

RESEARCH ARTICLE

Asian sand dust aggravates allergic rhinitis in guinea pigs induced by Japanese cedar pollen

Takamichi Ichinose¹, Kyoko Hiyoshi¹, Seiichi Yoshida¹, Hirohisa Takano², Kenichi Inoue², Masataka Nishikawa³, Ikuko Mori³, Hiroaki Kawazato⁴, Aiko Yasuda⁴, and Takayuki Shibamoto⁵

¹Department of Health Sciences, Oita University of Nursing and Health Sciences, Notsuharu, Oita, Japan, ²Pathophysiology Research Team, ³Environmental Chemistry Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan, ⁴Faculty of Medicine, Oita University, Hasama-machi, Oita, Japan, and ⁵Department of Environmental Toxicology, University of California, Davis, CA, USA

Abstract

Asian sand dust (ASD) contains microbial materials, sulfate (SO_4^{2-}), and nitrate (NO_3^-), and is derived from air pollutants in East China. ASD reportedly causes adverse respiratory health effects; a case in point is aggravated allergen-associated experimental lung eosinophilia. Guinea pigs were administered normal saline (control), ASD (0.3 mg/animal), ASD (0.6 mg/animal), Japanese cedar pollen (JCP) (0.2 mg/kg body weight), JCP + ASD (0.3 mg/animal), or JCP + ASD (0.6 mg/animal), into their nasal cavities at seven weekly intervals. The number of sneezes, amount of nasal secretions, and nasal obstructing response were measured as indices of nasal responses. Total immunoglobulin E (IgE) antibodies in serum and the number of eosinophils, histamine, and arachidonic acid metabolites in nasal cavity lavage fluids (NCLF) were also measured. ASD enhanced the JCP-associated nasal obstructing response, but not the number of sneezes or amount of nasal secretions. ASD enhanced JCP-associated cysteinyl leukotrienes (C_4 , D_4 , E_4) and histamine production in NCLF. ASD augmented the number of eosinophils in NCLF and total IgE in serum induced by JCP. ASD enhanced eosinophil recruitment in the nasal mucosa, and goblet cell proliferation in the nasal epithelium induced by JCP. These results suggest that ASD enhances the nasal allergic reaction induced by repeated JCP administration in guinea pigs.

Keywords: Asian dust; Japanese cedar pollen; pollinosis; guinea pig

Introduction

In the spring, the incidence of allergic rhinitis is caused by *Cryptomeria japonica* or Japanese cedar pollen (JCP). JCP is one of the most common causes of pollinosis in Japan, and its prevalence continues to increase (Kusunoki et al., 2002; Nakamura et al., 2002).

Asian dust storms, which also occur during the spring season, arise in arid and semi-arid areas of mid- and north western China (the Gobi Desert and the Ocher Plateau). Asian sand dust (ASD) aerosol spreads over large areas, including East China, the Korean Peninsula, and Japan (Duce et al., 1980; Husar et al., 2001; Kim et al., 2001). ASD contains various chemical species such as sulfate (SO_4^{2-}) or nitrate (NO_3^-) as well as microbiological materials derived from alkaline soil (Ichinose et al., 2005). Alkaline soil is known to capture acid gases, such as sulfur oxides (SO_2) and

nitrogen oxides (NO_2) (Choi et al., 2001; Mori et al., 2003), byproducts of the combustion of coal and other fossil fuels from industrialized East China.

The potentially hazardous effect of ASD on the incidence and severity of pollinosis is a major public concern in Japan. Many patients in Japan complain of an increase in pollinosis aggravation possibly caused by ASD. Recent experimental studies have reported the aggravating effect of ASD on allergen-induced experimental lung eosinophilia (Hiyoshi et al., 2005; Ichinose et al., 2006, 2008a, 2008b). Recent epidemiologic studies have also shown that Asian dust storm events coincide with an increase in daily admissions and clinic visits for asthma (Yang et al., 2005), allergic rhinitis (Chang et al., 2006), or conjunctivitis (Yang, 2006) in Taipei, Taiwan. Unfortunately, there is currently no scientific report on the relationship between pollinosis and ASD. Experimental

studies to confirm the epidemiological results, suggesting that ASD causes adverse respiratory health effects during a dust storm event, are appropriate.

Additionally, the seasonal overlap of JCP dispersal and ASD occurrence may also have an impact on allergic rhinitis in Japanese patients. However, the effects of ASD on allergic rhinitis caused by JCP are not clear. In the present study, the role of ASD in the nasal allergic reaction induced by repeated nasal administration of JCP in guinea pigs was investigated.

Materials and methods

Materials

JCP and aluminum hydroxide hydrate (alum) gel were purchased from Cosmo Bio. Co., Ltd. (Tokyo, Japan). Diff-Quik stain solution was purchased from International Reagents (Kobe, Japan). Decalcifying solution (EDTA (ethylenediaminetetraacetic acid) method), periodic acid–Schiff (PAS), and hematoxylin and eosin (H&E) stain solutions were bought from Wako Pure Chemicals (Osaka, Japan). A guinea pig-immunoglobulin E (IgE) enzyme-linked immunosorbent assay (ELISA) kit was purchased from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan).

Japanese cedar (*Cryptomeria japonica*) pollen (JCP) extract was obtained from Cosmo Bio Co., Ltd. The extract of freeze-dried powder contained 2 mg protein per vial.

Animals

A total of 36 male Hartley guinea pigs (weight about 290 g and age 5 wk) were bought from Japan SLC, Inc. (Hamamatsu, Japan). After body weight and absence of infection were checked for 2 weeks, the 7-week-old guinea pigs were fed a standard commercial diet, RC4 (Oriental Yeast Co. Ltd., Tokyo) and given water ad libitum. Guinea pigs were housed in stainless steel cages. 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. Animals used for experiments at 7 wk of age weighed about 400 g. 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.

Preparation of Asian sand dust (ASD)

The present study used ASD previously collected from surface soils in the Shapotou Desert located on the southern fringe of the Tengger Desert, where dust storms occur frequently in North Central China (Hiyoshi et al., 2005; Ichinose et al., 2006, 2008a, 2008b). As shown in Table 1, the oxide composition in this ASD sample was 60% SiO₂, 11.1% Al₂O₃, 4.1% Fe₂O₃, 1.8% Na₂O, 9.0% CaO, 2.5% MgO, 0.7% TiO₂, and 2.2% K₂O. Also, 8.6% of total oxides were lost at ignition as shown in a previous report (Ichinose et al., 2008a).

Airborne ASD collected by an air sampler was contaminated with various amounts of micro-materials, such as soot, sulfate, and nitrate derived from air pollutants. Airborne ASD is more toxic to a mouse lung than ASD collected from

Table 1. Oxide composition, water-soluble components, lipopolysaccharide (LPS), and β -glucan content of Asian sand dust (ASD).

Oxide composition	Water-soluble components, LPS, and β -glucan
SiO ₂ 60%	SO ₄ ²⁻ 900 μ g/g
Al ₂ O ₃ 11.1%	NO ₃ ⁻ < 500 μ g/g
Fe ₂ O ₃ 4.1%	Cl ⁻ < 500 μ g/g
Na ₂ O 1.8%	NH ₄ ⁺ < 500 mg/g
CaO 9.0%	LPS 3.66 pg/mg
MgO 2.5%	β -glucan 15.2 pg/mg
TiO ₂ 0.7%	
K ₂ O 2.2%	
Loss on ignition 8.6%	

Note: ASD collected from Shapotou Desert located on the southern fringe of the Tengger Desert.

surface soil (Ichinose et al., 2005). First of all, therefore, it is important to examine the effects caused by sand dust collected from desert surface soils without air pollutants. Therefore, ASD collected from surface soil was chosen over airborne ASD in the present study. The particle diameter of the samples (a total of 600 particles) was measured under a scanning electron microscope (JSM-5800; Jeol Ltd., Tokyo, Japan). The size distribution peak was observed at 6 μ m.

Analysis of sulfate, nitrate, lipopolysaccharide, and β -glucan in ASD

Sulfate (SO₄²⁻) and nitrate (NO₃⁻) in the samples were analyzed using an ion chromatograph (DX-100; Dionex, Sunnyvale, CA). The measurement of lipopolysaccharide (LPS) content was conducted by kinetic assay using Endospec ES test MK (Seikagaku Corp., Tokyo, Japan). The measurement of β -glucan was performed by β -glucan activity assay using Fungitec G test MK (Seikagaku Corp.) according to the manufacturer's protocol. In brief, approximately 2.5 mg of each particle sample was suspended in 1 ml of water (LPS and β -glucan free; Otsuka Co., Kyoto, Japan) for 1 hr in a screw tube and allowed to stand on the bench top at room temperature for 2 hrs, after which the supernatants were recovered and tested for LPS and β -glucan concentration. The limits of detection of LPS and β -glucan in the assays were < 0.001 endotoxin units (EU)/ml and 2 pg/ml, respectively.

Study protocol

Guinea pigs were divided into six groups ($n = 6$) according to treatment with two doses of ASD and/or JCP: normal saline (control), ASD (0.3 mg/animal) alone, ASD (0.6 mg/animal) alone, JCP (0.2 mg/kg body weight) alone, JCP (0.2 mg/kg body weight) + ASD (0.3 mg/animal), and JCP (0.2 mg/kg body weight) + ASD (0.6 mg/animal).

At 14 weeks, when the body weight of the guinea pigs was about 512 g, the tidal volume was approximately 1.8 ml and breathing rate was about 94 breaths per min. The amount of suspended particulate matter (SPM) deposited in the lungs of a single guinea pig per week (0.17 mg) was calculated using the inhaled value for SPM set by the Japanese national air quality standard (0.1 mg/m³), the tidal volume, and the

breathing rate. If 100% of 0.1 mg/m^3 —which is the Japanese national air quality standard for suspended particulate matter—is accumulated in a lung (0.17 mg), the instillation dose (0.3 mg/animal) of particles used in the present study would be 1.7 times the amount accumulated in a lung. In the case of the 'Human respiratory tract model for radiological protection' (James et al., 1994), the deposition rate into a nasal cavity was approximately 68% for a $6\text{-}\mu\text{m}$ diameter particle.

The 0.3-mg and 0.6-mg particle instillation doses in the present study were approximately 2.6 and 5.2 times the amount of the 68% deposition (approximately 0.115 mg), respectively. It has been reported that the SPM in Japan ranges from 0.3 to 0.5 mg/m^3 (Hayasaki et al., 2007; Mori et al., 2003) when Asian dust storm events occur. The SPM levels are 2–6 times higher than those of the national air quality standard for SPM. Therefore, the instillation doses of particles were set at 0.3 mg and 0.6 mg , which were close to the concentration levels of an actual environment.

These particles were suspended in normal saline (0.9% NaCl) for instillation (Otsuka Co.). JCP was dissolved in the same saline. One week before the first intranasal administration, 1 mg of JCP + 1.5 mg of alum ($\text{Al}(\text{OH})_3$) gel was dissolved in 1 ml of saline solution and injected intraperitoneally as a sensitization. JCP non-treated groups were injected intraperitoneally with normal saline. Guinea pigs were administered a total $80 \mu\text{l}$ of JCP and/or ASD solution into both anterior nares at seven weekly intervals. Sneezes, nasal secretions, and nasal congestions were measured as indices of nasal responses for 20 min after JCP administration for 6 weeks.

Evaluation of nasal responses

Immediately after intranasal administration, a guinea pig was placed in a two-chamber restrainer. The restrainer was placed behind the neck which kept the neck fixed and isolated from the body. The sneeze sound was picked up with a microphone (RP-3102E; National, Tokyo, Japan), amplified, and reproduced by a tape recorder equipped with a loudspeaker (CFD-D77; Sony, Tokyo, Japan). The number of sneezes was counted during every 20-min interval. The degree (clinical score) of nasal obstruction was graded as: (0) not present; (1) slight; (2) moderate; (3) marked; and (4) marked + cyanosis, according to the magnitude of the sound amplified and reproduced by the tape recorder. The terms were defined as follows. "Slight" was less than -10 dB of peak meter; "moderate" was $-10 \pm 2 \text{ dB}$; and "marked" was more than $+2 \text{ dB}$, when the recording ("REC") level of the tape recorder was 4. Cyanosis was judged by a change in color (purple) of the nose, ear, and finger of a forefoot as well as by a change in color of the whites of the eyes.

Nasal secretion from the nostrils was measured as an index of the exocrine activity of the nasal mucosa. The secretion was wiped off with a piece of weighed paper ($100 \times 120 \text{ mm}$: Kimwipe, S-200; Jujo-Kimberly, Tokyo, Japan). The paper was wrapped in a piece of weighed aluminum foil ($60 \times 150 \text{ mm}$) and then reweighed.

Sample preparation and analysis of cell contents in nasal cavity lavage fluids

The cell content in the nasal cavity lavage fluids (NCLF) of all guinea pigs was examined. Twenty minutes after the last intranasal administration, all groups (guinea pigs were 14 weeks old and their body weight was 660 g) were killed by exsanguination under deep anesthesia by intraperitoneal injection of pentobarbital. After the head of each guinea pig was removed, the connected part of the larynx and pharynx was cannulated. The nasal cavity was lavaged with two instillations of 2 ml of sterile saline at 37°C , instilled by syringe. The lavaged fluid was collected from the nostrils. The average volume retrieved was 85% of the amount instilled (3.4 ml). The fluids from two lavages were combined and then cooled to 4°C . The fluids were centrifuged at 2000 rpm for 10 min . Total cell counts were determined on a fresh fluid specimen using a hemocytometer. Differential cell counts were assessed on cytologic preparations. The slides were prepared using a Cytospin (Sakura Co., Ltd., Tokyo, Japan) and stained with Diff-Quik. A total of 250 cells were counted under oil immersion microscopy. The aliquots of the supernatants were stored at -80°C until analysis for histamine and arachidonic acid metabolites.

Pathological evaluation

After nasal cavity lavage, the lower jaw, skin, and musculature were removed, and the head was immersed in 10% neutral phosphate-buffered formalin. After fixation, the heads were decalcified in a decalcifying solution containing 0.5 M EDTA disodium salt ($\text{pH } 7.5$) for 6 months. The decalcified heads were rinsed in tap water. A tissue block was removed from the anterior nasal cavity by making two transverse cuts perpendicular to the hard palate immediately posterior to the upper incisors, according to the procedure described in a previous report (Kobayashi, 2000). The tissue block was embedded in paraffin and then $5\text{-}\mu\text{m}$ thick sections were cut from the anterior surface. Sections were stained with hematoxylin and eosin (H&E) to identify eosinophils with red granules that can distinguish them from neutrophils, and stained with periodic acid-Schiff (PAS) to identify goblet cells in the bronchial epithelium. Pathological observations were mainly performed in the epithelium and subepithelium of the respiratory region and olfactory region in the nasal cavity using a Nikon Eclipse light microscope (Nikon Co., Tokyo, Japan) (Figure 1). The number of eosinophils infiltrated into respiratory epithelium and subepithelium in the photo area shown in Figure 5 was counted with a video micrometer (VM-30; Olympus, Tokyo, Japan) according to the methods described in a previous report (Kobayashi, 2000). The number of goblet cells per $200 \mu\text{m}$ of surface epithelium in the photo area shown in Figure 6 was counted by a micrometer. The numbers of eosinophils and goblet cells were counted in four locations per animal. Results are expressed as the number of eosinophils and the number of goblet cells per $200 \mu\text{m}$ of surface epithelium.

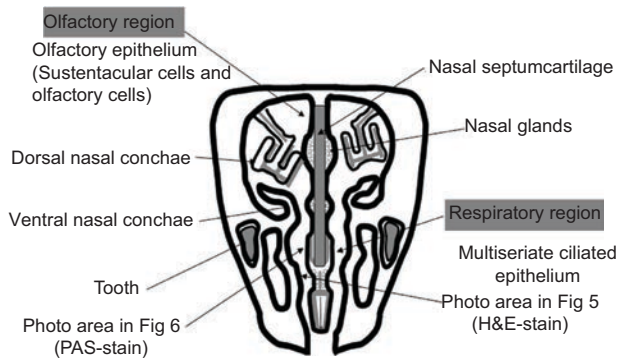


Figure 1. Area of pathological observation of inflammatory cells and epithelial cells in the nasal cavity. Goblet cell proliferation in the epithelium and eosinophil infiltration into the subepithelium of the respiratory region were observed.

Measurement of total IgE antibody

Total IgE antibody in serum was measured using a guinea pig IgE ELISA kit. The absorption at 450 nm (sub-wave length, 620 nm) for IgE antibody was measured using a microplate reader (SPECTRAFluor; Tecan, Salzburg, Austria).

Measurement of histamine and arachidonic acid metabolites in nasal cavity lavage fluids

Histamine in NCLF was measured using an ELISA kit (Oxford Biomedical Research, Inc., MI). The absorption at 490 nm (sub-wave length, 650 nm) for histamine was measured with a microplate reader (SPECTRAFluor; Tecan).

Prostaglandin E_2 (PGE_2), prostaglandin E metabolite (a more accurate index of PGE_2 biosynthesis), and cysteinyl leukotrienes (cysLTs)— LTC_4 , LTD_4 , and LTE_4 are collectively referred to cysLTs—in NCLF were measured using an ELISA kit (Cayman Chemical Co, Ann Arbor, MI). These absorptions at 412 nm were measured using a microplate reader as above.

Statistical analysis

Statistical analyses of cell contents in NCLF and total IgE antibody in serum, as well as sneezes, nasal secretion, and nasal obstructing response as indices of nasal responses, were conducted using Fisher's projected least significant differences (P/SD) test in an analysis of variance (ANOVA) (Statview; Abacus Concepts, Inc., Berkeley, CA). Differences among groups were determined as statistically significant at a level of $p < 0.05$.

Results

Water soluble components, LPS, and β -glucan in ASD

The concentration of SO_4^{2-} was 900 $\mu\text{g/g}$ in ASD samples. The concentrations of NO_3^- , Cl^- , and NH_4^+ were less than 500 $\mu\text{g/g}$ in ASD. The content of LPS in ASD samples was 3.66 pg/mg , and the content of β -glucan was 15.20 pg/mg .

Effects of ASD on nasal responses with JCP

The effect of ASD upon the sneezing response induced by repeated nasal JCP administration is shown in Figure 2A.

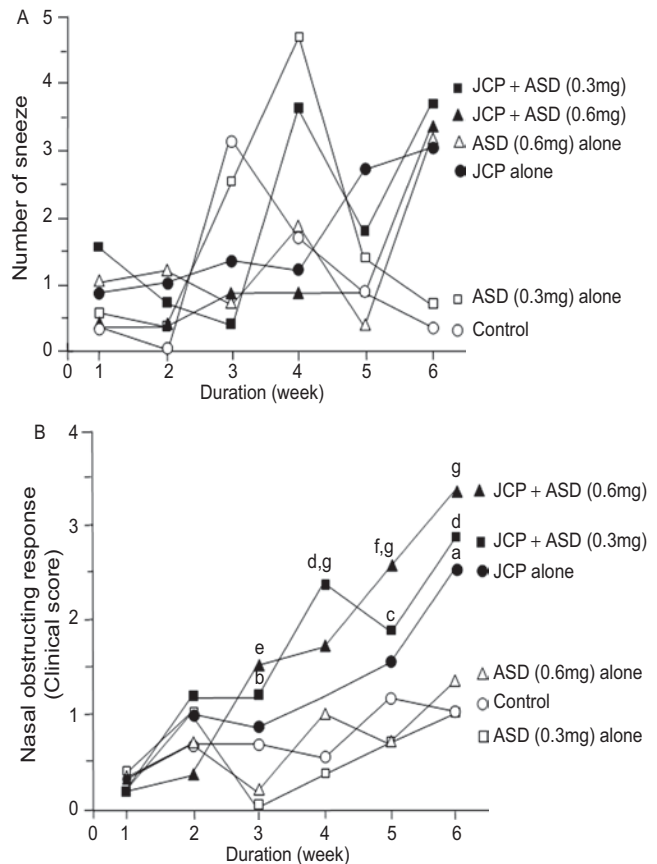


Figure 2. Effects of Asian sand dust (ASD) on nasal responses with Japanese cedar pollen (JCP). The number of sneezes, nasal secretions, and nasal obstruction were measured as indices of nasal responses for 20 min after weekly JCP administration. (A) Number of sneezes; (B) nasal obstructing response. Data are the mean for six animals. ^a $p < 0.001$ compared to control; ^b $p < 0.05$, ^c $p < 0.01$, and ^d $p < 0.001$ compared to ASD (0.3 mg); ^e $p < 0.01$ and ^f $p < 0.001$ compared to ASD (0.6 mg); ^g $p < 0.05$ compared to JCP.

The ASD (0.3mg)-only group clearly had increased sneezing response at the fourth administration, but then this response decreased after the fifth administration compared with the control group. Also, the JCP + ASD (0.3mg) group exhibited considerable increased sneezing at the fourth administration, which then subsided somewhat at the fifth administration but increased to the same level as the fourth administration at the sixth administration. The same phenomenon was observed in the case of the ASD (0.6mg)-only group. All groups except the ASD (0.3mg) group exhibited certain effects against sneezing at the sixth administration. However, it is difficult to explain why the control group had the highest value at the third administration.

The nasal obstructing response is shown in Figure 2B. Generally, the addition of JCP to ASD samples increased nasal obstruction throughout the test period. The administration of JCP + ASD (0.3mg) significantly increased the nasal obstructing response at the third ($p < 0.05$), fourth ($p < 0.001$), fifth ($p < 0.01$), and sixth ($p < 0.001$) administration by about 12-fold, 9.2-fold, 3.0-fold, and 3.1-fold, respectively, compared with ASD (0.3mg) alone. The same administration increased the response at the fourth ($p < 0.05$)

administration by 2.3-fold, compared with JCP alone. The administration of JCP + ASD (0.6 mg) increased the nasal obstructing response at the third ($p < 0.05$), fifth ($p < 0.001$), and sixth ($p < 0.001$) administration by 12-fold, 4.5-fold, and 2.8-fold, respectively, compared with ASD (0.6 mg) alone. The same administration increased the nasal obstructing response at the fourth ($p < 0.05$) and sixth ($p < 0.05$) administration by 1.7-fold and 1.4-fold, respectively, compared with JCP alone.

Effects of ASD/JCP on cell numbers in nasal cavity lavage fluids

The numbers of total cells, eosinophils, and epithelial cells found in NCLF after JCP and ASD treatment are shown in Figure 3A. ASD (0.3 mg), ASD (0.6 mg), and JCP alone caused no appreciable increase of total cells compared with the control. JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) increased the total number of cells considerably, by 2.95-fold and 2.65-fold, respectively. When JCP was mixed with ASD, both ASD samples showed a significant increase in the total number of cells. Eosinophils were formed in the greatest amounts, ranging from $(0.88 \pm 0.38) \times 10^4/\text{ml}$ (ASD, 0.3 mg) to $(27.7 \pm 7.39) \times 10^4/\text{ml}$ (JCP + ASD, 0.3 mg), among the different cells. Among the samples with a single component, JCP alone increased eosinophils the most from $(0.78 \pm 0.25) \times 10^4/\text{ml}$ (control) to $(8.88 \pm 5.43) \times 10^4/\text{ml}$. When JCP was mixed into ASD, eosinophils for both ASD samples increased significantly. For example, JCP + ASD (0.3 mg) increased eosinophils by 35.5-fold compared with the control. Also, compared with ASD alone, JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) increased eosinophils by 31.5-fold ($p < 0.001$) and 13.5-fold ($p < 0.01$), respectively. Compared with JCP alone, JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) increased eosinophils by 3.12-fold ($p < 0.01$) and 2.64-fold ($p < 0.05$), respectively. It is obvious that the JCP and ASD had a synergistic effect on the increase in cell numbers.

The numbers of neutrophils and lymphocytes found in NCLF after treatment with JCP and ASD are shown in Figure 3B. These cell numbers were at very low levels compared with eosinophil numbers. JCP + ASD (0.6 mg) increased neutrophils by 6.56-fold ($p < 0.05$) and 7.81-fold ($p < 0.05$), compared with ASD (0.6 mg) alone and JCP alone, respectively. No samples exhibited any appreciable positive effect on the numbers of lymphocyte cells; rather, they exhibited somewhat negative effects.

Effects of ASD/JCP on amounts of histamine and arachidonic acid metabolites in nasal cavity lavage fluids

The concentrations of histamine and prostaglandin E metabolite (PGEM) in NCLF after treatment are shown in Figure 4A. JCP alone increased histamine from $11.6 \pm 1.01 \text{ pg/ml}$ (control) to $34.6 \pm 7.65 \text{ pg/ml}$, whereas the samples of ASD alone did not show any appreciable effect. On the other hand, when JCP and ASD were mixed, the amount of histamine increased significantly compared with control. JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) increased histamine

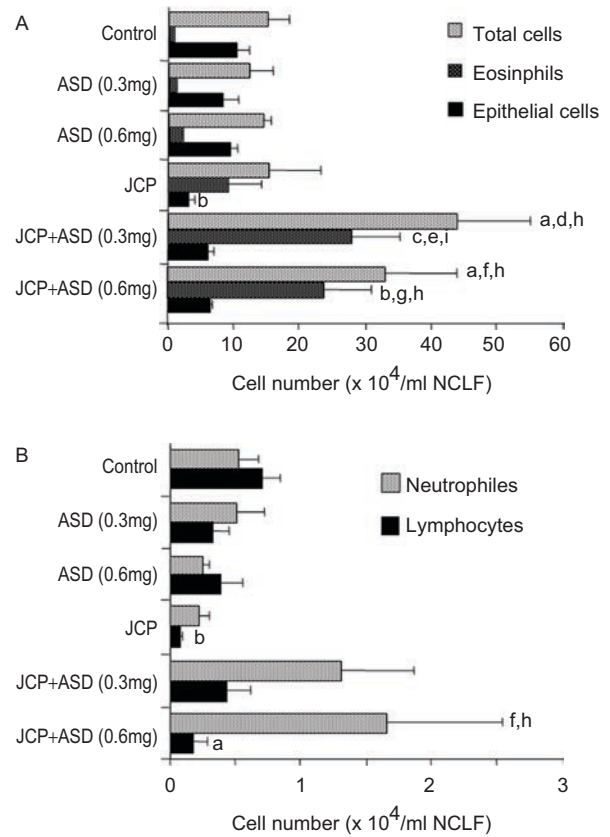


Figure 3. Cellular profile in nasal cavity lavage fluids (NCLF). Values are mean $\pm$ SE ($n = 6$, six animals). ^a $p < 0.05$, ^b $p < 0.01$, and ^c $p < 0.001$ compared with control; ^d $p < 0.01$ and ^e $p < 0.001$ compared with ASD (0.3 mg); ^f $p < 0.05$ and ^g $p < 0.01$ compared with ASD (0.6 mg); ^h $p < 0.05$ and ⁱ $p < 0.01$ compared with JCP.

by 4.90-fold ($p < 0.01$) and 4.23-fold ($p < 0.05$), respectively, compared with the control. The results indicate a clear enhancing effect of JCP with ASD on histamine formation.

The samples of ASD alone did not show any appreciable effect on PGEM concentrations, but JCP alone increased PGEM from $4.77 \pm 1.13 \text{ pg/ml}$ (control) to $11.8 \pm 0.93 \text{ pg/ml}$ ($p < 0.001$). The addition of JCP to ASD samples had moderate effects on PGEM concentrations in NCLF compared with control: 2.75-fold increase by JCP + ASD (0.3 mg) ($p < 0.001$) and 2.81-fold by JCP + ASD (0.6 mg) ($p < 0.001$). Compared with JCP alone, JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) did not cause a significant increase of PGEM.

The concentrations of cysLTs and PGE_2 in NCLF after treatment are shown in Figure 4B. The samples of ASD alone did not show any effect on the concentration of either cysLTs or PGE_2 . On the other hand, JCP alone increased cysLTs from $273 \pm 45.8 \text{ pg/ml}$ (control) to $2208 \pm 487 \text{ pg/ml}$ ($p < 0.001$). The addition of ASD to JCP increased cysLTs concentrations significantly compared with the control: 10.9-fold by JCP + ASD (0.3 mg) ($p < 0.001$) and 9.7-fold by JCP + ASD (0.6 mg) ($p < 0.001$). Compared with JCP alone, JCP + ASD (0.3 mg) significantly increased cysLTs concentrations ($p < 0.05$).

All samples exhibited a moderate increase in PGE_2 concentrations compared with the control (118.25 pg/ml), ranging from $200 \pm 27.6 \text{ pg/ml}$ (ASD alone, 0.6 mg) to $348 \pm 34.8 \text{ pg/ml}$

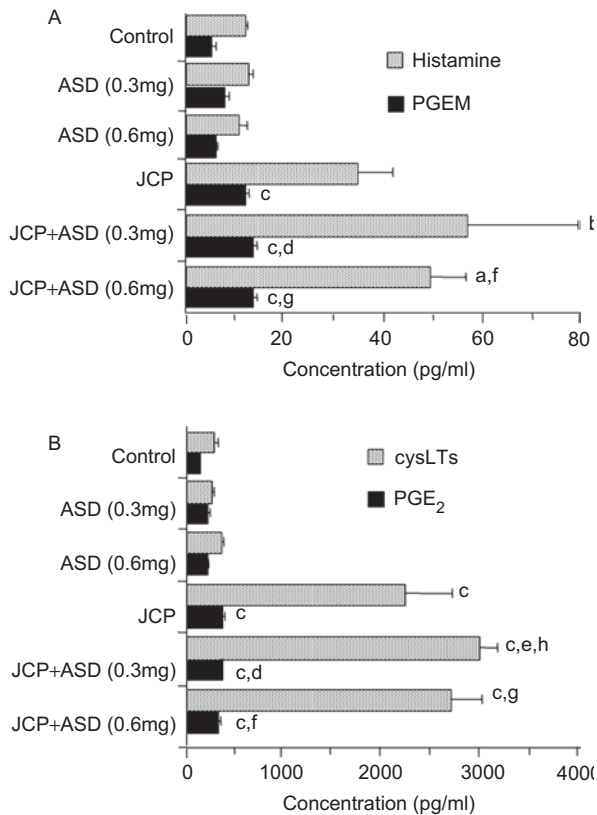


Figure 4. Concentrations of histamine and arachidonic acid metabolites in NCLF. Values are mean $\pm$ SE ($n = 6$, six animals). ^a $p < 0.05$, ^b $p < 0.01$, and ^c $p < 0.001$ compared to control; ^d $p < 0.01$ and ^e $p < 0.001$ compared to ASD (0.3 mg); ^f $p < 0.05$ and ^g $p < 0.01$ compared to ASD (0.6 mg); ^h $p < 0.05$ compared to JCP.

(JCP alone) ($p < 0.001$). JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) increased PGE₂ by 2.92-fold ($p < 0.001$) and 2.55-fold ($p < 0.001$) compared with the control. However, the addition of ASD to JCP did not have additional effects on PGE₂ concentrations compared with JCP alone.

Pathologic changes in nasal mucosa by JCP and ASD

Focal lesions were found in the epithelium and subepithelium mainly in the respiratory region after treatment with JCP and/or ASD. ASD (0.3 mg) and ASD (0.6 mg) alone caused slight infiltration of eosinophils into the nasal subepithelium (Figure 5B and C). JCP alone caused slight to moderate eosinophil infiltration into the subepithelium (Figure 5D). However, the combination treatments caused moderate to marked eosinophil infiltration into the subepithelium (Figure 5E and F). Eosinophils were also observed in the nasal epithelium (Figure 5E and F). The photo area with H&E stain in Figure 5 is shown in Figure 1.

Figure 6 shows the epithelium of the nasal septum. ASD (0.3 mg) and ASD (0.6 mg) alone caused very slight goblet cell proliferation in the nasal epithelium (Figure 6B and C). JCP alone induced slight to moderate goblet cell proliferation in the nasal epithelium (Figure 6D). JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) promoted marked goblet cell proliferation in the epithelium. Sometimes hyperplasia of goblet

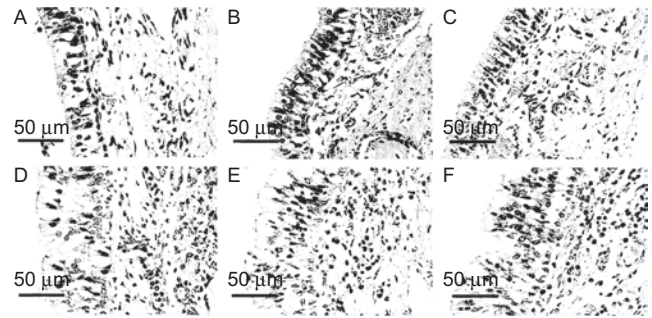


Figure 5. Photographs of the nasal epithelium and subepithelium in the respiratory region of a guinea pig (H&E stain). (A) Control group; (B) ASD (0.3 mg)-alone group; (C) ASD (0.6 mg)-alone group; (D) JCP-alone group; (E) JCP + ASD (0.3 mg) group; (F) JCP + ASD (0.6 mg) group. Nasal tissues were taken 20 min after the last intranasal administration. (A) No pathological changes can be seen in the nasal epithelium. (B, C) Slight infiltration of eosinophils into the nasal subepithelium. (D) Slight to moderate eosinophil infiltration into the subepithelium. (E, F) Edematous changes in the subepithelium and moderate to marked infiltration of eosinophils into the epithelium and subepithelium.

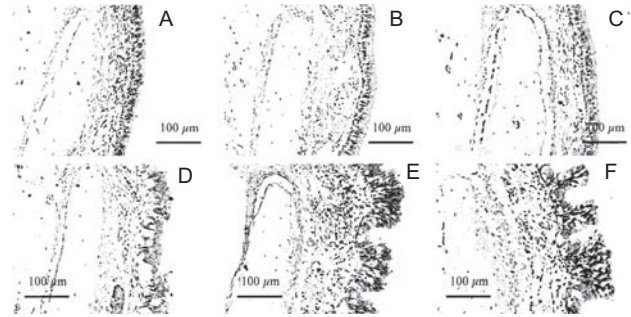


Figure 6. Photographs of nasal mucosa in the respiratory region of a guinea pig (PAS stain, $\times 100$). (A) Control group; (B) ASD 0.3 mg group; (C) ASD 0.6 mg group; (D) JCP group; (E) JCP + ASD (0.3 mg) group; (F) JCP + ASD (0.6 mg) group. Nasal tissues were taken 20 min after the last intranasal administration. (D) Slight to moderate goblet cell proliferation in the nasal epithelium. (E) Marked goblet cell proliferation in the epithelium. Hyperplasia of goblet cells, tall cells, and the formation of epithelial foveolae by goblet cell proliferation were observed. (F) Detachment of goblet cells from the nasal epithelium. (E, F) Edematous changes in the subepithelium.

cells and its tall cells were observed (Figure 6E). Detachment of goblet cells from the nasal epithelium (Figure 6F) and the formation of epithelial foveolae by goblet cell proliferation were observed in the combination treatment groups, especially in the JCP + ASD (0.6 mg) group (Figure 6F). The photo area with PAS stain is shown in Figure 1.

Many inflammatory cells were also observed in the subepithelium. No focal lesions were found in the olfactory epithelia or subepithelia of the combination treatment groups.

Enhancement of eosinophil and goblet cell numbers in nasal mucosa by ASD

As shown in Table 2, JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) increased the number of eosinophils in the nasal epithelium and subepithelium by 2.11-fold ($p < 0.001$) and 1.58-fold ($p < 0.001$), respectively, compared with JCP alone.

Table 2. Effects of ASD on eosinophil infiltration and goblet cell proliferation in nasal mucosa induced by JCP.

Group	Animals (n)	Eosinophils (/10 ⁴ μ m ²)	Goblet cells (/200 μ m)
Control	6	0.63 $\pm$ 0.49	0.16 $\pm$ 0.26
ASD 0.3 mg	6	5.13 $\pm$ 1.47 [†]	2.33 $\pm$ 2.25
ASD 0.6 mg	6	8.75 $\pm$ 4.28 [†]	2.75 $\pm$ 1.67
JCP	6	14.2 $\pm$ 2.41 [†]	9.75 $\pm$ 2.64 [†]
JCP + ASD 0.3 mg	6	29.9 $\pm$ 4.90 ^{†,*,‡}	17.7 $\pm$ 3.86 ^{†,*,‡}
JCP + ASD 0.6 mg	6	22.4 $\pm$ 2.84 ^{†,*,§}	14.2 $\pm$ 4.16 ^{†,*,§}

Note: Six groups of guinea pigs were administered with normal saline (control), ASD (0.3 mg/animal), ASD (0.6 mg/animal), JCP (0.2 mg/kg body weight), JCP + ASD (0.3 mg/animal), or JCP + ASD (0.6 mg/animal), into the nasal cavities at seven weekly intervals. Nasal tissues were taken 20 min after the last intranasal administration. Numbers of eosinophils and goblet cells were counted in four locations per animal. Results are expressed as number of eosinophils per 10⁴ μ m² and number of goblet cells per 200 μ m of surface epithelium (mean $\pm$ SD). [†] p < 0.001 vs. control; ^{*} p < 0.01, ^{**} p < 0.0001 vs. JCP; [‡] p < 0.001 vs. ASD 0.3 mg; [§] p < 0.01 versus ASD 0.6 mg.

Also, JCP + ASD (0.3 mg) and JCP + ASD (0.6 mg) increased the number of goblet cells in the nasal epithelium by 1.81-fold (p < 0.001) and 1.46-fold (p < 0.01), respectively, compared with JCP alone.

Enhancement of total IgE antibody by ASD

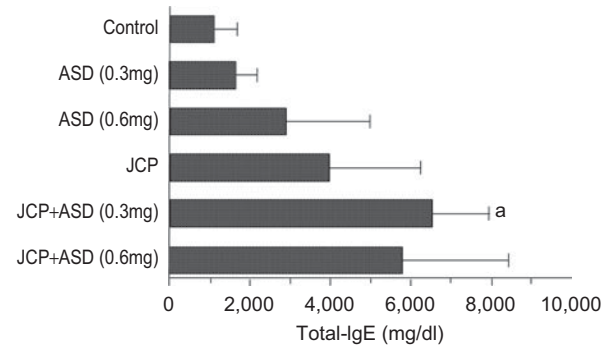
The production of total IgE antibody in serum is shown in Figure 7. The combination treatment with JCP increased the total IgE antibody, but not significantly compared with the control or a corresponding single treatment, except in the case of JCP + ASD (0.3 mg) (p < 0.05).

Discussion

Wind-borne ASD, containing microbiological materials, chemical species (SO₄²⁻, NO₃⁻), and silica, may cause serious respiratory health problems in humans (Choi et al., 2001; Mori et al., 2003). Our previous reports demonstrated that ASD aggravated allergen-associated experimental lung eosinophilia (Hiyoshi et al., 2005; Ichinose et al., 2006).

This study was performed to clarify the effects of ASD on allergic rhinitis caused by JCP. We demonstrated that ASD aggravated nasal allergic reactions induced by JCP. These allergic reactions include eosinophil infiltration in the nasal subepithelium along with goblet cell proliferation and nasal obstructing response. This is the first study to show physiologic and pathologic evidence that ASD augmentation exacerbates the nasal allergic reaction caused by JCP.

It has been reported that irritant gases such as diesel exhaust (DE), containing DE particles and many gaseous components (e.g. NO₂, NO, SO₂, and formaldehyde), increase the sneezing response (Kobayashi et al., 1997, 1998) and nasal secretion. In the present study, however, ASD alone did not exhibit any effects on those responses, suggesting that the ASD tested did not contain particles irritating to

**Figure 7.** Production of total IgE antibody in serum. Blood was taken 20 min after the last intranasal administration. Data are shown as mean $\pm$ SE (n = 6). ^a p < 0.05 compared to control.

nasal mucosa. However, as shown in Figure 5E and F, ASD augmented eosinophil infiltration into the nasal epithelium and subepithelium induced by JCP administration. ASD also increased the number of eosinophils induced by JCP in NCLF (Figure 3). Infiltrating eosinophils may release toxic granular proteins—including major basic protein, eosinophil cationic protein, and eosinophil peroxidase—which could damage or desquamate nasal epithelial cells as observed in patients with asthma (Frigas & Gleigh, 1986). The proliferation of goblet cells in the nasal epithelium may be caused by this phenomenon. As shown in Figure 6E and F, ASD enhanced the JCP-associated goblet cell proliferation in the nasal epithelium.

Our previous studies have shown that ASD enhances eosinophil recruitment induced by ovalbumin (OVA) or mite-allergen in the submucosa of the airway. The airway showed goblet cell proliferation in the bronchial epithelium along with eosinophil-relevant cytokines and chemokines—such as interleukin-5 (IL-5), monocyte chemotactic protein-3 (MCP-3), and eotaxin—in bronchoalveolar lavage fluids (BALF). The nasal epithelial changes in this study were similar to changes in the bronchial epithelium in our previous studies (Ichinose et al., 2008a, 2008b).

The IgE-mast cell reaction in the subepithelium is considered to play an important role in augmentation of the nasal allergic reaction. A chemical mediator, such as histamine, released from mast cells by antigen-antibody reactions induces nasal epithelial permeability, edematous changes, and infiltration of inflammatory cells into the epithelium (White, 1990). In the present study, although JCP-specific IgE antibody was not measured, combination treatment with JCP increased total IgE antibody in serum as well as histamine in NCLF compared with JCP alone.

Arachidonic acid metabolites play an important role in nasal allergic rhinitis. LTD₄ has especially strong blockage effects on nasal (Okuda et al., 1988) and mucus secretion from goblet cells in the respiratory epithelium (Hoffstein et al., 1990). In the present study, ASD enhanced the JCP-associated production of cysLTs (LTC₄, LTD₄, LTE₄), which are slow-reacting substances of anaphylaxis released from mast cells (Cruse et al., 2005) and leukocytes (Samuelsson et al., 1987). The release of chemical mediators (histamine and

cysLTs), which were enhanced by JCP + ASD, into the nasal microenvironment may cause the aggravation of symptoms in allergic rhinitis. The nasal challenge of the antigen increased cyclooxygenase metabolites such as prostaglandin D_2 and E_2 in the early phase of allergic rhinitis (Brown et al., 1987). Prostaglandin E_2 (PGE_2) has been reported to be associated with vascular permeability (Hirasawa et al., 1986) and inflammatory pain (Pulichino et al., 2006). However, ASD did not enhance the production of JCP-associated PGE_2 or its metabolite (PGEM) in the present study.

JCP + ASD (0.6mg) attenuated the enhanced expressions of histamine and cysLTs, and the eosinophil numbers in NCLF compared with JCP + ASD (0.3mg). The causes of these phenomena are not clear. It might be speculated that they were increased at an earlier stage in the JCP + ASD (0.6mg) group than in the JCP + ASD (0.3mg) group. It seems that a reactive substrate in the JCP + ASD (0.6mg) group had decreased by the end of the experiment. If extrapolating these results to humans, a high concentration of ASD may accelerate the development and aggravation of pollinosis caused by JCP. Therefore, investigations of the time-course of these indices are appropriate in future experiments.

As mentioned earlier, ASD contains various chemical species derived from acid gases, such as sulfate (SO_4^{2-}) from sulfur oxides (SO_2) and nitrate (NO_3^-) from nitrogen oxides (NO_2). These species are air pollutants generated in China (Choi et al., 2001; Mori et al., 2003). ASD also contains microbial materials (Ichinose et al., 2005). Our previous study reported that microbial materials adsorbed onto the ASD were related to the enhancement of eosinophil recruitment via chemical mediators (IL-5, MCP-3) in the alveoli (Ichinose et al., 2008a), but not SO_4^{2-} (Ichinose et al., 2006). The major inorganic chemical in the ASD in this study was SiO_2 (silica), which was derived mainly from feldspar and quartz (Nishikawa et al., 2000). Some previous studies have reported that crystalline silica (standard quartz DQ12 with particle sizes less than $5\ \mu m$) or amorphous silica (Mancino & Ovary, 1980; Mancino et al., 1984) was able to stimulate the production of IgE and IgG1 antibody in a single $1\text{-}\mu g$ dose of ovalbumin in mice, as does $Al(OH)_3$.

In the present study, the adjuvant effect of ASD on total IgE and the aggravating effects of ASD on allergic inflammation in the nasal mucosa of guinea pigs might be at least partially due to microbial materials, silica, and quartz.

The experimental design of the present study was established based on the assumption that simultaneous exposure to ASD and JCP in the seasonal overlap causes the activation of an allergic reaction and increased prevalence and symptom exacerbation of pollinosis. Therefore, simultaneous treatment with ASD and JCP was performed in the study. Because the instillation doses of ASD tested in the present study were close to the atmospheric levels in Japan when Asian dust storm events occur, the results of the present study may mimic cases reported by many patients, suggesting increased prevalence and pollinosis aggravation caused by ASD.

It was reported that in Taipei, Taiwan, the effects of dust storms on clinical visits for allergic rhinitis were prominent (19% increase) 2 days after the event (Chang et al., 2006). The experimental design in the present study was not influenced by ASD after the establishment of pollinosis by JCP. The ASD tested was not airborne ASD, and thus had not been contaminated with toxic materials in the air. In the future, epidemiological investigation to confirm the possible influence of airborne ASD after establishment of allergic rhinitis by antigen is appropriate.

Conclusions

The present study showed that ASD had an aggravating effect on JCP-associated eosinophilic nasal inflammation and nasal obstruction. Atmospheric exposure to molds, bacteria, and silica-carrying ASD may be one aggravating factor in the pollinosis caused by Japanese cedar pollen (JCP) during the spring in Japan. The results of the present study warn that wind-borne sand dust can have ill effects on the human upper respiratory system.

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