

Asian sand dust enhances ovalbumin-induced eosinophil recruitment in the alveoli and airway of mice

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Abstract

Asian sand dust (ASD) containing sulfate (SO_4^{2-}) reportedly causes adverse respiratory health effects but there is no experimental study showing the effect of ASD toward allergic respiratory diseases. The effects of ASD and ASD plus SO_4^{2-} toward allergic lung inflammation induced by ovalbumin (OVA) were investigated in this study. ICR mice were administered intratracheally with saline; ASD alone (sample from Shapotou desert); and ASD plus SO_4^{2-} (ASD- SO_4); OVA + ASD; OVA + ASD- SO_4 . ASD or ASD- SO_4 alone caused mild neutrophilic inflammation in the bronchi and alveoli. ASD and ASD- SO_4 increased pro-inflammatory mediators, such as Keratinocyte chemoattractant (KC) and macrophage inflammatory protein (MIP)-1 alpha, in bronchoalveolar lavage fluids (BALF). ASD and ASD- SO_4 enhanced eosinophil recruitment induced by OVA in the alveoli and in the submucosa of the airway, which has a goblet cell proliferation in the bronchial epithelium. However, a further increase of eosinophils by addition of SO_4^{2-} was not observed. The two sand dusts synergistically increased interleukin-5 (IL-5) and monocyte chemotactic protein-1 (MCP-1), which were associated with OVA, in BALF. However, the increased levels of IL-5 were lower in the OVA + ASD- SO_4 group than in the OVA + ASD group. ASD caused the adjuvant effects to specific-IgG1 production by OVA, but not to specific-IgE. These results suggest that the enhancement of eosinophil recruitment in the lung is mediated by synergistically increased IL-5 and MCP-1. IgG1 antibodies may play an important role in the enhancement of allergic reaction caused by OVA and sand dust. However, extra sulfate may not contribute to an increase of eosinophils.

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1. Introduction

Wind erosion in arid and semi-arid areas of middle and northwestern China forms the Asian sand dust (ASD) aerosol. This ASD spreads over large areas, including East China, the Korean Peninsula, and Japan.

Sometimes the aerosol is transported across the Pacific Ocean to the United States (Duce et al., 1980; Kim et al., 2001; Husar et al., 2001). The sand dust aerosol originates in the sandstorms occurring in the Gobi Desert and the Ocher Plateau in spring. Both the daily observations and atmospheric concentrations of the dust aerosol have been increasing steadily in the eastern Asia region in recent years (Zhuang et al., 2001; Mori et al., 2003). ASD contains various chemical species such as

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Sulfate (SO_4^{2-}) or nitrate (NO_3^-) derived from alkaline soil which captures acid gases, such as sulfur oxides (SO_2) and nitrogen oxides (NO_2) (Choi et al., 2001; Mori et al., 2003). These gases are byproducts formed from coal and other fossil fuels combusted in industrialized eastern China.

Recent epidemiologic studies have shown that ASD events are associated with an increase in daily mortality in Seoul, Korea (Kwon et al., 2002) and Taipei, Taiwan (Chen et al., 2004). ASD has also caused cardiovascular and respiratory problems in Seoul, Korea (Kwon et al., 2002). Therefore, experimental studies confirm that the epidemiological results—adverse respiratory health effects caused by ASD during a dust storm event—are in order. Although ASD causes neutrophilic lung inflammation and injury in a pulmonary hypertensive rat (Lei et al., 2004), there is no experimental study showing the effect of ASD or $\text{ASD} + \text{SO}_4^{2-}$ toward allergic respiratory diseases.

In the present study, a standard sample of ASD from a desert, where dust storms occur frequently, was prepared to clarify the concentrations and composition of elements and minerals as well as to clarify their chemical reactions with acid gases such as SO_2 or NO_2 in sand dust. Investigation on the reaction of the standard sample or experimental samples with acid gases, is important in clarifying their effects on the respiratory system of natural ASD present in the atmosphere. In the present study, the effects of a standard sample (ASD) or experimental sample (sulfate was added) on allergic inflammation were investigated. The investigation included pathologic changes in the mice lungs, cytological alteration in bronchoalveolar lavage fluids (BALF), changes of inflammatory cytokines and chemokines in BALF.

2. Materials and methods

2.1. Animals

A total of 96 male ICR mice (5 weeks of age) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). ICR male mice (5 weeks of age) reported to be moderately responsive to airway inflammation caused by OVA were used (Ichinose et al., 2003). After body weight and absence of infection were checked for 1 week, mice were used at 6 weeks of age. They were fed a commercial diet CE-2 (CLEA Japan, Inc., Tokyo, Japan) and given water ad libitum. Mice were housed in plastic cages lined with soft wood 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 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 Sciences in Oita, Japan.

2.2. Preparation of particles

ASD was collected from surface soils in the Shapotou Desert, on the southern fringe of the Tengger Desert, where dust storms occur frequently. There were 100 sampling points at site in the deserts, where 0–6 cm of surface sand was sampled. The space between sites was 10 m². After collection from the site, the sands were sieved with a 250-mesh screen. The particles which were less than 10 µm in diameter were collected by a separation system with a wind tunnel (16 m-length) under controlled conditions (2 m/s wind speed). The experimental dust sample ($\text{ASD} + \text{SO}_4$) was prepared as follows: a mixture of the original sieved Shapotou fine particles (1 g) and SO_2 gas (100 ppm) in a 1000 mL glass bottle was allowed to stand for 2 days. The particle diameter of the samples (a total of 600 particles) was measured under a scanning electron microscope (JSM-5800 JEOL Ltd., Tokyo, Japan). This procedure was repeated three times. The mean distribution peak of particle diameter in ASD was observed at 6 µm.

2.3. Analysis of sulfate, nitrate and elements, lipopolysaccharide, and, β -glucan in particles

Sulfate (SO_4^{2-}) and nitrate (NO_3^-) in the samples were analyzed using an ion chromatograph (DX-100, Dionex, Sunnyvale, CA, USA). 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, MA). LPS and β -glucan in particles were measured according to the “Bacterial Endotoxins Test” (Japan Ministry of Health and Labor, 2001).

2.4. Study protocol

ICR mice were divided into 6 groups ($n = 16$) according to treatment with two kinds of particles: Saline; OVA alone; ASD alone; ASD plus SO_4 ($\text{ASD} + \text{SO}_4$); OVA + ASD; and OVA + $\text{ASD} + \text{SO}_4$. These particles were suspended in normal saline (0.9% NaCl) for instillation (Otsuka Co, Kyoto, Japan). OVA was dissolved in the same saline. The instillation dose of particles was 0.1 mg per mouse. The instillation dose of OVA was set to 1 µg per mouse according to the previous report (Ichinose et al., 2003). 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 of normal saline (Otsuka Co, Kyoto, Japan) per mouse. The

instillation was repeated 4 times at 2-week intervals for the induction of allergic inflammation using the method previously reported (Ichinose et al., 2003). One day after the last intratracheal administration, all groups were killed by exsanguination under deep anesthesia by i.p. injection of pentobarbital at 12 weeks of age.

2.5. Pathological evaluation and bronchoalveolar lavage

Eight out of 16 mice from each group were used for pathologic examination. The lungs were fixed by 10% neutral phosphate-buffered formalin, and then stained with haematoxylin and eosin (H & E) to evaluate the degree of infiltration of eosinophils or lymphocytes in the airway from proximal to distal. The lung samples were also stained with periodic acid-shiff (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, Japan). The degree of infiltration of eosinophils and lymphocytes in the airway or proliferation of goblet cells in the bronchial epithelium was graded as the method previously reported (Ichinose et al., 2003): (0), not present; (1), slight; (2), mild; (3), moderate; (4), moderate to marked; and (5), marked. 'Slight' was defined as less than 10% of the airway with eosinophilic inflammatory reaction or with goblet cells stained with PAS; 'mild' as 20–30% of the airway; 'moderate' as 40–50%; 'moderate to marked' as 60–70%, and 'marked' as more than 80% of the airway. (Ichinose et al., 2003). The remaining eight mice were used for examination of free cell contents from BALF. BAL and cell counts were conducted by a previously reported method (Takano et al., 1997). The BAL supernatants were stored at -80°C until analysis of cytokines and chemokines.

2.6. Quantitation of cytokines and chemokines in BALF

The cytokine protein levels in the BALF were determined using enzyme-linked immunosorbent assays (ELISA). Interleukin (IL)-5, IL-12, interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and granulocyte macrophage-colony stimulating factor (GM-CSF) were measured using an ELISA kit from Endogen, Inc (Cambridge, MA). Keratinocyte chemoattractant (KC), monocyte chemotactic 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).

2.7. Measurement of IgG1 and IgE antibodies

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

2.8. Statistical analysis

Statistical analyses on the pathologic evaluation in the airway, cytokine and chemokine proteins in BALF were conducted using Fisher's protected least significant differences (P/SD) test in ANOVA (Statview; Abacus Concepts, Inc., Berkeley, CA). Differences among groups were determined as statistically significant at a level of $P < 0.05$. The correlation coefficients among the number of inflammatory cells in BALF and the levels of cytokines and chemokines were calculated for each mouse ($n = 64$) using Fisher's z -transformation (Statview; Abacus Concepts, Inc., Berkeley, CA).

3. Results

Table 1 shows concentration of sulfate, nitrate, elements, LPS, and β -glucan in the sand dusts. The concentrations of SO_4^{2-} were 900 $\mu\text{g/g}$ in ASD sample and 3500 $\mu\text{g/g}$ in ASD- SO_4 sample. The concentrations of NO_3^- were less than 500 $\mu\text{g/g}$ in both the samples. The amounts of LPS and β -glucan in the both samples were consistent. The element contents in the ASD were 0.28 g/g for Si; 0.058 g/g for Al; 0.054 g/g for Ca; 0.029 g/g for Fe; and 0.017 g/g for K.

Two sand dust samples caused bronchitis and alveolitis (Fig. 1). Two sand dust samples caused slight accumulation of lymphocytes in the submucosa of the airway (Table 2). The treatment with OVA alone caused slight infiltration of eosinophils and lymphocytes in the submucosa of the airway with slight goblet cell proliferation in the bronchial epithelium (Table 2). OVA + ASD and OVA + ASD- SO_4 caused a mild to

Table 1
Concentrations of sulfate (SO_4^{2-}), nitrate (NO_3^-), lipopolysaccharide (LPS), and β -glucan in particles

Particles	SO_4^{2-} ($\mu\text{g/g}$)	NO_3^- ($\mu\text{g/g}$)	LPS (EU/mg)	β -glucan (pg/mg)
ASD ^a	900	<500	1.9	12.0
ASD- SO_4 ^a	3500	<500	1.7	12.9

^aASD: ASD collected from Shapotou desert; ASD- SO_4 : ASD plus SO_4^{2-} .

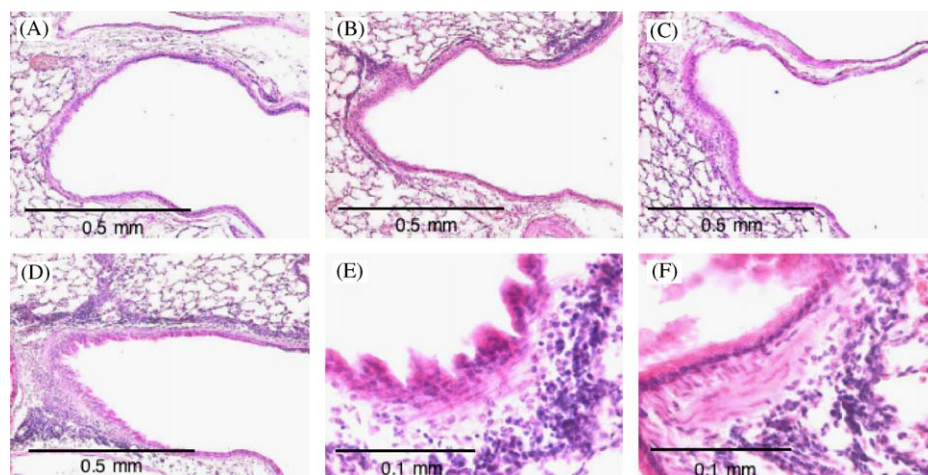


Fig. 1. Effects of Asian sand dust (ASD) on pathological changes in the lungs. No pathological changes in the main bronchus treated with saline (A). Slight infiltration of inflammatory cells in the connective tissue around the main bronchus treated with ASD alone (B). Slight proliferation of goblet cells that have mucus stained pink with PAS solution in the main bronchus treated with OVA alone (C). Marked proliferation of goblet cells in the airway epithelium and the infiltration of inflammatory cells into connective tissue around the main bronchus treated with OVA + ASD (D). (A–D) PAS stain. Numerous eosinophils and lymphocytes in the lamina propria of the airway treated with OVA + ASD (E) and treated with ASD-SO₄ (F) HE stain.

Table 2
Evaluation of pathologic changes in the murin airway

Group ^a	Pathologic changes		
	Lymphocytes	Eosinophils	Proliferation of goblet cells
Control	0.13 ± 0.23	0	0
ASD	1.31 ± 0.53 [‡]	0	0.19 ± 0.26
ASD-SO ₄	1.19 ± 0.70 [‡]	0	0.13 ± 0.23
OVA	1.81 ± 0.70 [‡]	0.90 ± 0.83 [†]	1.19 ± 1.07 [‡]
OVA + ASD	3.75 ± 0.46 ^{‡,§,}	3.25 ± 0.37 ^{‡,§,}	3.63 ± 0.16 ^{‡,§,}
OVA + ASD-SO ₄	3.19 ± 0.70 ^{‡,§,}	2.63 ± 0.95 ^{‡,§,}	3.19 ± 0.76 ^{‡,§,}

[†] $P < 0.05$ vs. control; [‡] $P < 0.001$ vs. control; [§] $P < 0.001$ vs. OVA; ^{||} $P < 0.001$ vs. ASD; ^{*} $P < 0.001$ vs. ASD-SO₄.

^aSix groups of mice (8 each) were treated intratracheally with normal saline (Control), ASD (Asian sand dust), ASD-SO₄ (ASD + SO₄), ovalbumin (OVA), OVA + ASD and OVA + ASD-SO₄ for 6 weeks. Lung tissues were taken 24 h after the last intratracheal instillation. All values are mean ± SD ($n = 8$).

moderate eosinophil infiltration in the airway (Table 2). The changes were significantly different ($P < 0.001$) from OVA or sand dust alone. The magnitude of eosinophil infiltration was lower in OVA + ASD-SO₄ than in OVA + ASD (Table 2). OVA + ASD and OVA + ASD-SO₄ also caused a moderate to marked accumulation of lymphocytes in the airway (Table 2). The degree of the accumulation was lower in OVA + ASD-SO₄ than in OVA + ASD (Table 2). OVA + ASD and OVA + ASD-SO₄ also increased proliferation of goblet cells ($P < 0.001$) in the airway compared with OVA or sand dust alone (Table 2).

Table 3 shows the cellular profile in BALF. ASD significantly increased the cell number of neutrophils

($P < 0.01$) compared with the control. ASD-SO₄ also increased the cell number ($P < 0.05$). The cell number was lower in the ASD-SO₄ group than in the ASD group. OVA + ASD further increased the cell number compared with ASD ($P < 0.001$), but OVA + ASD-SO₄ did not. ASD, ASD-SO₄, and OVA only caused no significant increase of eosinophils compared with the control. The cell number increased in the OVA + ASD and OVA + ASD-SO₄ treated groups compared with the OVA alone group ($P < 0.001$) or OVA non-treated groups ($P < 0.001$). The cell numbers in the OVA + ASD group and the OVA + ASD-SO₄ group were similar. A synergistic phenomenon was observed in the increase of eosinophils in the OVA + ASD group and in the OVA + ASD-SO₄ group, compared with those in the OVA alone group or the OVA non-treated groups. ASD-SO₄ increased the cell number of lymphocytes compared with the control ($P < 0.05$). OVA + ASD increased the cell number compared with ASD only ($P < 0.001$).

ASD increased protein levels of GM-CSF, KC, and MIP-1 α ($P < 0.001$) in BALF (Tables 4 and 5) in reference to the control. ASD-SO₄ also increased KC ($P < 0.05$) and MIP-1 α ($P < 0.001$) in BALF (Table 5). The protein levels of GM-CSF, KC and MIP-1 α in the ASD-SO₄ group were lower than those in the ASD group. OVA + ASD increased protein levels of IL-5 ($P < 0.01$) in BALF (Table 4) compared with OVA alone. However, the increased level of IL-5 was lower in the OVA + ASD-SO₄ group than in the OVA + ASD group. OVA + ASD and OVA + ASD-SO₄ increased MCP-1 ($P < 0.05$) in BALF (Table 5) compared with OVA alone. OVA + the two sand dusts did not increase the protein levels of eotaxin. OVA + ASD decreased

Table 3
Cellular profile in bronchoalveolar lavage fluids

Group ^a	Cell number (× 10 ⁴ /total BALF)				
	Total cells	Macrophages	Eosinophils	Neutrophils	Lymphocytes
Control	23.4 ± 6.40	23.3 ± 6.40	0	0.04 ± 0.04	0.02 ± 0.03
ASD	69.3 ± 38.8 [‡]	29.8 ± 9.79	0.80 ± 0.53	37.4 ± 29.8 [‡]	1.19 ± 1.25
ASD-SO ₄	73.8 ± 8.73 [§]	28.4 ± 6.73	0.71 ± 0.89	42.2 ± 8.00 [§]	1.66 ± 0.73 [†]
OVA	39.1 ± 12.0	33.4 ± 9.89	1.22 ± 1.83	3.85 ± 3.97	0.65 ± 0.53
OVA + ASD	115 ± 28.5 ^{¶,††}	38.0 ± 10.3	17.3 ± 13.9 ^{¶,††}	56.1 ± 23.1 ^{¶,**}	3.24 ± 0.82 ^{¶,††}
OVA + ASD-SO ₄	105 ± 36.8 ^{¶,‡‡}	40.7 ± 12.3 ^{‡‡}	18.7 ± 9.85 ^{¶,§§}	42.4 ± 21.9 [¶]	2.87 ± 2.58 [¶]

[†]*P* < 0.05 vs. control; [‡]*P* < 0.01 vs. control; [§]*P* < 0.001 vs. control; [¶]*P* < 0.001 vs. OVA; [¶]*P* < 0.001 vs. OVA; ^{**}*P* < 0.01 vs. ASD; ^{††}*P* < 0.001 vs. ASD; ^{‡‡}*P* < 0.05 vs. ASD-SO₄; ^{§§}*P* < 0.001 vs. ASD-SO₄.

^aSix groups of mice (8 each) were treated intratracheally with normal saline (Control), ASD (Asian sand dust), ASD-SO₄ (SFP + SO₄), ovalbmin (OVA), OVA + ASD and OVA + ASD-SO₄ for 6 weeks. Bronchoalveolar lavage (BAL) was conducted 24 h after the last intratracheal instillation. All values are mean ± SD (*n* = 8).

Table 4
Expression of cytokines in bronchoalveolar lavage fluid

Group ^a	Cytokines (pg protein/total BAL supernatants)				
	IL-5	IL-12	IFN-γ	TNF-α	GM-CSF
Control	0	201 ± 39.2	705 ± 76.5	41.5 ± 9.03	2.11 ± 0.21
ASD	1.82 ± 0.89	915 ± 244	713 ± 110	46.6 ± 7.07	6.16 ± 1.31 [‡]
ASD-SO ₄	2.00 ± 0.98	945 ± 107	904 ± 116	54.6 ± 10.5	3.25 ± 0.15
OVA	11.4 ± 4.23	184 ± 39.8	392 ± 90.0	17.8 ± 4.71 [†]	3.60 ± 0.37
OVA + ASD	117 ± 49.5 ^{¶,††}	842 ± 160	352 ± 57.2	14.4 ± 4.48 ^{**}	4.48 ± 0.75
OVA + ASD-SO ₄	55.9 ± 10.4	566 ± 65.3	435 ± 90.0 ^{‡‡}	17.5 ± 3.52 ^{§§}	4.25 ± 0.50

[†]*P* < 0.05 vs. control; [‡]*P* < 0.001 vs. control; [§]*P* < 0.01 vs. OVA; [¶]*P* < 0.01 vs. OVA; [¶]*P* < 0.001 vs. OVA; ^{**}*P* < 0.01 vs. ASD; ^{††}*P* < 0.001 vs. ASD; ^{‡‡}*P* < 0.05 vs. ASD-SO₄; ^{§§}*P* < 0.001 vs. ASD-SO₄.

^aSix groups of mice (8 each) were treated intratracheally with normal saline (Control), ASD (Asian sand dust), ASD-SO₄ (SFP + SO₄), ovalbmin (OVA), OVA + ASD and OVA + ASD-SO₄ for 6 weeks. Bronchoalveolar lavage (BAL) was conducted 24 h after the last intratracheal instillation. All values are mean ± SE (*n* = 8).

Table 5
Expression of chemokines in bronchoalveolar lavage fluid

Group ^a	Chemokines (pg protein/total BAL supernatants)				
	Eotaxin	KC	RANTES	MCP-1	MIP-1α
Control	0	59.8 ± 5.06	0	1.53 ± 0.75	0.50 ± 0.50
ASD	0	321 ± 59.0 [†]	24.8 ± 16.2	123 ± 66.3	27.3 ± 8.42 [‡]
ASD-SO ₄	0	196 ± 15.3 [†]	17.3 ± 1.48	48.8 ± 11.9	19.3 ± 1.75 [‡]
OVA	0	115 ± 15.2	0	11.6 ± 3.11	2.51 ± 1.15
OVA + ASD	24.0 ± 11.8	279 ± 36.3 [¶]	25.3 ± 12.1	330 ± 86.0 [§]	13.1 ± 2.24 ^{§,††}
OVA + ASD-SO ₄	22.1 ± 8.46	271 ± 16.2 [¶]	12.8 ± 4.21	383 ± 122 ^{§,††}	15.8 ± 2.56 [§]

[†]*P* < 0.05 vs. control; [‡]*P* < 0.001 vs. control; [§]*P* < 0.05 vs. OVA; [¶]*P* < 0.01 vs. OVA; [¶]*P* < 0.001 vs. OVA; ^{**}*P* < 0.01 vs. ASD; ^{††}*P* < 0.05 vs. ASD-SO₄.

^aSix groups of mice (8 each) were treated intratracheally with normal saline (Control), ASD (Asian sand dust), ASD-SO₄ (SFP + SO₄), ovalbmin (OVA), OVA + ASD and OVA + ASD-SO₄ for 6 weeks. Bronchoalveolar lavage (BAL) was conducted 24 h after the last intratracheal instillation. All values are mean ± SE (*n* = 8).

TNF-α (*P* < 0.01) and MIP-1α (*P* < 0.01) in BALF compared with ASD alone (Tables 4 and 5). OVA + ASD-SO₄ decreased IFN-γ (*P* < 0.05, *P* < 0.001) and TNF-α (*P* < 0.001) in BALF compared with ASD-SO₄ alone (Table 4).

The number of eosinophils in BALF was correlated with the amounts of BAL-IL-5 (*r* = 0.813, *P* < 0.0001), BAL-Eotaxin (*r* = 0.676, *P* < 0.0001). The amounts of IL-5, eotaxin and MCP-1 in BALF were significantly correlated with each other (*r* = 0.765, *P* < 0.0001). The

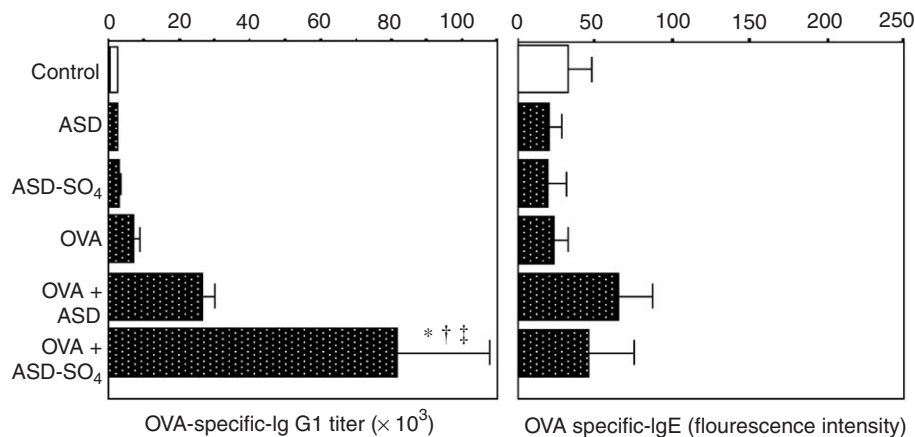


Fig. 2. Effects of sand dusts on OVA-specific IgE and OVA-specific IgG1 production. All values were expressed as mean $\pm$ SE. The values in IgG1 were 2274 $\pm$ 256, control; 2399 $\pm$ 287, ASD; 3055 $\pm$ 373, ASD-SO₄; 7083 $\pm$ 1895, OVA; 26460 $\pm$ 3460, OVA + ASD; and 81625 $\pm$ 25831, OVA + ASD-SO₄. The values in IgE were 32.5 $\pm$ 14.8, control; 20.5 $\pm$ 8.24, ASD; 19.7 $\pm$ 12.3, ASD-SO₄; 20.1 $\pm$ 8.84, OVA; 64.9 $\pm$ 22.6, OVA + ASD; and 47.4 $\pm$ 31.6, OVA + ASD-SO₄. * P < 0.001 vs. control; † P < 0.001 vs. OVA; ‡ P < 0.001 vs. ASD.

number of lymphocytes in BALF was correlated with the amounts of BAL-IL-5 ($r = 0.546$, $P < 0.0001$), as well as the numbers of eosinophils in BALF ($r = 0.699$, $P < 0.0001$). The correlation was observed between the amounts of BAL-IFN- γ and BAL-IL-12 ($r = 0.823$, $P < 0.0001$).

The OVA-specific-IgG1 titer was significantly increased in the OVA + ASD-SO₄ group ($P < 0.001$) compared with the OVA-only group (Fig. 2). The OVA-specific-IgE in the OVA-treated groups caused no increase compared with the OVA-only group.

4. Discussion

The wind-borne ASD containing chemical species, such as SO₄²⁻ and NO₃⁻ (Choi et al., 2001; Mori et al., 2003) may cause serious respiratory health problems in humans. This study demonstrated that ASD and ASD-SO₄ alone caused bronchitis and alveolitis. It also enhanced eosinophil recruitment in the alveoli and the airway with goblet cell proliferation induced by OVA.

Mineral dusts, such as mineral fibers (crocidolite asbestos) and quartz (crystalline silica), are known to cause cytotoxic and genotoxic effects toward pneumocytes (Liu et al., 2000; Schins et al., 2002). ASD used in the present study has a mineral composition that includes montmorillonite, vermiculite, mica, gypsum, chlorite, kaolinite, calcite, feldspar, and quartz (Quan et al., 1996). The major element in the ASD tested in this study was Si, which was derived mainly from feldspar and quartz (Nishikawa et al., 2000). 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 and Sietsma, 1994). The present study suggests that the inflammatory response caused by ASD tested was due to the mineral particles or the microbiological materials. The various biological components in BALF may well reflect an event in the lungs. Regarding the neutrophil relevant chemokines, MIP-1 α is involved in neutrophil accumulation and lung permeability via LPS (Standiford et al., 1995). KC elements, which act as IL-8 in humans, are well known to cause the recruitment and activation of neutrophils (Akahoshi et al., 1994). In the present study, the number of neutrophils in BALF was significantly correlated with the amounts of protein in MIP-1 α or KC in BALF. Therefore, the mineral particles and/or the microbiological materials adsorbed into particles may cause the recruitment of neutrophils via the expressions of MIP-1 α and KC. On the other hand, further increase of neutrophils by addition of SO₄²⁻ was not observed in BALF of the ASD-SO₄ group. Therefore, neutrophil recruitment may not be due to SO₄²⁻. This hypothesis is supported by the results that the protein levels of MIP-1 α and KC in the ASD-SO₄ group were lower than those in the ASD group. This phenomenon may occur because the surface of mineral particles or microbiological materials was coated with extra sulfate.

Allergic asthma is clinically characterized as eosinophilic airway inflammation and airway hyper-responsiveness. Pathologically, OVA alone caused the slight infiltration of eosinophils and lymphocytes along with goblet cell proliferation in the airway. This phenomenon is associated with human asthma. In the present study, the mild to moderate eosinophil infiltration in the airway was observed in the OVA + ASD and the OVA + ASD-SO₄ groups. However, addition of SO₄²⁻

decreased the magnitude of eosinophil infiltration in the airway. Sulfate, therefore, may not be a factor that causes the increases of eosinophils.

As the eosinophil relevant cytokine and chemokines, IL-5 is recognized as a key mediator in bronchial asthma, which affects eosinophilic inflammation (Foster et al., 1996; Robinson et al., 1993). MCP-1 induces chemotaxis in eosinophils via CCR2 receptors expressed on cell surfaces (Dunzendorfer et al., 2001). In the present study, the addition of ASD to OVA caused synergistic increases of IL-5 and MCP-1 in BALF. A clear correlation was observed between the number of eosinophils in BALF and the amounts of IL-5 or MCP-1 in BALF. Therefore these cytokine and chemokine may play an important role in the enhancement of eosinophil recruitment in the lungs caused by OVA + sand dusts. The increased level of IL-5, however, was lower in the OVA + ASD-SO₄ group than in the OVA + ASD group, suggesting that extra sulfate is not related to the enhancement of IL-5.

Eotaxin also is an eosinophil-specific chemokine, which implicates the recruitment of eosinophils in allergic inflammation (Rothenberg et al., 1997). However, the combination of OVA and sand dusts did not lead to increased levels of eotaxin. No effects of OVA + sand dusts on the production of RANTES and GM-CSF in BALF were observed. Therefore, RANTES and GM-CSF may not be involved in the enhancement of eosinophils by sand dusts.

Interleukin-12 (IL-12) is an activator in the differentiation of Th1-lymphocytes (Hsieh et al., 1993). IFN- γ is a Th1-type cytokine (Adamthwaite and Cooley, 1994). IFN- γ caused macrophage activation and TNF- α production (Ikezumi et al., 2003).

LPS or β -glucan caused an increase of these cytokines (Heinzel et al., 1994; Young et al., 2001). In the present study, two sand dust samples increased IL-12 by 4.55–4.7 times that of control. Therefore, the increase may be due to the microbiological materials.

OVA treatment suppressed the expression of Th-1 lymphocyte relevant cytokines, such as IL-12, IFN- γ or TNF- α in the BALF. On the other hand, the two sand dust samples caused a synergistic increase of Th-2 cytokine, such as IL-5 related to OVA. IL-4, IL-10 and IL-13 released from Th-2 lymphocytes as well as IL-5 are well known to suppress IL-12, IFN- γ or TNF- α (D'Andrea et al., 1993, 1995). The suppression of Th-1 lymphocyte relevant cytokines could be due to these Th-2 cytokines. Th-2 predominant reactions may be promoted by the combination of OVA and sand dust. IL-12 prevents antigen-induced eosinophil recruitment into mouse airways by inhibiting IL-5 production (Iwamoto et al., 1996). Since microbiological materials, such as LPS, cause an induction of IL-12 (Heinzel et al., 1994), microbiological materials in ASD may not be related to IL-5 enhancement. Therefore, mineral parti-

cles in ASD could be responsible for triggering IL-5 enhancement.

In the present study, the adjuvant effect of sand dusts toward the OVA-specific IgG1 production was detected. Therefore, the antibody may play an important role in the aggravation of allergic inflammation caused by the sand dusts tested.

In conclusion, the present study has shown that ASD caused neutrophilic lung inflammation, in which the mineral particles (such as feldspar or quartz but not extra sulfate) and/or the microbiological materials may be involved. ASD also enhanced eosinophilic lung inflammation related to OVA, but not extra sulfate. The enhancement may be mediated synergistically between IL-5 and MCP-1. Therefore, the sand dusts containing mineral particles or microbiological materials probably implicated the pathogenesis of human respiratory disorders during a dust event. Because wind-borne sand dust causes serious problems to human respiratory health, not only in East Asia but also in many other areas, including West African cities, southern Europe (Sahara dust from Africa), the American southwest, southeast Washington state, and eastern Australia (Guerzoni and Chester, 1996; Hefflin et al., 1994; Rutherford et al., 1999; Taylor, 2002), the experimental findings in the present study may be a warning about the ill effects of wind-borne sand dust on the human respiratory system.

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References

- Adamthwaite, D., Cooley, M.A., 1994. CD8 + T-cell subsets defined by expression of CD45 isoforms differ in their capacity to produce IL-2, IFN-gamma and TNF-beta. *Immunology* 81, 253–260.
- Akahoshi, T., Endo, H., Kondo, H., Kashiwazaki, S., Kasahara, T., Mukaida, N., Harada, A., Matsushima, K., 1994. Essential involvement of interleukin-8 in neutrophil recruitment in rabbits with acute experimental arthritis induced by lipopolysaccharide and interleukin-1. *Lymphokine Cytokine Res.* 13, 113–116.
- Becher, R., Hetland, R.B., Refsnes, M., Dahl, J.E., Dahlman, H.J., Schwarze, P.E., 2001. Rat lung inflammatory responses after in vivo and in vitro exposure to various stone particles. *Inhal. Toxicol.* 13, 789–805.

- Chen, Y.S., Sheen, P.C., Chen, E.R., Liu, Y.K., Wu, T.N., Yang, C.Y., 2004. Effects of Asian dust storm events on daily mortality in Taipei, Taiwan. *Environ. Res.* 95, 151–155.
- Choi, J.C., Lee, M., Chun, Y., Kin, J., Oh, S., 2001. Chemical composition and source signature of spring aerosol in Seoul, Korea. *J. Geophys. Res.* 106, 18067–18074.
- D'Andrea, A., Aste-Amezaga, M., Valiante, N.M., Ma, X., Kubin, M., Trinchieri, G., 1993. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J. Exp. Med.* 178, 1041–1048.
- D'Andrea, A., Ma, X., Aste-Amezaga, M., Paganin, C., Trinchieri, G., 1995. Stimulatory and inhibitory effects of interleukin (IL)-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor alpha production. *J. Exp. Med.* 181, 537–546.
- Duce, A.R., Unni, C.K., Ray, B.J., Prospero, J.M., Merrill, J.T., 1980. Long-range atmospheric transport of soil dust from Asia to the tropical north pacific: temporal variability. *Science* 209, 1522–1524.
- Dunzendorfer, S., Kaneider, N.C., Kaser, A., Woell, E., Frade, J.M., Mellado, M., Martinez-Alonso, C., Wiedermann, C.J., 2001. Functional expression of chemokine receptor 2 by normal human eosinophils. *J. Allergy Clin. Immun.* 108, 581–587.
- Foster, P.S., Hogan, S.P., Ramsay, A.J., Matthaei, K.I., Young, I.G., 1996. Interleukin 5 deficiency abolishes eosinophilia, airway hyperactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183, 195–201.
- Guerzoni, S., Chester, R. (Eds.), 1996. *The Impact of Desert Dust across the Mediterranean*. Kluwer Academic Publishers, The Netherlands.
- Hearn, V.M., Sietsma, J.H., 1994. Chemical and immunological analysis of the *Aspergillus fumigatus* cell wall. *Microbiology* 140, 789–795.
- Hefflin, B.J., Jalaludin, B., McClure, E., Cobb, N., Johnson, C.A., Jecha, L., Etzel, R.A., 1994. Surveillance for dust storms and respiratory diseases in Washington State, 1991. *Arch. Environ. Health* 49, 170–174.
- Heinzel, F.P., Rerko, R.M., Ling, P., Hakimi, J., Schoenhaut, D.S., 1994. Interleukin 12 is produced in vivo during endotoxemia and stimulates synthesis of gamma interferon. *Infect. Immun.* 62, 4244–4249.
- Hsieh, C.S., Macatonia, S.E., Tripp, C.S., Wolf, S.F., O'Garra, A., Murphy, K.M., 1993. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 260, 47–49.
- Husar, R.B., Tratt, D.B., Schichtel, B.A., Falke, S.R., Li, F., Jaffe, D., Gasso, S., Gill, T., Laulainen, N.S., Lu, F., Reheis, M.C., Chun, Y., Westphal, D., Holben, B.N., Gueymard, C., McKendry, I., Kuring, N., Feldman, G.C., McClain, C., Frouin, R.J., Merrill, J., DuBois, D., Vigonola, F., Sugimoto, N., Malm, W.C., 2001. Asian dust events of April 1998. *J. Geophys. Res.* 106, 18316–18330.
- Ichinose, T., Takano, H., Sadakane, K., Yanagisawa, R., Kawazato, H., Sagai, M., Shibamoto, T., 2003. Differences in airway-inflammation development by house dust mite and diesel exhaust inhalation among mouse strains. *Toxicol. Appl. Pharmacol.* 187, 29–37.
- Ikezumi, Y., Atkins, R.C., Nikolic-Paterson, D.J., 2003. Interferon-gamma augments acute macrophage-mediated renal injury via a glucocorticoid-sensitive mechanism. *J. Am. Soc. Nephrol.* 14, 888–898.
- Iwamoto, I., Kumano, K., Kasai, M., Kurasawa, K., Nakao, A., 1996. Interleukin-12 prevents antigen-induced eosinophil recruitment into mouse airways. *Am. J. Respir. Crit. Care Med.* 154, 1257–1260.
- Japan Ministry of Health and Labor: Bacterial endotoxins test., 2001. In: *The Japanese Pharmacopoeia*, 14th ed. Tokyo. (<http://www.mhlw.go.jp/topics/bukyoku/iyaku/yakkyoku/>).
- Kim, B.G., Han, J.S., Park, S.U., 2001. Transport SO₂ and aerosol over the Yellow sea. *Atmos. Environ.* 35, 727–737.
- Kwon, H.J., Cho, S.H., Chun, Y., Lagarde, F., Pershagen, G., 2002. Effects of the Asian dust events on daily mortality in Seoul, Korea. *Environ. Res.* 90, 1–5.
- Lei, Y.C., Chan, C.C., Wang, P.Y., Lee, C.T., Cheng, T.J., 2004. Effects of Asian dust event particles on inflammation markers in peripheral blood and bronchoalveolar lavage in pulmonary hypertensive rats. *Environ. Res.* 95, 71–76.
- Liu, W., Ernst, J.D., Courtney, B.V., 2000. Phagocytosis of crocidolite asbestos induces oxidative stress, DNA damage, and apoptosis in mesothelial cells. *Am. J. Respir. Cell Mol. Biol.* 23, 371–378.
- Mori, I., Nishikawa, M., Tanimura, T., Quan, H., 2003. Change in size distribution and chemical composition of Kosa (Asian dust) aerosol during long-range transport. *Atmos. Environ.* 37, 4253–4263.
- Nikaido, H., 1969. Structure of cell wall lipopolysaccharide from *Salmonella typhimurium*. I. Linkage between o side chains and R core. *J. Biol. Chem.* 244, 2835–2845.
- Nishikawa, M., Quan, H., Morita, M., 2000. Preparation and Evaluation of Certified Reference Materials for Asian Mineral Dust. *Global Environ. Res.* 1, 103–113.
- Quan, H., Huang, Y., Nishikawa, M., Liu, X., Mori, I., Iwasaka, Y., Wei, Q., Qiao, S., 1996. Preparation of artificial kousa aerosol with two original desert sands. *J. Environ. Chem.* 6, 225–231.
- Robinson, D.S., Ying, S., Bentley, A.M., Meng, Q., North, J., Durham, S.R., Kay, A.B., Hamid, Q., 1993. Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. *J. Allergy Clin. Immunol.* 92, 397–403.
- Rothenberg, M.E., MacLean, J.A., Pearlman, E., Luster, A.D., Leder, P., 1997. Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. *J. Exp. Med.* 185, 785–790.
- Rutherford, S., Clark, E., McTainsh, G., Simpson, R., Mitchell, C., 1999. Characteristics of rural dust events shown to impact on asthma severity in Brisbane, Australia. *Int. J. Biometeorol.* 42, 217–225.
- Schins, R.P., Duffin, R., Hohr, D., Knaapen, A.M., Shi, T., Weishaupt, C., Stone, V., Donaldson, K., Borm, P.J., 2002. Surface modification of quartz inhibits toxicity, particle uptake, and oxidative DNA damage in human lung epithelial cells. *Chem. Res. Toxicol.* 15, 1166–1173.
- Standiford, T.J., Kunkel, S.L., Lukacs, N.W., Greenberger, M.J., Danforth, J.M., Kunkel, R.G., Strieter, R.M., 1995. Macrophage inflammatory protein-1 alpha mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. *J. Immunol.* 155, 1515–1524.
- Takano, H., Yoshikawa, T., Ichinose, T., Miyabara, Y., Imaoka, K., Sagai, M., 1997. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. *Am. J. Respir. Crit. Care Med.* 156, 36–42.
- Taylor, D.A., 2002. Dust in the wind. *Environ. Health Perspect.* A 110, 80–87.
- Young, S.H., Ye, J., Frazer, D.G., Shi, X., Castranova, V., 2001. Molecular mechanism of tumor necrosis factor-alpha production in 1–3 beta-glucan (zymosan)-activated macrophages. *J. Biol. Chem.* 276, 0781–0787.
- Zhuang, G.S., Guo, J.H., Yuan, H., Zhao, C.Y., 2001. The compositions, sources, and size distribution of the dust storm from China in spring of 2000 and its impact on the global environment. *Chinese Sci. Bull.* 46, 895–901.