $\pm$ 2.43 ^b	68.2 $\pm$ 5.86 ^b	0.26 $\pm$ 0.05	0.46 $\pm$ 0.07	0.12 $\pm$ 0.50
<i>D. farinae</i> + SD-1	8	211 $\pm$ 13.2 ^{c, d}	67.6 $\pm$ 7.96 ^c	21.3 $\pm$ 1.47 ^{c, d}	110 $\pm$ 83.4 ^{c, d}	11.5 $\pm$ 0.82 ^{c, d}
<i>D. farinae</i> + SD-2	8	195 $\pm$ 18.7 ^{d, e}	76.0 $\pm$ 8.25 ^e	26.4 $\pm$ 2.13 ^{d, e}	95.2 $\pm$ 11.9 ^{d, e}	3.22 $\pm$ 0.69 ^{d, e}

^a Six groups of mice were treated intratracheally with normal saline (control), SD-1 (sample from Maowusu desert), SD-2 (sample from Shapotou desert), *Dermatophagoides farinae* (*D. farinae*), *D. farinae* + SD-1, and *D. farinae* + SD-2, 4 times at 2-wk intervals. Bronchoalveolar lavage (BAL) was obtained 24 h after the last intratracheal instillation. The values are mean $\pm$ SE ($n = 8$).

^b Significant at $p < .05$ vs. control.

^c Significant at $p < .05$ vs. SD-1.

^d Significant at $p < .05$ vs. *D. farinae*.

^e Significant at $p < .05$ vs. SD-2.

TABLE 3. Expression of Cytokines in Bronchoalveolar Lavage Fluid (BALF)

Group ^a	Animals (n)	Cytokines (pg protein/total BAL F)			
		IL-5	IFN- γ	TNF- α	GM-CSF
Control	8	0	71.4 $\pm$ 14.2	12.9 $\pm$ 4.90	1.26 $\pm$ 0.31
SD-1	8	2.62 $\pm$ 1.54	439 $\pm$ 69.8 ^b	82.4 $\pm$ 14.1 ^b	14.6 $\pm$ 2.18 ^b
SD-2	8	1.50 $\pm$ 0.50	175 $\pm$ 33.6	36.2 $\pm$ 5.49 ^b	16.0 $\pm$ 1.97 ^b
<i>D. farinae</i>	8	6.60 $\pm$ 2.89	130 $\pm$ 38.0	36.0 $\pm$ 6.36 ^b	3.23 $\pm$ 1.19
<i>D. farinae</i> + SD-1	8	23.5 $\pm$ 10.5 ^{b, c}	475 $\pm$ 170 ^b	28.2 $\pm$ 1.94 ^c	7.67 $\pm$ 1.85 ^{b, c}
<i>D. farinae</i> + SD-2	8	60.1 $\pm$ 11.8 ^{b, d, e}	169 $\pm$ 38.3	21.7 $\pm$ 1.72	8.65 $\pm$ 2.42 ^{b, c}

^a Six groups of mice were treated intratracheally with normal saline (control), SD-1 (sample from Maowusu desert), SD-2 (sample from Shapotou desert), *Dermatophagoides farinae* (*D. farinae*), *D. farinae* + SD-1, and *D. farinae* + SD-2, 4 times at 2-wk intervals. Bronchoalveolar lavage (BAL) was conducted 24 h after the last intratracheal instillation. The values are mean $\pm$ SE ($n = 8$).

^b Significant at $p < .05$ vs. control.

^c Significant at $p < .05$ vs. SD-1.

^d Significant at $p < .05$ vs. SD-2.

^e Significant at $p < .05$ vs. *D. farinae*.

TABLE 4. Expression of chemokines in bronchoalveolar lavage fluid (BALF)

Group ^a	Animals (n)	Chemokines (pg protein/total BAL F)			
		Eotaxin	RANTES	MCP-1	MIP-1 α
Control ^a	8	0	0.91 $\pm$ 0.39	5.35 $\pm$ 2.97	1.36 $\pm$ 0.38
SD-1	8	0	56.6 $\pm$ 6.51 ^b	41.0 $\pm$ 51.3	43.6 $\pm$ 4.04 ^b
SD-2	8	0	42.8 $\pm$ 6.73 ^b	97.8 $\pm$ 20.9	28.2 $\pm$ 2.08 ^b
<i>D. farinae</i>	8	0	3.20 $\pm$ 0.71	7.01 $\pm$ 2.97	2.98 $\pm$ 0.95
<i>D. farinae</i> + SD-1	8	24.4 $\pm$ 9.45 ^{b, c, d}	46.3 $\pm$ 16.4 ^{b, d}	602 $\pm$ 170 ^{b, c, d}	37.2 $\pm$ 5.43 ^{b, d}
<i>D. farinae</i> + SD-2	8	32.5 $\pm$ 9.44 ^{b, d, e}	44.2 $\pm$ 15.6 ^{b, d}	781 $\pm$ 137 ^{b, d, e}	30.6 $\pm$ 5.34 ^{b, d}

^a Six groups of mice were treated intratracheally with normal saline (control), SD-1 (sample from Maowusu desert), SD-2 (sample from Shapotou desert), *Dermatophagoides farinae* (*D. farinae*), *D. farinae* + SD-1, and *D. farinae* + SD-2, 4 times at 2-wk interval. Bronchoalveolar lavage (BAL) was conducted 24 h after the last intratracheal instillation. The values are mean $\pm$ SE ($n = 8$).

^b Significant at $p < .05$ vs. control.

^c Significant at $p < .05$ vs. SD-1.

^d Significant at $p < .05$ vs. *D. farinae*.

^e Significant at $p < .05$ vs. SD-2.

compared with *D. farinae* alone (Tables 3 and 4). *Dermatophagoides farinae* with SD-2 significantly elevated levels of IL-5 compared with *D. farinae* alone (Tables 3). However, *D. farinae* with SD-1 or SD-2 significantly decreased GM-CSF compared with SD alone. *Dermatophagoides farinae* with SD-1 significantly decreased TNF- α compared with SD-1 alone. A synergistic or cumulative phenomenon was observed in the rise of IL-5, eotaxin, and MCP-1 in the combined groups compared with the *D. farinae* alone and SD alone.

However, these increased levels in the combined groups were higher in the *D. farinae* + SD-2 group than in the *D. farinae* + SD-1 group.

Enhancement of *D. farinae*-Specific IgG1 Production by Sand Particles

Figure 2 shows the titers of *D. farinae*-specific IgG1 in the serum. The *D. farinae*-specific IgG1 titers were significantly increased in the *D. farinae* + SD-2 group compared with the *D. farinae*-only group. The *D. farinae*-specific IgE antibodies were lower than 1 titer in all groups (data not shown).

DISCUSSION

The major element found in the two different particles tested was Si, which was derived mainly from feldspar and quartz (Quan et al., 1996). Both feldspar and quartz particles exhibited similar potency in inflammatory responses in vivo and in the release of inflammatory cytokines in vitro (Becher et al., 2001). On the other hand, LPS and β -glucan were adsorbed on the sand dust particles. LPS is a glycolipid of gram-negative bacteria (Nikaido, 1969), and β -glucan is the major structural component of fungi walls (Hearn & Sietsma, 1994). These microbial materials induced neutrophilic pulmonary inflammation (Takano et al., 2002; Young et al., 2001). The present study suggests that a part of the inflammatory responses produced by the sand dust samples tested may be due

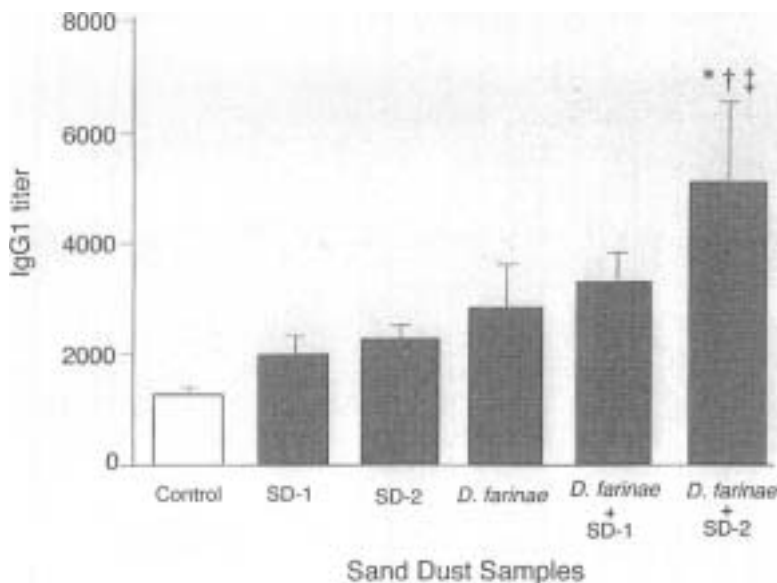


FIGURE 2. Effects of sand dusts on *D. farinae*-specific IgG1 production. The values are mean $\pm$ SE ($n = 8$). Asterisk indicates significant at $p < .05$ vs. control; $\dagger$, significant at $p < .05$ vs. SD-2; $\ddagger$, significant at $p < 0.05$ vs. *D. farinae*.

to mineral particles. The concentrations and compositions of elements and minerals were consistent among the samples tested in the present study. The content of LPS was similar among the samples tested. The content of β -glucan was twofold higher in SD-1 than SD-2. However, the inflammatory response was different between the two test samples. In the present study, the number of neutrophils in BALF correlated with the content of β -glucan. Therefore, differences in inflammatory response in BALF to the two sand dusts may be due to variation in amounts of β -glucans in the sand dusts.

The various biological components in BALF may reflect an event in the lungs. IFN- γ induced macrophage activation, TNF- α production, and macrophage-mediated tissue injury (Ikezumi et al, 2003). IFN- γ and TNF- α were involved in the shock and tissue injury produced by LPS (Mayeux, 1997; Heremans & Billiau, 1989; Josephs et al., 2000). IFN- γ was induced by β -glucan, and TNF- α was also produced in macrophages by β -glucan (Sakurai et al., 1994; Young et al., 2003). In the present study, a part of the increase of IFN- γ or TNF- α in BALF by the sand dust alone might be associated with LPS or β -glucan. However, the induction levels in IFN- γ and TNF- α were 2.5- and 2.3-fold higher in SD-1 than in SD-2. Therefore, the differences in induction levels of the two cytokines in the two test samples could be due to the different amounts of β -glucans in the sand dusts. Especially, SD-1 containing higher amounts of β -glucan than SD-2 could promote TNF- α production in macrophages via IFN- γ , and consequently produce tissue injury and neutrophilic inflammation in the lungs. RANTES is implicated in the recruitment of neutrophils (Pan et al., 2000). MIP-1 α is involved in neutrophil accumulation and lung permeability via LPS (Standiford et al., 1995). GM-CSF promotes neutrophil survival (Cox et al., 1992), and activates neutrophils by enhancing expressions of the toll-like receptor TLR-2, which is the receptor for zymosan (β -glucans) and peptidoglycan, or TLR-4, which is a receptor for gram-negative bacteria and LPS (Kurt-Jones et al., 2002). In the present study, RANTES, MIP-1 α , or GM-CSF increased by sand dust alone may be involved in neutrophilic inflammation. The increased levels of IFN- γ and TNF- α in BALF of the groups without *D. farinae* were consistent with the concentration of β -glucan rather than with LPS. β -Glucan adsorbed onto sand dust, especially onto SD-1, might promote TNF- α production in macrophages via IFN- γ , and consequently produce tissue injury and inflammation in the lungs.

Allergic asthma is clinically characterized as a chronic inflammatory eosinophilic bronchitis and airway hyperresponsiveness. Pathologically, *D. farinae* induced the infiltration of eosinophils and lymphocytes in the airway, along with goblet-cell proliferation. This phenomenon is associated with human asthma. In the present study, the infiltration of eosinophils in the airway was more severe in the *D. farinae* + sand dust groups than with *D. farinae* only. The infiltration of eosinophils produced by SD-2 with lower amounts of β -glucan was much greater than that induced by SD-1 with higher amounts of β -glucan (Table 1).

As the eosinophil-relevant cytokine and chemokine, IL-5 is recognized as a key mediator in bronchial asthma, which affects eosinophilic inflammation and airway hyperresponsiveness (Foster et al., 1996; Robinson et al., 1993). Eotaxin is an

eosinophil-specific chemokine, which may induce the recruitment of eosinophils in antigen-induced tissue eosinophilia (Rothenberg et al., 1997). MCP-1 induces chemotaxis in eosinophils via CCR2 receptors expressed on the cell surfaces (Dunzendorfer et al., 2001). The addition of sand dusts to *D. farinae* synergistically or cumulatively increased IL-5, eotaxin, and MCP-1 in BALF (Tables 2 and 3). The production levels of IL-5, eotaxin, and MCP-1 in BALF correlated with eosinophil numbers in BALF and the airway. These results suggest that the increased cytokine and chemokines in BALF play an important role in the enhancement of eosinophil infiltration in the airway produced by *D. farinae* + sand dusts.

The levels of these cytokines and chemokines in BALF enhanced by *D. farinae* + SD-2 were higher than those induced by *D. farinae* + SD-1. IFN- γ is well known to act as a suppressor on the production of Th-2 cytokine (IL-5) and inhibit antigen-induced eosinophil recruitment (Iwamoto et al., 1993). Therefore, the expressions of these eosinophil relevant inflammatory mediators in BALF of the *D. farinae* + SD-1 group may be suppressed by the increased IFN- γ . The differences in eosinophil numbers in BALF and the airway in responses to the two sand dusts may be due to differences in the induction levels of IFN- γ .

GM-CSF, RANTES, and MIP-1 α are also related for survival, recruitment, activation, and degranulation of eosinophils (Alam et al., 1993; Hallsworth et al., 1992; Lukacs et al., 1995; Rot et al., 1992). However, no effects of *D. farinae* + sand dusts on the production of GM-CSF, RANTES, and MIP-1 α in BALF were observed. Therefore, these cytokines and chemokines may not be involved in the enhancement of eosinophils by sand dusts. They seem to be involved in inflammation induced by sand dust only. The production of TNF- α was also suppressed by the combined treatments. IL-4, IL-10, and IL-13 released from Th-2 lymphocytes inhibited the production of TNF- α from monocytes or macrophages (D'Andrea et al., 1995; Moore et al., 1993). In the present study, these cytokines were not measured. However, the suppression of TNF- α could be induced by these Th-2 cytokines.

In the present study, the adjuvant effect of SD-2 toward *D. farinae*-specific IgG1 production was detected. However, the treatment dose (1 μ g) of *D. farinae* did not affect the production of allergen-specific IgE antibody. It has been reported that antigen-specific IgG1 antibodies might contribute to antigen-specific eosinophil degranulation via Fc γ RII on the eosinophil surface (Kaneko et al., 1995). Therefore, the degranulation of antigen-specific eosinophils, especially in the *D. farinae* + SD-2 group, may be induced by an increased antigen-specific-IgG1 antibody.

In conclusion, the present study showed that Asian sand dusts enhanced an infiltration of eosinophils in the airway related to *D. farinae*. The enhancement may be mediated by synergistically or cumulatively increased IL-5, eotaxin, and MCP-1. The results suggest that sand dust (mineral dust) in the atmosphere is implicated in the aggravation of human asthma. The experimental findings in the present study suggest that a warning concerning Asian sand dust effects on allergic respiratory disorders in the eastern Asia region may be warranted.

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