



Sensitization to Asian dust and allergic rhinoconjunctivitis



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ABSTRACT

Objective: Asian dust storms frequently occur in northeast Asia and the dust occasionally even spreads as far as North America during spring. Asian dust can be harmful to human health and the environment, and thus has become one of the most serious problems for Asian countries. In the present study, we evaluated sensitization to Asian dust in Japanese patients with rhinoconjunctivitis.

Methods: In March 2011, a prospective, non-randomized, cross-sectional study was conducted in 10 patients with allergic rhinoconjunctivitis (allergic group), 3 patients with atopic keratoconjunctivitis (atopic group), and 10 age- and sex-matched healthy control subjects (control group). Skin prick tests (SPT) were performed with untreated Asian dust, Asian dust extract, heat-sterilized Asian dust, silicon dioxide (SiO₂), and phosphate-buffered saline (PBS). A panel of 14 allergen extracts was also tested, comprising extracts of pollens (cedar, orchard grass, ragweed, and mugwort), house dust (house dust mixture and *Dermatophagoides pteronyssinus*), animal dander (cat and dog), fungi (*Alternaria tenuis*, *Candida*, and *Aspergillus*), and foods (milk, egg, and wheat). Then the SPT-positive rate and the mean wheal diameter for each allergen were compared among the three groups.

Results: The SPT-positive rates for untreated Asian dust, Asian dust extract, and sterilized Asian dust were significantly higher in the allergic and atopic groups than in the control group (all $p < 0.05$). In the allergic group, there were significant differences of the SPT-positive rates for untreated Asian dust (70%), Asian dust extract (50%), sterilized Asian dust (20%), SiO₂ (20%), and PBS (0%) ($p = 0.0068$). The SPT response to untreated Asian dust was correlated with the mean wheal diameters for four plant pollens ($r = 0.71$, $p = 0.0104$) and for three fungi ($r = 0.57$, $p = 0.0426$). Multivariate logistic regression analysis showed that significant predictors of the SPT reaction to untreated Asian dust were the mean wheal diameter for the four plant pollen (odds ratio = 2.54, $p = 0.0138$) and that for the three fungi (odds ratio = 1.84, $p = 0.0273$).

Conclusions: Asian dust may act as an adjuvant to promote allergic disease induced by inhaled allergens such as pollen and fungi.

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1. Introduction

“Asian dust” or “yellow sand” is blown from the deserts of Mongolia, China, and Kazakhstan during the spring months. The dust is carried eastward by the prevailing winds and passes over Korea and Japan. Occasionally, it even reaches North America. The environmental damage related to Asian dust storms laced with industrial pollutants from China has increased and social concern about allergic diseases caused by Asian dust (including

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cardiovascular and respiratory symptoms) has been increasing over the last decade (Chan et al., 2008; Chan and Ng, 2011; Kwon et al., 2002). It is well known that symptoms of pollinosis and allergies become progressively worse during the Asian dust season (Chang et al., 2006; Park et al., 2005; Watanabe et al., 2011; Yang et al., 2005). In Japan, Asian dust levels reach a peak during the spring, while the levels of pollens that cause pollinosis show two peaks (a higher peak in late spring and a lower peak in autumn) (Fujishima et al., 1995; Mimura et al., 2004, 2005). These reports are in agreement with the clinical finding that pollinosis worsens in the spring when Asian dust is in the atmosphere (Tanaka et al., 2012). Thus, Asian dust may also play an important role in the aggravation of pollinosis during spring in addition to various airborne pollens.

Recently, Fujishima et al. (2013) demonstrated that diesel exhaust fumes, a major source of air pollution, induce inflammation of cultured human conjunctival cells and are a risk factor for allergic conjunctivitis. It is also possible that allergic conjunctivitis may be triggered and worsened by Asian dust because pollutants in the dust, as well as pollens, fungi, and diesel exhaust particles, can come into direct contact with the conjunctiva and eyelid margin. However, no studies have investigated the association between Asian dust and allergic rhinoconjunctivitis. One reason why there has been no evidence published about the influence of Asian dust on allergic rhinitis and conjunctivitis may be that the dust contains various potential allergens, including metals, pollen, bacteria, fungal spores, and diesel exhaust particles. Another reason is that there are no antibodies for Asian dust and it is difficult to measure IgE directed against the dust to diagnose dust-related allergy.

Against this background, the present cross-sectional study was designed to evaluate sensitization to Asian dust in patients with rhinoconjunctivitis. The primary aim was to compare sensitization to Asian dust among control subjects and patients with allergic rhinoconjunctivitis or atopic conjunctivitis by using the skin prick test (SPT). We also compared the SPT-positive rate for untreated Asian dust, Asian dust extract, and sterilized Asian dust without microorganisms, as well as the association between sensitization to Asian dust and reactions to various allergens, in order to assess the adjuvant effect of the dust for allergens related to the development of allergic rhinoconjunctivitis.

2. Materials and methods

2.1. Study design

This prospective, nonrandomized, cross-sectional case study was conducted at the Hospital of the University of Tokyo Graduate School of Medicine and its affiliated hospitals. The study was approved by the Institutional Review Boards of Tokyo University and Tokyo Women's Medical University and was performed in accordance with the Helsinki Declaration. Informed consent was obtained from each subject prior to enrollment in the study.

2.2. Subjects

Patients were excluded from the study if they had dry eyes syndrome or a history of cataract surgery, corneal refractive surgery, or infectious conjunctivitis. In addition, we also excluded all subjects older than 20 years of age. All subjects underwent a Schirmer 1 test, with the Schirmer paper test strips placed over the lid margin at the junction of the lateral and middle thirds of the lower eyelid for 5 min. All subjects had a Schirmer 1 test score greater than 10 mm and had normal meibomian glands. None of subjects wore contact lenses and none of them had used either topical or systemic drugs during the preceding 6 weeks.

Then three groups examined in this study are shown in Table 1. The allergic group was designated as group 1 and consisted of 10 consecutive outpatients with

Table 1

Comparisons among the healthy control subjects (control group), patients with seasonal rhinoconjunctivitis (allergic group), and patients with atopic keratoconjunctivitis (atopic group).

	Control group	Allergic group	Atopic group	P value
Number of patients	10	10	3	
Age (years)	15.5 ± 1.9	14.4 ± 2.6	15.7 ± 1.7	NS*
Male/female	5/5	5/5	2/1	NS**
Schirmer I test	13.1 ± 2.3	14.9 ± 2.3	14.3 ± 1.7	NS*
Having smokers in the family	2	3	0	NS**

Values correspond to the number of patients or the mean ± standard deviation. NS = not significant.

* One-way analysis of variance and Scheffe's multiple comparison test.

** Chi-square test of independence or Fisher's exact probability test.

acute seasonal allergic conjunctivitis that only occurred during the spring. They comprised of 5 males and 5 females with a mean age of 14.4 ± 2.6 years (range: 12–19 years). In this group, all treatment was performed from March 8 to March 21, 2011. Group 2 was the age- and sex-matched control group, comprising of 5 males and 5 females with a mean age of 15.5 ± 1.9 years (range: 13–19 years) who were healthy non-smoking volunteers with no history of allergic diseases. Group 3 was the atopic keratoconjunctivitis group (atopic group), comprising 2 males and 1 female with a mean age of 15.7 ± 1.7 years (range: 14–18 years) who had been diagnosed with atopic keratoconjunctivitis.

All of the patients lived in central Tokyo, Japan. Tokyo is situated at a longitude of 149° east and a latitude of 35° north. The climate is generally warm and humid. The temperature ranges between 0.2 °C and 37.5 °C, with an annual mean of 17.0 °C (Japan Meteorological Agency). This study was conducted between March 8 and March 21, 2011. During this 2-week period, the density of airborne suspended particulate matter (SPM) in Tokyo was as high as 520 ± 226 µg/m³/24 h (Ministry of the Environment, Government of Japan, <http://www.env.go.jp/en/>). In Japan, 35 µg/m³/h is classified as "Hazardous" by the Ministry of the Environment.

2.3. Definition of allergic rhinitis

Two definitions of allergic rhinitis were considered (Lee et al., 2003; Mimura et al., 2010a). Physician-diagnosed allergic rhinitis was defined by an affirmative patient or parental answer to the question: "Have you ever been diagnosed by a physician as having allergic rhinitis?" Information on symptoms of allergic rhinitis was obtained by patients completing a questionnaire after giving a positive response to the question: "Have you ever had a problem with sneezing, or a runny or blocked nose, when you did not have a cold or the flu?"

2.4. Definition of allergic conjunctivitis

A single ophthalmologist (T.M.) diagnosed allergic conjunctivitis based on symptoms such as ocular itching and tearing, as well as the findings on slit lamp examination (conjunctival follicles, papillae, and erythema) in accordance with the published guidelines for the diagnosis of conjunctivitis (Benezra, 1994). Atopic keratoconjunctivitis was diagnosed based on the criteria published by Hogan: chronic conjunctivitis and progressive keratitis associated with atopic dermatitis and a hereditary allergic tendency (Hogan, 1952; Ozcan et al., 2007). All of the patients with atopic keratoconjunctivitis had atopic dermatitis, eyelid eczema, blepharitis, and allergic conjunctivitis (Nivenius et al., 2012; Rachdan et al., 2012). All of them were also diagnosed as having allergic conjunctivitis by measuring the total tear IgE level with the Allerwatch[®] test (Hitachi Chemical Co., Ltd., Tokyo, Japan; and Wakamoto Pharmaceutical Co., Ltd., Tokyo, Japan) (Inada et al., 2009; Mimura et al., 2010a, 2010b, 2011a, 2011b, 2012, 2013).

2.5. Preparing Asian dust Extracts

Sampling was performed in Nerima-ku, Tokyo on March 6, 2011. Two transparent plastic containers measuring 10 cm in height and 50 cm in diameter were left outdoors for 24 h, after which Asian dust (approximately 1.8 g) was collected from the containers. The concentrations of air pollutants and pollen recorded in Tokyo during the study period are listed in Table 2 and Fig. 1. Data on atmospheric conditions were obtained from the Ministry of the Environment (<http://www.env.go.jp/en/>), the National Institute for Environmental Studies (<http://www.nies.go.jp/>), and the Japan Meteorological Agency (<http://www.jma.go.jp/jma/index.html>).

We prepared the following three types of samples from the collected Asian dust for performing the SPT. (1) Unchanged Asian dust (Asian dust): The dust was diluted to 0.1 mg/ml in phosphate-buffered saline (PBS; Wako Pure Chemical Industries, Osaka, Japan). The resulting solution could contain pollen, microbes, and diesel exhaust particles eluted from the Asian dust. (2) Asian dust extract: Asian dust solution (0.1 mg/ml diluted in PBS) was vortexed for 5 min and then filtered through a 5-µm syringe filter (Nihon Millipore, Tokyo, Japan) to remove pollen (30–40 µm in diameter) and large particles, but smaller micro-organisms such as bacteria (0.5–10 µm), fungi (0.5–30 µm), and viruses (0.02–0.3 µm) could pass through the filter and may have been contained in the Asian dust extract. (3) Heat-sterilized Asian dust: Dry Asian dust was heated to 200 °C for 10 min in an oven to destroy endotoxin and kill all microbes. After heating, 0.5 mg of the dust was diluted in 5 ml of PBS and was subjected to vigorous vortexing for 5 min. Then 0.1 mg/ml of the sterilized Asian dust solution was filtered through a 0.45-µm pore size filter (Millipore) to remove any remaining bacteria. The resulting sterilized Asian dust solution did not contain any bacteria or pollen.

We also prepared PBS solution as a negative control and a solution of silicon dioxide (SiO₂, Wako Pure Chemical Industries) (0.1 mg/1 mL PBS). Asian dust has a high content of SiO₂ (24–32%), which is the main constituent of the dust (Zhang et al., 2003). The water solubility of SiO₂ is 0.12 mg/1.0 mL at room temperature and is strongly dependent on the crystalline form and the ambient temperature (Fournier and Rowe, 1977).

Table 2
Concentrations of air pollutants and pollens during the study period.

	Sampling of Asian dust	Skin prick test
	March 6, 2011. ^a Average for 24 h	March 8–21, 2011. ^b Average for 14 days
Oxidants (ppb)	23.0 ± 15.0	34.1 ± 5.9
Nitric oxide (ppb)	3.3 ± 3.8	2.4 ± 1.9
Nitrogen dioxide (ppb)	25.6 ± 10.7	18.1 ± 6.4
Nitrogen oxide (ppb)	28.9 ± 12.2	20.2 ± 7.9
SPM (μg/m ³ /h)	24.0 ± 13.1	21.9 ± 9.7
Carbon monoxide (0.1 ppm)	4.5 ± 1.2	3.4 ± 1.0
Methane (0.01 ppmC)	197.7 ± 3.4	196.3 ± 2.8
NMH (0.01 ppmC)	20.9 ± 5.3	16.7 ± 3.9
Total hydrocarbons (0.01 ppmC)	218.6 ± 8.3	212.9 ± 6.2
Wind velocity (0.1 m/s)	6.7 ± 3.8	12.6 ± 7.2
Temperature (0.1 °C)	80.5 ± 45.5	79.9 ± 30.2
Humidity (0.10%)	522.9 ± 147.6	601.6 ± 146.2
Pollen (grains/m ³ /h)	284.3 ± 164.0	226.8 ± 192.4

Data recorded in Nerima-ku (Tokyo, Japan) were obtained from the Japanese Ministry of Environment.

SPM: Suspended particulate matter, NMH: Non-methane hydrocarbons.

^a The average values for 24 h are shown.

^b The average for 14 days after calculating mean value for each day.

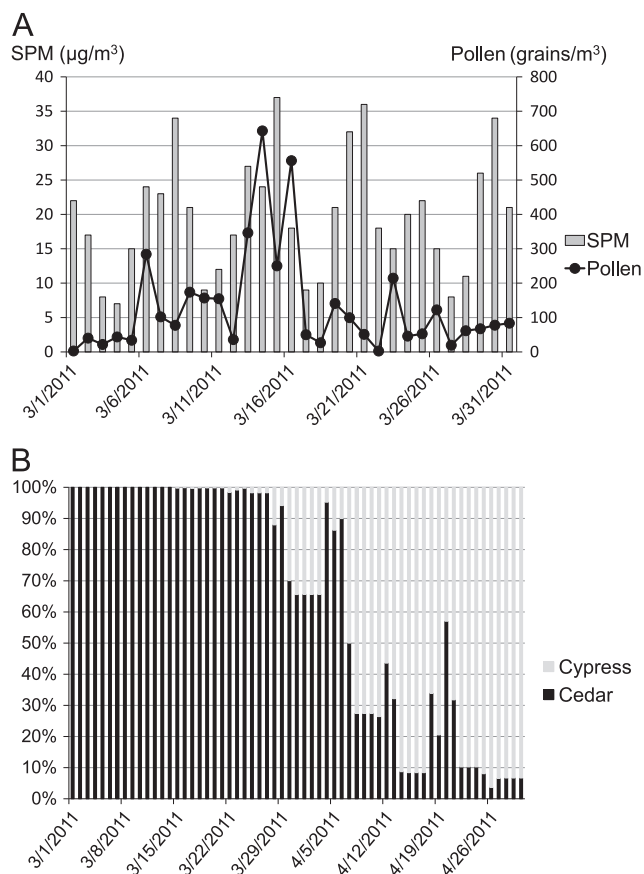


Fig. 1. Airborne suspended particulate matter (SPM) and pollen count during the study. (A) The 24-hour average SPM (< 10 μm) concentration (μg/m³) and average airborne pollen count (number of grains/m³) at each monitoring site in May 2011. (B) Dispersion of cedar and cypress pollens monitored by the Japan Meteorological Agency in Tokyo (May 2011).

2.6. Skin prick test

We performed SPTs with single-use sterile lancets (Yayoi Corporation, Tokyo, Japan) and a standard panel of 14 allergen extracts for pollens (cedar, orchard grass, ragweed, and mugwort), house dust (house dust mixture and *D. pteronyssinus*, animal dander (cat and dog), fungi (*A. tenuis*, *Candida*, and *Aspergillus*), and foods (milk, egg, and wheat). All extracts were used at a concentration of 1:20 wt/vol and all were obtained from Torii Pharmaceutical Co., Ltd. (Tokyo, Japan). The allergens included in this test panel are considered to be the main causes of allergic diseases in Japan. We also used our own Asian dust-related allergen solutions (Asian dust, Asian dust extract, sterilized Asian dust, and SiO₂). Physiological saline (isotonic sodium chloride solution: Otsuka Pharmaceutical Co., Tokyo, Japan) was employed as a negative control. Each SPT was performed by placing a drop of the extract directly on the forearm and introducing it into the epidermis with the lancet puncture. Evaluation was done at 15 min after application and the test was considered positive if the mean wheal diameter was at least 3 mm.

2.7. Statistical analysis

The chi-square test or Fisher's exact test was used to assess differences between percentages or categorical variables. One-way analysis of variance and Scheffe's multiple comparison test were used to compare mean values among the three groups. Pearson's two-tailed correlation coefficients were calculated to investigate the relationships between continuous variables. Factors significantly associated with SPT diameter for Asian dust were selected by forward stepwise multiple logistic regression analysis. Explanatory variables included the mean wheal diameter in each allergen category (pollen, house dust, animal dander, fungi, and food). The level of significance was set at $p < 0.05$. Statistical analysis was performed with SAS System 9.1 software (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

3.1. Prevalence of a positive SPT and mean wheal diameter for each allergen

Table 3 shows the prevalence of a positive SPT for each allergen and Fig. 2 shows the mean wheal diameter for each allergen. Both the SPT positive rate and the mean wheal diameter for each allergen were greater in the allergic and atopic groups compared with the control group (Table 3 and Fig. 2). In the allergic group, the highest SPT positivity rate was observed for cedar pollen, house dust, and

Table 3
Comparisons of SPT reactions among the control, allergic, and atopic groups.

Allergens	Control group	Allergic group	Atopic group	P value
Plant pollens				
Cedar	1 (10%)	8 (80%)	3 (100%)	0.0015
Orchard grass	1 (10%)	6 (60%)	3 (100%)	0.0016
Ragweed	0 (0%)	6 (60%)	2 (67%)	0.0087
Mugwort	0 (0%)	6 (60%)	3 (100%)	0.0016
House dust				
House dust	2 (20%)	8 (80%)	3 (100%)	0.0068
<i>D. pteronyssinus</i>	1 (10%)	8 (80%)	3 (100%)	0.0068
Animal dander				
Cat	0 (0%)	4 (40%)	3 (100%)	0.0029
Dog	0 (0%)	2 (20%)	1 (33%)	0.2214
Fungi				
<i>Alternaria tenuis</i>	0 (0%)	2 (20%)	1 (33%)	0.2214
<i>Candida</i>	0 (0%)	1 (10%)	1 (33%)	0.1952
<i>Aspergillus</i>	0 (0%)	2 (20%)	1 (33%)	0.2214
Foods				
Milk	0 (0%)	0 (0%)	2 (67%)	0.0007
Egg	0 (0%)	0 (0%)	0 (0%)	–
Wheat	0 (0%)	0 (0%)	1 (33%)	0.0307

Values indicate the number of patients. Chi-square test of independence or Fisher's exact probability test among three groups.

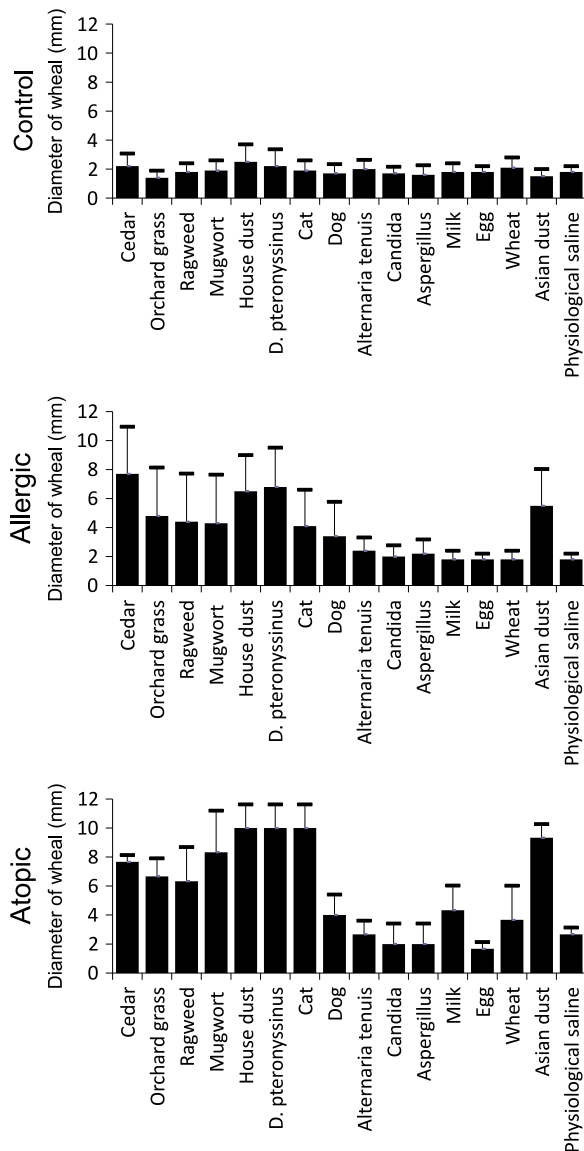


Fig. 2. Skin prick test (SPT) responses to various allergens in the control, allergic, and atopic groups. Mean wheal sizes are shown (mm).

D. pteronyssinus (all 80%), followed by orchard grass, ragweed, and mugwort (60%), and then cat dander (20%) (Table 3).

3.2. Prevalence of sensitization to Asian dust-related allergens

The prevalence of sensitization to Asian dust-related allergens is shown in Table 4. A significant difference was found with respect to the positive rate for Asian dust (0%, 70%, and 100%, $\chi^2=14.5$, $p=0.0007$), Asian dust extract (0%, 50%, and 100%, $\chi^2=12.0$, $p=0.0025$), and sterilized Asian dust (0%, 20%, and 67%, $\chi^2=7.2$, $p=0.0270$) among the control, allergic, and atopic groups, respectively (2 degrees of freedom [df] in Fisher's exact probability test). In contrast, no significant differences were detected in the SPT positive rates for SiO_2 and physiological saline (Table 4). Comparison of the various Asian dust allergens showed a significant difference of the SPT positivity rate among five solutions, including the four Asian dust allergens and physiological saline, in the allergic group ($\chi^2=14.15$, $\text{df}=2$, $p=0.0068$, Fisher's exact probability test), with the rate being highest for Asian dust, followed by Asian dust extract, sterilized Asian dust, SiO_2 , and physiological saline in descending order (Table 4).

Table 4

Skin prick test (SPT) results for Asian dust-related allergens in the control, allergic, and atopic groups.

SPT	Control group	Allergic group	Atopic group	P value*
Asian dust	0 (0%)	7 (70%)	3 (100%)	0.0007
Asian dust extract	0 (0%)	5 (50%)	3 (100%)	0.0025
Sterilized Asian dust	0 (0%)	2 (20%)	2 (67%)	0.0270
Silicon dioxide (SiO_2)	0 (0%)	2 (20%)	1 (33%)	0.2214
Physiological saline	0 (0%)	0 (0%)	0 (0%)	–
P value**	–	0.0068	0.5194	

Values represent the number of patients. Chi-square test of independence or Fisher's exact probability test among *three groups or **five SPT results.

Table 5

Correlation of a positive reaction to Asian dust with the SPT reaction to various allergens in the allergic group ($n=10$).

Variable	Correlation coefficient		
	R	(95% CI)	P value
Cedar	0.77	(0.27–0.94)	0.0048
Orchard grass	0.59	(–0.06 to 0.89)	0.0365
Ragweed	0.53	(–0.15 to 0.87)	0.0562
Mugwort	0.55	(–0.13 to 0.88)	0.0509
House dust	0.31	(–0.40 to 0.78)	0.1940
<i>D. pteronyssinus</i>	0.22	(–0.48 to 0.75)	0.2729
Cat	0.12	(–0.55 to 0.70)	0.3730
Dog	0.36	(–0.34 to 0.81)	0.1500
<i>Alternaria tenuis</i>	0.64	(0.02–0.91)	0.0221
<i>Candida</i>	0.51	(–0.18 to 0.86)	0.0668
<i>Aspergillus</i>	0.40	(–0.30 to 0.82)	0.1248
Milk	–0.39	(–0.82 to 0.31)	0.8699
Egg	0.00	(–0.63 to 0.63)	0.5000
Wheat	0.26	(–0.44 to 0.77)	0.2319

Correlations between the median SPT wheal diameters for Asian dust and 14 other allergens in the allergic group ($N=10$) were calculated by the two-tailed Pearson's product moment formula. R =Pearson's correlation coefficient; CI=confidence interval.

3.3. Correlations between SPT reaction for Asian dust and various allergens

Table 5 shows the correlations between the mean SPT wheal diameters for Asian dust and for the 14 panel allergens in the allergic group. The reaction to Asian dust was significantly correlated with the reactions to all of the plant pollens, including cedar ($r=0.77$, $p=0.0048$), orchard grass ($r=0.59$, $p=0.0365$), ragweed ($r=0.53$, $p=0.0562$), and mugwort ($r=0.55$, $p=0.0509$, Pearson's correlation coefficient analysis). A weak correlation was also seen between the reaction to Asian dust and those to fungi, including *A. tenuis* ($r=0.64$, $p=0.0221$), *Candida* ($r=0.51$, $p=0.0668$), and *Aspergillus* ($r=0.40$, $p=0.1248$, Pearson's correlation coefficient analysis), although the relations were not necessarily significant.

Table 6 shows the correlations between the mean SPT wheal diameter for Asian dust and the mean SPT wheal diameters of the panel allergens, as well as the multivariate odds ratios for the skin reaction to Asian dust in the allergic group. The 14 allergens were categorized into the following 5 groups: pollen (cedar, orchard grass, ragweed, and mugwort), house dust (house dust mixture, and *D. pteronyssinus*), animal dander (cat and dog), fungi (*A. tenuis*, *Candida*, and *Aspergillus*), and foods (milk, egg, and wheat). When the average SPT wheal diameter was calculated for each allergen group, the wheal diameter for Asian dust was correlated with those for pollen ($r=0.71$, $p=0.0104$) and fungi ($r=0.57$, $p=0.0426$, Pearson's correlation coefficient analysis). When all variables were assessed simultaneously by multivariate logistic regression

Table 6
Correlations between the SPT reactions to Asian dust and various allergens, as well as multivariate odds ratios for a reaction to asian dust in the allergic group ($n=10$).

Variable	Correlation coefficient			Multivariate analysis		
	R	(95% CI)	P Value	OR	(95% CI)	P value
Pollens	0.71	(0.15 to 0.93)	0.0104	2.54	(0.26 to 1.19)	0.0138
House dust	0.27	(−0.44 to 0.77)	0.2294	1.35	(−0.30 to 0.78)	0.2400
Animal dander	0.24	(−0.46 to 0.76)	0.2484	0.48	(−1.61 to 0.11)	0.0598
Fungi	0.57	(−0.09 to 0.88)	0.0426	1.84	(0.35 to 3.19)	0.0273
Foods	−0.09	(−0.68 to 0.57)	0.5933	1.26	(−3.70 to 6.48)	0.3926

Average SPT wheal diameters were calculated for pollens (cedar, orchard grass, ragweed, and mugwort), house dust (house dust and *D. pteronyssinus*), animal dander (cat and dog), fungi (*alternaria tenuis*, *candida*, and *aspergillus*), and foods (mild, egg, and wheat). Correlations between the wheal diameter for Asian dust and various wheal diameters in the SPT were calculated using the two-tailed Pearson's product moment formula. Independent determinants of the wheal diameter for Asian dust were investigated by multiple logistic regression analysis. R=Pearson's correlation coefficient; CI=confidence interval; OR=odds ratio.

analysis with stepwise selection of variables, the significant predictors of an SPT reaction to Asian dust were the SPT diameter for plant pollen (odds ratio=2.54, $p=0.0138$) and that for fungi (odds ratio=1.84, $p=0.0273$).

4. Discussion

SPT positivity for Asian dust showed a higher prevalence in the allergic and atopic groups compared with the control group (Fig. 2). Asian dust may contain the spores of pathogenic fungi as well as pollens, and it caused a higher SPT-positive rate (70%) compared with Asian dust extract (50%), sterilized Asian dust (20%), and silicon dioxide (20%) (Table 4). In addition, the SPT wheal diameter for Asian dust showed a strong correlation with those for pollens (cedar, orchard grass, ragweed, mugwort) and fungi (*A. tenuis*, *Candida*, *Aspergillus*) (Table 5). Multivariate analysis also showed a significant association of the response to Asian dust with those to pollens and fungi (Table 6). These results suggest that Asian dust, as well as airborne allergens such as pollen and fungi adherent to the dust, may have a role in the development of allergic rhinoconjunctivitis.

In Japan, Asian dust storms are frequent during the spring (Kimura, 2012). In that season, Asian dust storms aggravate respiratory symptoms in adults with asthma (Hashizume et al., 2010; Otani et al., 2011; Watanabe et al., 2011). Thus, we planned to perform the current study during March, which is the season for Asian dust storms. We utilized the early warning and monitoring systems based on Asian dust modeling by the Japanese Ministry of the Environment. Clinical research on the effects of Asian dust in relation to rhinoconjunctivitis was found to be difficult to conduct. The first reason was that we had to collect and prepare the Asian dust solutions for the SPT immediately before our clinical study. The second reason was that we had to complete the study while Asian dust levels in the air were constant for 2 weeks. If the Asian dust season was too short, our study could not have been accomplished. The third reason is that Asian dust is composed of minerals and metals such as calcium, silicon, and aluminum, but not protein (Ichinose et al., 2008a, 2008b). For this reason, we could not obtain a specific antibody to Asian dust for research and could not measure specific serum immunoglobulin targeting Asian dust in the clinical setting.

The SPT-positive rate for Asian dust was higher in the allergic and atopic groups than in the control group (Fig. 2, Table 4). This result suggests that allergens contained in Asian dust cause allergic skin reactions.

Comparing the SPT-positive rate of various forms of Asian dust, the positive rate was highest for unchanged Asian dust followed by Asian dust extract, sterilized Asian dust, and silicon dioxide (Table 4). The unchanged form of Asian dust contains various

airborne particles, such as pollutants, aerosols, pollens, and fungi. In contrast, the Asian dust extract was obtained by filtering out large particles such as pollen from Asian dust, while sterilized Asian dust was obtained by heating Asian dust to eliminate bacterial and fungal contamination. Silicon dioxide contains no pollen or fungi. Using these different allergens, our findings clearly indicated that components of Asian dust, such as pollen and fungi are related to the adjuvant effect of Asian dust on allergic reactions.

Next, we examined the correlation between the SPT reactions to Asian dust and various other allergens to assess its adjuvant effect (Table 5). As a result, the SPT reaction to Asian dust showed a strong correlation with those to pollens and fungi, indicating that these allergens may be involved in the development of the Asian-dust related allergic reactions as adjuvants within the dust. Indeed, multivariate analysis also showed that these allergens are main risk factors for an allergic reaction to Asian dust (Table 6).

It has been reported that allergic symptoms and asthma become worse during Asian dust storms (Chang et al., 2006; Park et al., 2005; Watanabe et al., 2011; Yang et al., 2005), which occur sporadically during the spring. The reason why allergic symptoms become worse during Asian dust storms may be that pollen is also dispersed during the same season in Japan. Pollen is undoubtedly the most important cause of seasonal allergic rhinitis and conjunctivitis. As seen in Fig. 1(A), the highest total pollen count was detected on March 14, 2011, when this research was conducted. Most of the pollen dispersed in this season is cedar pollen, after which the cypress pollen season starts (Fig. 1(B)). The SPT reaction to Asian dust was also highly correlated with those to autumnal airborne allergens (such as orchard grass, ragweed, and mugwort) as well as to the springtime allergen cedar pollen (Table 5). Our previous studies have shown that positivity for specific serum IgE antibodies directed against cedar pollen is associated with sensitivity to other pollens such as cypress, ragweed, and mugwort (Mimura et al., 2004, 2005). These results suggest that various allergens may cross-react and have common epitopes.

Asian dust also contains viable bacteria and fungal spores. At Fusarium on the west coast of Korea, four molds (*Fusarium*, *Aspergillus*, *Penicillium*, and *Basipetospora*) were identified from samples of SPM taken during the Asian dust season in the spring of 2000 (Yeo and Kim, 2002). Another study done in Korea revealed that Asian dust collected between April 2007 and March 2008 contained spores of *Aquabacterium*, *Flavobacteriales*, *Prevotellaceae*, and other bacteria, whereas samples collected when there were no dust storms contained *Propionibacterium*, *Bacillus*, *Acinetobacter*, and others (Lee et al., 2009). In southern Taiwan, Asian dust collected between December 2000 and April 2001 contained higher concentrations of Basidiomycota, *Penicillium/Aspergillus*, *Nigrospora*, *Arthrinium*, *Curvularia*, *Rusts*, *Stemphylium*, *Cercospora*,

and *Pithomyces* spores than samples collected when there were no dust storms (Wu et al., 2004). Fungal spores can cause asthma and other respiratory allergic reactions (Gonianakis et al., 2006; Hasnain et al., 1985; Singh and Mathur, 2012). In the present study, multivariate analysis demonstrated a statistically significant relationship between the SPT reaction to Asian dust and that to fungi (Table 6). This suggests that Asian dust may enhance the allergic reaction to airborne fungal spores and have an adjuvant effect on allergic disease.

Asian dust is widely suspected to be an important factor in the pathogenesis of atopic dermatitis (Watanabe et al., 2011). Our study also demonstrated a higher SPT-positive rate for Asian dust in the atopic group (Table 4, Fig. 2). The fungi, mites, and other potential allergens contained in Asian dust may be involved in the development of atopic dermatitis. Asian dust is particularly rich in calcium carbonate (CaCO_3), but contains little iron (Cao et al., 2005a, 2005b). A solution of CaCO_3 shows alkalinity, so Asian dust may act like an exfoliant to open skin pores and remove epithelium, thus provoking cutaneous inflammation in patients with atopic dermatitis.

A limitation of this study was the small number of patients investigated. Asian dust storms cause very short-term peaks of dust levels in Japan, which occurred on a total of only 14 days in 2011 (Japan Meteorological Agency). Additionally, our two-week study of Asian dust events within a limited area made it extremely difficult to recruit more patients for this clinical trial.

In conclusion, the present study demonstrated that there was a stronger allergic reaction to Asian dust in the allergic and atopic groups than in the control group, and the SPT-positive rate for Asian dust was higher than that for sterilized Asian dust in the allergic group. The SPT reaction to Asian dust was strongly associated with the reactions to pollen and fungi. Exposure to Asian dust may contribute to the pathophysiology of allergic rhinoconjunctivitis as an adjuvant to antigenic pollens and fungal elements.

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