

ENVIRONMENT

Effects on asthma and induction of interleukin-8 caused by Asian dust particles collected in western Japan

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Abstract

Objective: Asian dust storms (ADS) contain various airborne particles that may augment airway inflammation by increasing the level of interleukin-8. The objective of the study was to investigate the association of exposure to an ADS with worsening of symptoms of adult asthma and the effect of ADS particles on interleukin-8 transcriptional activity. **Methods:** The subjects were 112 patients with mild to moderate asthma who recorded scores for their daily upper and lower respiratory tract symptoms and measured morning peak expiratory flow (PEF) from March to May 2011. Interleukin-8 transcriptional activity was assessed in THP-G8 cells that were exposed to airborne particles collected during days of ADS exposure. **Results:** Of the 112 patients, 31 had comorbid allergic rhinitis (AR) and/or chronic sinusitis (CS), and had worsened scores for upper respiratory tract symptoms on ADS days compared to non-ADS days. Scores for lower respiratory tract symptoms during ADS days were higher than non-ADS days in all patients. Three patients also had unscheduled hospital visits for exacerbation of asthma on ADS days. However, there was no significant difference in daily morning PEF between ADS and non-ADS days. Airborne particles collected on ADS days induced interleukin-8 transcriptional activity in THP-G8 cells compared to the original soil of the ADS. **Conclusion:** Exposure to an ADS aggravates upper and lower tract respiratory symptoms in patients with adult asthma. ADS airborne particles may increase airway inflammation through enhancement of interleukin-8 transcriptional activity.

Keywords

Asian dust storms, interleukin-8, luciferase assay, respiratory tract, THP-G8 cells

History

Received 4 October 2013
Revised 20 February 2014
Accepted 9 March 2014
Published online 27 March 2014

Introduction

Exposure to particulate matter is associated with respiratory and cardiovascular morbidity or mortality [1,2]. In pediatric asthma patients, Strickland et al. found that short-term exposure to air pollutants increased the number of emergency department visits, even with exposure at relatively low concentrations [3]. Desert dust, which is considered to be harmless, can increase the prevalence of asthma and the occurrence of asthma symptoms [4–6]. Asian dust storms (ADS) originating in the deserts of Mongolia, northern China,

and Kazakhstan often disperse dust over East Asia from spring until late autumn. In Japan, the highest frequency of ADSs has occurred from March to May in recent years, based on information from the Japan Meteorological Agency. An ADS can occasionally be large enough to reach the west coast of the United States [7,8] and Uno et al. [9] showed that an ADS can spread around the globe.

Recent studies have shown an association of ADSs with an increased risk of exacerbation of asthma [10–13]. Our previous telephone surveys also showed that an ADS can aggravate symptoms and pulmonary dysfunction in adult patients with asthma [14,15]. However, it has been suggested that an ADS is not significantly associated with the risk of hospitalization for asthma or the incidence of asthma attacks in Taipei [16,17]. Min et al. showed that yellow sand aggravated upper respiratory tract symptoms in adult patients

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with asthma [18], but few studies have examined the association of ADS exposure with upper respiratory tract symptoms in adult asthma.

In animal models, airborne particles originating from ADSs in Mongolia and northern China have been shown to increase pulmonary inflammation and infiltration [19–21]. The mechanism is unclear, but Sierra-Vargas et al. showed that neutrophils migrate to the lung during acute inflammation induced by exposure to air pollutants [22]. Air pollutants also increase the concentration of interleukin (IL)-8 in bronchial lavage fluid (BALF) and IL-8 mRNA expression in bronchial biopsy tissue obtained from healthy subjects [23]. IL-8 increases in the blood of asthma patients during exacerbation [24], and thus is thought to be a key cytokine in exacerbation of asthma.

In this study, we investigated the relationship between exposure to an ADS and upper and lower respiratory tract symptoms and respiratory function in adult patients with controlled asthma. An IL-8 luciferase assay in a stable THP-1-derived IL-8 reporter cell line was used to investigate the effect of airborne ADS particles.

Materials and methods

Patients

A total of 184 outpatients aged >18 years old with asthma were recruited into the study from December 2010 to January 2011. The patients were residents in four different locations, Yonago City, Matsue City, Toyooka City, and Kanda Town, which are distributed in a rural area of under 200 km diameter in western Japan. Of these patients, 112 were included in the analysis based on the following inclusion criteria (Figure 1): (1) mild to moderate asthma, as defined by the National Heart, Lung, and Blood Institute [25], (2) controlled asthma within 3 months before March 2011, based on the Global Initiative for Asthma (GINA) definition [26], (3) recorded

daily respiratory symptoms and PEF for >90% of days from March to May, 2011, and (4) no hospitalization except for asthma from March to May, 2011. On the basis of GINA criteria, asthma was defined as positive if a case met (1) and (2) or (3) of the following criteria: (1) a history of intermittent wheezing; (2) airway hyper-responsiveness to methacholine; and (3) reversible airflow limitation (12% and 200 ml variability in FEV₁). The Research Ethics Committees of each participating institution approved the study and all patients gave written informed consent.

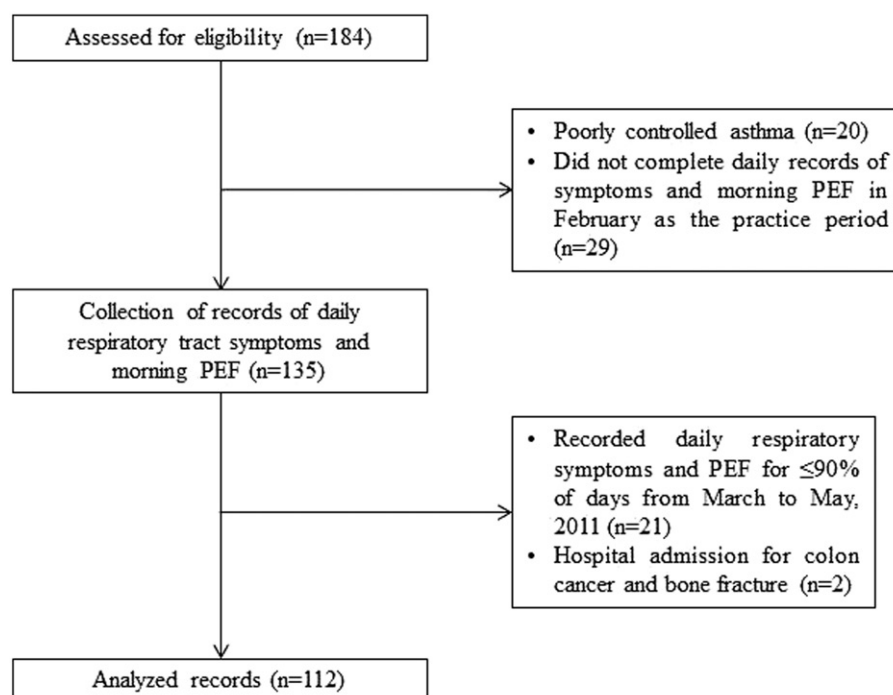
Definition of the period of ADS exposure and monitoring of air pollutants

The period of ADS exposure was determined using information from the Japan Meteorological Agency based on a criterion of visibility <10 km due to dust arising from the deserts of East Asia, as determined by meteorological satellites. Concentrations of suspended particulate matter (SPM), sulfur dioxide (SO₂), nitrogen dioxide (NO₂), and photochemical oxidants (O_x) were confirmed based on monitoring at many locations in Japan by the Japanese Ministry of the Environment. In this study, we used these data for concentrations of SPM, SO₂, NO₂, and O_x at the four study locations.

Recording of daily upper and lower respiratory tract symptoms and PEF

From February to May 2011, all patients recorded their daily respiratory tract symptoms and measured their morning PEF using a peak flow meter (Mini-Wright, Harlow, England, American Thoracic Society scale). February was the practice period. Scores for upper respiratory tract symptoms such as stuffiness, sneezing, and lower respiratory tract symptoms such as cough, sputum, dyspnea, and wheezing were recorded in a diary using 0 = absent, 1 = mild, and 2 = severe for each

Figure 1. Flowchart showing the disposition of subjects in the study.



symptom. Unscheduled hospital visits, fever and pharyngeal pain, and use of oral corticosteroids for exacerbation of asthma on ADS days were also recorded by the patients. Respiratory tract infection was defined as being present if patients had pharyngeal pain and/or fever, which was defined as a body temperature $>37.5^{\circ}\text{C}$.

The total scores for each upper and lower respiratory tract symptom was calculated by summing the score for each day. The period of ADS exposure was determined to be 1–3 May 2011 using information from the Japanese Ministry of the Environment. The results were analyzed over a period of one month because climate conditions differ markedly between March and May in Japan. Thus, the control period of non-ADS exposure was defined as 10–30 April 2011. Patients with worsened symptoms associated with ADS were defined as those with wheezing or a severe score for more than two symptoms out of cough, sputum, and dyspnea without pharyngeal pain and/or fever during the ADS period. Patients recorded the best PEF value from three attempts within 30 min of waking up and before taking inhaled corticosteroids (ICS), β_2 -agonists or oral drugs. The minimum morning PEF (% recent best) was determined based on the lowest daily morning prebronchodilator PEF [27]. The recent best value is defined as the best PEF from February to May 2011 in each patient. The lowest PEF values were also determined for the ADS (1 to 3 May 2011) and non-ADS (10 to 30 April 2011) periods.

Preparation of airborne particles collected on ADS days

Soil from the China Loess Plateau (CJ-1), the original ADS soil in the Tengger Desert and Huining located in Gansu Province, was obtained from the National Institute for Environmental Studies (Ibaraki, Japan) in 2002. This reference material was certified by the National Institute for Environmental Studies and the National Research Center for Environmental Analysis and Measurement (Beijing, China). Dust particles were collected in Yonago City on ADS days (1–3 May 2011) using a large acrylic basin with a collection area of 5000 cm^2 and a depth of 30 cm (custom made by Denyo Inc., Tokyo, Japan). CJ-1 and the collected dust were sterilized at 121°C for 30 min in an autoclave (Tomy SX-300; Tomy Co., Tokyo, Japan) and stored in a freezer at -20°C to prevent growth of bacteria and fungi. For stimulation of THP-G8 cells, airborne particles collected on ADS days were diluted to various concentrations with distilled deionized water. Supernatant extracted from water-soluble airborne particles was also collected after the particles were kept on ice for 1 h.

IL-8 promoter-luciferase gene reporter assay

THP-G8 cells are a THP-1-derived reporter cell line that express stable luciferase orange (SLO) and stable luciferase red (SLR) genes under the control of the IL-8 and glyceraldehyde 3-phosphate dehydrogenase promoters, respectively [28]. The THP-G8 cell line was kindly provided by the Department of Dermatology, Tohoku University, Graduate School of Medicine (Sendai, Japan) and was cultured according to a previous report [28]. To measure

the changes of SLO and SLR luciferase activity after exposure to CJ-1 soil and ADS airborne particles, THP-G8 cells (5×10^4 cells/ $100\text{ }\mu\text{l}$ /well) in 96-well black plates (Greiner Bio-One GmbH, Frickenhausen, Germany) were stimulated for 5 h with solvent only (negative control), 100 ng/ml lipopolysaccharide (LPS) (Wako Pure Chemicals, Osaka, Japan), and various concentrations of CJ-1 soil and ADS airborne particles. The IL-8 transcriptional activity of THP-G8 cells was evaluated at maximum induction after stimulation by 100 ng/ml LPS for 5 h [28]. Luciferase activity was determined using a microplate luminometer with a Phelios multicolor detection system (Atto Co., Tokyo, Japan) using Tripluc luciferase assay reagent (Toyobo Co., Osaka, Japan). IL-8 transcriptional activity was assessed from normalized SLO luciferase activity (nSLO-LA), which was calculated as SLO-LA divided by SLR-LA, and the fold induction of nSLO-LA was calculated as the nSLO-LA level of treated cells divided by that of untreated cells [28].

Measurement of concentrations of IL-8 and endotoxin

IL-8 concentrations in culture supernatants were determined using an enzyme-linked immunosorbent assay (ELISA) kit for IL-8 (R&D Systems, Minneapolis, MN, USA). Samples were run in triplicate and read using an automated ELISA reader (Bio-Rad Model 680, Bio-Rad, Philadelphia, PA). The range of the assay was 31.2 to 2000 pg/ml. The endotoxin concentration in ADS airborne particles was measured with a Chromogenic LAL endotoxin assay kit (GenScript, Piscataway, NJ, USA). The range of the assay was 0.01 to 1 EU/ml. The pH of ADS airborne particles was measured with a pH meter (MP220; Mettler Toledo, Schwerzenbach, Switzerland).

Statistical analysis

Results are shown as the mean $\pm$ standard deviation (SD). SPSS Statistics software (Japanese ver. 16.0 for Windows; IBM Japan, Tokyo, Japan) was used for statistical analysis. A Mann–Whitney U test was used for comparison of air pollution data between the ADS and non-ADS periods. A χ^2 test was used for comparison of categorical data between patients with and without comorbid allergic rhinitis and chronic sinusitis. Pulmonary function in these two groups was analyzed by Mann–Whitney U test. Comparisons of the average score for daily upper and lower respiratory tract symptoms between the ADS and non-ADS periods were analyzed by Wilcoxon signed rank test. The difference in average PEF in the ADS and non-ADS periods was analyzed by *t*-test. Significance was defined as $p < 0.05$ in all analyses.

Results

Patient characteristics

A flowchart showing the progression of subjects through the study is shown in Figure 1. Of the initial 184 outpatients with asthma, 20 were excluded due to poorly controlled asthma and 29 declined to record daily respiratory symptoms and morning PEF during the practice period in February. Of the

remaining 135 patients, 21 did not record daily respiratory symptoms and morning PEF for >90% of days from March to May and 2 were excluded due to hospital admissions for colon cancer and bone fracture. This left 112 patients with asthma who were eligible for analysis. The characteristics of these patients are shown in Table 1. Of the 112 patients, 31 (28%) were also diagnosed with allergic rhinitis and/or chronic sinusitis by an otolaryngologist. There was a significant difference in age between patients with and without comorbid allergic rhinitis and/or chronic sinusitis. There was no difference in sex, smoking status, dose of ICS, and prevalence of treatment with long-acting β_2 -agonists between the two groups.

Air pollution on ADS and non-ADS days

In the 2011 season, ADS exposure occurred from May 1 to 3 in the four locations in the study, which are located within a radius of 200 km in a rural area of western Japan. A control non-ADS period was defined from 10–30 April 2011, just before the ADS period. The daily average levels of air pollutants in the ADS and non-ADS periods in each location are shown in Table 2. The average and daily maximum SPM differed significantly between ADS and non-ADS days in the four locations. In contrast, there were no significant differences in the levels of SO_2 , NO_2 , and O_x between the ADS and non-ADS days in any of the locations.

Table 1. Characteristics of patients with or without comorbid allergic rhinitis (AR) and/or chronic sinusitis (CS).

	All patients	Patients without comorbid AR and/or CS	Patients with comorbid AR and/or CS
Number	112	81	31
Age (year)	61.4 $\pm$ 16.3	64.3 $\pm$ 14.7*	55.5 $\pm$ 18.0*
Gender (male/female)	43/69	31/50	12/19
Allergic rhinitis/Chronic sinusitis/Both	23/5/3	0/0/0	23/5/3
Allergic conjunctivitis	3	1	2
Atopic dermatitis	4	2	2
Smoking status Never/Ex/Current	101/8/3	72/6/3	29/2/0
FVC (L)	3.09 $\pm$ 0.91	3.00 $\pm$ 0.86	3.35 $\pm$ 0.96
FEV ₁ (L)	2.45 $\pm$ 0.80	2.34 $\pm$ 0.75	2.66 $\pm$ 0.86
%FEV ₁ (%)	110.5 $\pm$ 18.5	106.4 $\pm$ 17.7	112.0 $\pm$ 23.5
FEV ₁ % (%)	79.2 $\pm$ 7.4	78.6 $\pm$ 6.7	79.1 $\pm$ 8.7
Inhaled corticosteroid	111	80	31
Low dose	27	15	12
Medium dose	73	60	13
High dose	11	5	6
Long-acting β_2 -agonists	42	32	10
Leukotriene receptor antagonist	60	36	24
Theophylline	24	21	3
Histamine antagonist	26	1	25
Intranasal corticosteroid	12	0	12

Data is presented as the mean $\pm$ S.D.

FEV₁; forced expiratory volume in 1 s, %FEV₁; percentage of predicted FEV₁, FVC; forced vital capacity.

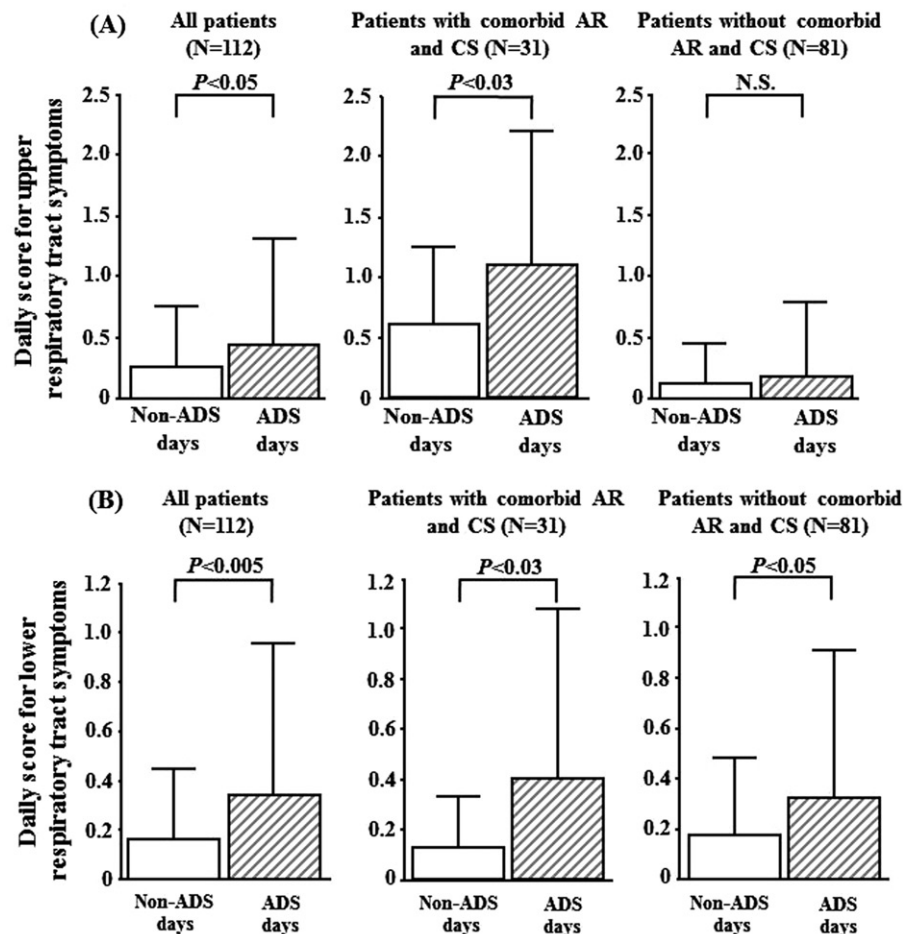
* $p < 0.05$ by Mann–Whitney U for comparison of patients with and without comorbid allergic rhinitis and/or chronic sinusitis

Table 2. Daily average of air pollution levels in four locations during the ADS days (1–3 May 2011) and non-ADS days (10–30 April 2011).

	Yonago City		Matsue City	
	ADS days	Non-ADS days	ADS days	Non-ADS days
Av SPM, $\mu\text{g}/\text{m}^3$	80.3 $\pm$ 29.1*	19.2 $\pm$ 1.5	64.0 $\pm$ 28.2*	17.2 $\pm$ 1.5
Av daily maximum SPM, $\mu\text{g}/\text{m}^3$	124.3 $\pm$ 2.1*	38.3 $\pm$ 4.3	92.3 $\pm$ 13.8*	29.4 $\pm$ 2.4
Av SO_2 , ppb	1.0 $\pm$ 0.0	1.2 $\pm$ 0.1	0.6 $\pm$ 0.1	1.0 $\pm$ 0.1
Av daily maximum SO_2 , ppb	2.0 $\pm$ 0.0	2.6 $\pm$ 0.4	1.1 $\pm$ 0.2	2.1 $\pm$ 0.3
Av NO_2 , ppb	6.3 $\pm$ 3.1	6.1 $\pm$ 2.0	2.0 $\pm$ 1.1	2.5 $\pm$ 1.2
Av daily maximum NO_2 , ppb	13.3 $\pm$ 8.5	15.3 $\pm$ 7.4	3.5 $\pm$ 1.9	5.9 $\pm$ 3.4
Av O_x , ppb	37.7 $\pm$ 5.0	38.3 $\pm$ 8.1	46.1 $\pm$ 12.4	53.1 $\pm$ 8.0
Av daily maximum O_x , ppb	52.3 $\pm$ 2.1	52.2 $\pm$ 13.3	61.5 $\pm$ 6.5	69.1 $\pm$ 11.6
	Toyooka City		Kanda Town	
	ADS days	Non-ADS days	ADS days	Non-ADS days
Av SPM, $\mu\text{g}/\text{m}^3$	100.6 $\pm$ 6.5*	22.2 $\pm$ 2.4	109.0 $\pm$ 35.8*	28.8 $\pm$ 11.4
Av daily maximum SPM, $\mu\text{g}/\text{m}^3$	162 $\pm$ 14.9*	32.0 $\pm$ 11.8	160.0 $\pm$ 17.8*	57.6 $\pm$ 33.2
Av SO_2 , ppb	0.30 $\pm$ 0.06	0.71 $\pm$ 0.90	1.3 $\pm$ 0.6	2.1 $\pm$ 1.2
Av daily maximum SO_2 , ppb	0.67 $\pm$ 0.33	1.3 $\pm$ 1.2	3.7 $\pm$ 2.1	5.9 $\pm$ 4.1
Av NO_2 , ppb	3.86 $\pm$ 0.28	4.76 $\pm$ 0.48	10.0 $\pm$ 8.7	15.5 $\pm$ 10.7
Av daily maximum NO_2 , ppb	13.0 $\pm$ 2.1	11.6 $\pm$ 3.7	26.0 $\pm$ 17.6	39.2 $\pm$ 22.4
Av O_x , ppb	45.2 $\pm$ 6.5	58.5 $\pm$ 2.1	37.0 $\pm$ 11.4	42.5 $\pm$ 14.4
Av daily maximum O_x , ppb	59.3 $\pm$ 6.4	67.0 $\pm$ 10.8	52.7 $\pm$ 13.7	63.8 $\pm$ 13.2

Data is presented as the mean $\pm$ S.D., Av; average, * $p < 0.05$, ADS days versus non-ADS days.

Figure 2. Scores for upper (A) and lower (B) respiratory tract symptoms in periods without (10–30 April 2011) and with (10–3 May 2011) an Asian Dust Storm (ADS) in all patients, and in patients with and without comorbid allergic rhinitis (AR) and/or chronic sinusitis (CS). Scores for upper respiratory tract symptoms such as stuffiness, sneezing, and lower respiratory tract symptoms such as cough, sputum, dyspnea, and wheezing were defined as 0 = absent, 1 = mild, and 2 = severe for each symptom.



Respiratory symptom scores and PEF

In all patients, scores for upper respiratory tract symptoms were significantly higher on ADS days compared to non-ADS days (0.45 ± 0.08 vs. 0.26 ± 0.05 , $p < 0.05$; Figure 2A). Similarly, patients with comorbid allergic rhinitis and/or chronic sinusitis had significantly increased scores for upper respiratory tract symptoms on ADS days compared to non-ADS days (Figure 2A). In contrast, in patients without comorbid allergic rhinitis and/or chronic sinusitis, there was no significant difference in these scores on ADS and non-ADS days.

Scores for lower respiratory tract symptoms in all patients were also significantly higher on ADS days compared to non-ADS days (0.35 ± 0.61 vs. 0.16 ± 0.28 , $p < 0.005$; Figure 2B). There were significant differences in the scores for these symptoms between ADS and non-ADS days in patients with and without comorbid allergic rhinitis and chronic sinusitis (Figure 2B). Three patients made unscheduled hospital visits and took oral corticosteroids due to exacerbation of asthma without respiratory tract infection during the ADS period. In contrast, no patients needed oral steroids for exacerbation of asthma on non-ADS days, except for one event of exacerbation of asthma due to respiratory tract infection.

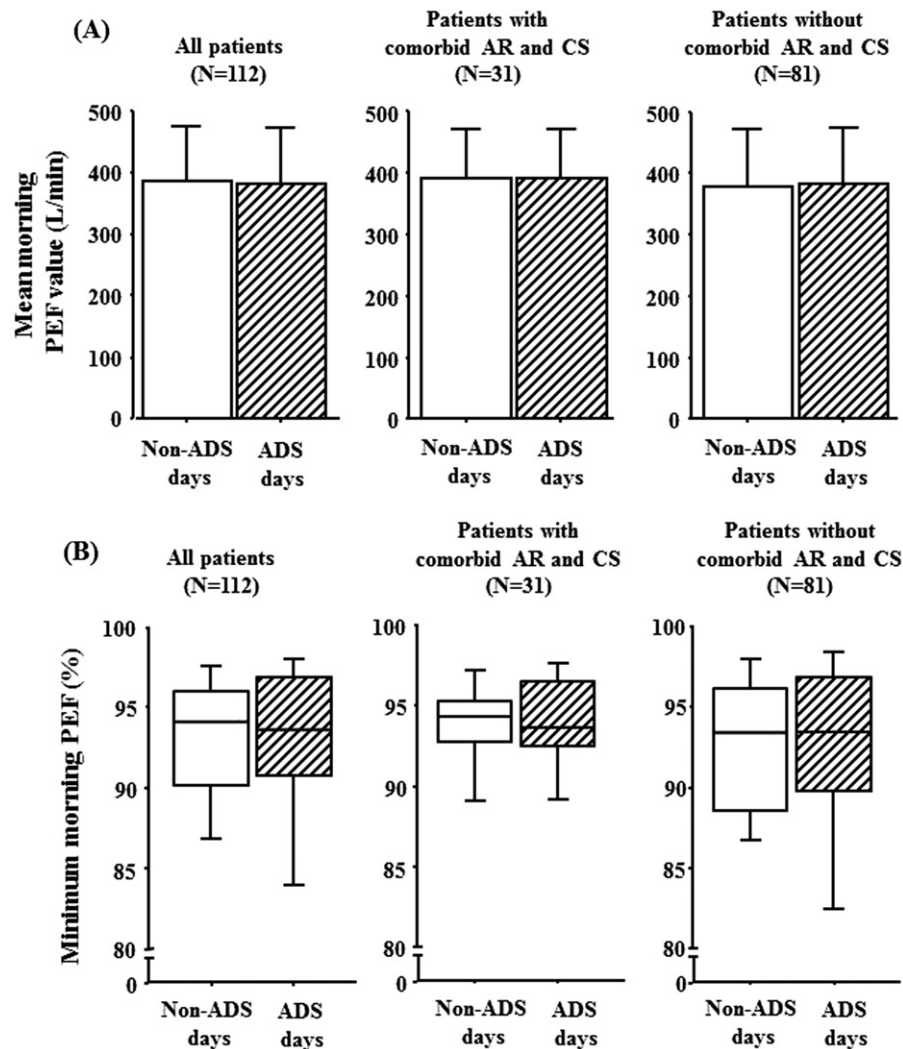
The mean morning PEF values were 384.9 ± 89.6 and 382.3 ± 90.2 l/min on ADS and non-ADS days (Figure 3A), with no significant difference between the periods. There were also no significant differences in mean morning PEF on

ADS days compared to non-ADS days in patients with and without comorbid allergic rhinitis and/or chronic sinusitis (Figure 3A). The respective minimum morning PEF values were $92.4 \pm 5.9\%$ and $92.7 \pm 4.6\%$ in all patients, $93.4 \pm 3.2\%$ and $93.2 \pm 4.2\%$ in patients with comorbid allergic rhinitis and/or chronic sinusitis, and $92.3 \pm 5.1\%$ and $92.0 \pm 6.4\%$ in patients without comorbid allergic rhinitis and/or chronic sinusitis (Figure 3B), also without a significant difference.

IL-8 transcriptional activity and IL-8 secretion in THP-G8 cells

The pH values of ADS airborne particles (400 μ g/ml) and CJ-1 soil (400 μ g/ml) were 7.9 and 8.4, respectively. THP-G8 cells were stimulated with CJ-1 soil after adjustment of the pH of the soil to 7.8 with 0.1 N sodium hydroxide. nSLO-LA was not increased before adjusting the pH (data not shown). Exposure of pH-adjusted CJ-1 soil ($n = 6$) also did not increase nSLO-LA in THP-G8 cells (Figure 4). In contrast, nSLO-LA was increased in exposure of cells to ADS airborne particles ($n = 6$) in a dose-dependent manner (Figure 5). Stimulation of THP-G8 cells with supernatant of ADS airborne particles caused nSLO-LA to increase to 1.3 ± 0.1 at 100 μ g/ml and to 3.1 ± 0.2 at 400 μ g/ml (Figure 5). However, the increase of nSLO-LA was smaller with the supernatant compared with the ADS airborne particles. The concentrations of IL-8 in supernatants of THP-G8 cells stimulated with vehicle, LPS ($n = 6$, 100 ng/ml), CJ-1 soil ($n = 6$, 400 μ g/ml) and ADS airborne particles ($n = 6$,

Figure 3. Morning peak expiratory flow (PEF) (A) and minimum morning PEF (% recent best) (B) in a period without (10–30 April 2011) and with (1–3 May 2011) an Asian Dust Storm (ADS) in all patients, and in patients with and without comorbid allergic rhinitis (AR) and/or chronic sinusitis (CS). The best PEF value was that recorded in three attempts within 30 min of waking up and before taking inhaled corticosteroids (ICS), β_2 -agonists or oral drugs. The minimum morning PEF (% recent best) is defined as the lowest daily morning prebronchodilator PEF [26].



400 $\mu\text{g/ml}$) were 0.03 ± 0.002 , 38.1 ± 1.7 , 0.30 ± 0.004 , and 29.7 ± 1.5 $\mu\text{g/ml}$, respectively (Figure 5).

Endotoxin concentration in airborne particles collected on ADS days

The endotoxin concentrations were 0.192 EU/ml in ADS airborne particles at 400 $\mu\text{g/ml}$ and 0.074 EU/ml in CJ-1 soil at 400 $\mu\text{g/ml}$. However, LPS at 100 ng/ml was out of the range of the assay, so we measured at 100 pg/ml of LPS. The endotoxin concentration was 0.885 EU/ml.

Discussion

This study showed that exposure to an ADS aggravated upper and lower respiratory tract symptoms in adult patients with controlled asthma, with three patients having unscheduled hospital visits for exacerbation of asthma without respiratory tract infections on ADS days. IL-8 transcriptional activity in THP-G8 cells was stimulated by airborne particles collected on ADS days. These results suggest that asthma might be exacerbated by ADS exposure due to enhanced airway inflammation mediated by increased IL-8 in the airway.

Our previous telephone surveys showed that exposure to an ADS can aggravate lower respiratory tract symptoms and respiratory function in asthma patients [14,15]. In this study,

we assessed both lower and upper respiratory tract symptoms on ADS and non-ADS days, and found that adult patients with asthma had worsened upper and lower respiratory tract symptom scores on ADS days. Three patients also had unscheduled hospital visits for asthma, although none were hospitalized, suggesting that ADS exposure has effects on symptoms in patients with controlled asthma. The worsening of upper respiratory tract symptoms in patient with asthma, and especially in those with comorbid allergic rhinitis and/or chronic sinusitis, on ADS days, is consistent with the findings in Min et al. [18]. In the present study, there were no significant differences in the mean morning PEF and the minimum morning PEF (% recent best) between ADS and non-ADS days, despite the worsening of symptoms. The lack of detection of a decrease in PEF on ADS days may have been due to the lower average level of SPM on these days in 2011 compared to our previous studies [14,15]. Alternatively, peak flow may not be a sensitive parameter for detecting subtle changes in asthma [29] and the patients in this study had well-controlled asthma.

IL-8 is a key chemokine in airway inflammation induced by air pollutants [23,24] and Honda et al. found that components adhered to ASD can increase release of IL-6 and IL-8 from airway epithelial cells [30]. An IL-8 promoter luciferase assay in THP-G8 cells, the so-called IL-8 Luc

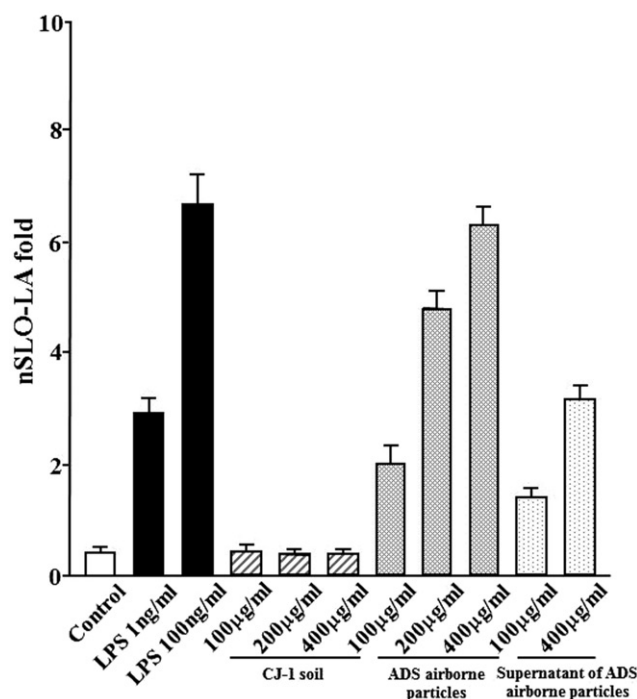


Figure 4. IL-8 transcriptional activity measured using an IL-8 luciferase assay in a stable THP-1-derived IL-8 reporter cell line. Cells were treated with solvent only (negative control), LPS (positive control), CJ-1 soil, airborne particles, and supernatant extracted from airborne particles collected during an ADS. IL-8 transcriptional activity was assessed from normalized SLO luciferase activity (nSLO-LA), which was calculated as SLO-LA divided by SLR-LA. The fold induction of nSLO-LA was calculated as the nSLO-LA of treated cells divided by that of untreated cells [27].

assay, was used in the study because it has high sensitivity for evaluation of the IL-8 level using a small amount of material. Sand dust particles of ADS in Japan contain various chemicals, metals, microorganisms and ionic components [31,32]. To examine exacerbation of respiratory function by sand dust particles, we measured the difference in production of IL-8 between ADS airborne particles and the origin CJ-1 soil of the ADS. We found that ADS airborne particles promoted transcriptional activity and production of IL-8 in THP-G8 cells, with a significant increase in IL-8 transcriptional activity in THP-G8 cells treated with ADS airborne particles compared to those treated with original ADS soil. This difference may be caused by substances such as chemicals, metals and microorganisms carried by the ADS, in agreement with the findings of Honda et al. [30].

A large amount of precipitate was formed when an aqueous solution of airborne particles collected on ADS days was kept on ice for 1 h. The supernatant containing water-soluble ADS airborne particles also augmented IL-8 transcriptional activity in THP-G8 cells. This suggests that materials attached to or contained in the soil augmented this activity. Thus, ADS particles may exacerbate aggravation of asthma when chemicals, metals and microorganisms adhere to these particles.

Patients with asthma are more sensitive to the effects of environmental endotoxins (LPS) compared to healthy subjects, and inhaled LPS is associated with airway neutrophil inflammation in patients with asthma and in healthy subjects [33,34]. Our results showed that ADS airborne particles

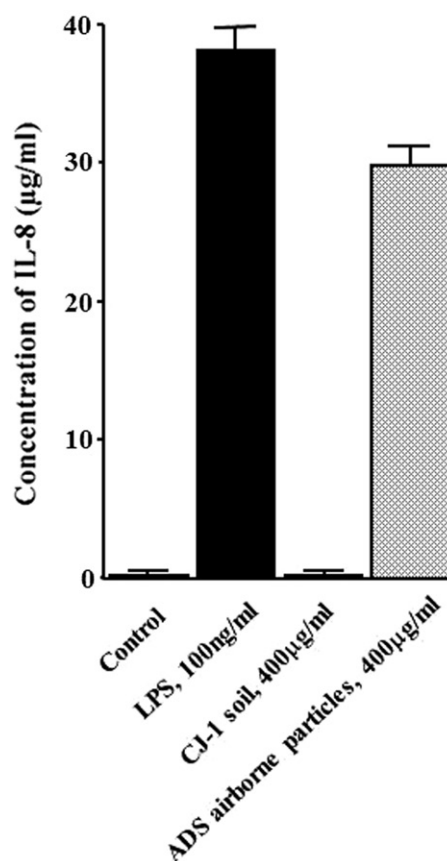


Figure 5. Concentration of IL-8 in supernatants of a stable THP-1-derived IL-8 reporter cell line stimulated with solvent only (negative control), LPS ($n=6$, 100 ng/ml), CJ-1 ($n=6$, 400 µg/ml), and dust particles collected during an ADS ($n=6$, 400 µg/ml). The concentration of IL-8 was measured using an ELISA kit. Samples were run in triplicate. The range of the assay was from 31.2 to 2000 pg/ml.

contained endotoxin, as also found previously [32], but the original soil did not do so, which suggests that endotoxin might have contributed to augmentation of the IL-8 transcriptional activity in THP-G8 cells.

There are several limitations in the study. First, we were not able to collect the same amount of airborne particles on non-ADS days because the level of SPM was very low on these days. Therefore, we were unable to compare stimulation of IL-8 transcription by airborne particles on ADS days with that by particles collected on non-ADS days. Second, we were not able to analyze the composition of airborne particles and the supernatant. Third, we did not assess the amount of pollen, which can affect upper respiratory tract symptoms in rhinitis patients from January to March, distinct from the period of ADS exposure. Future work is required to collect more airborne particles using two high-volume air samplers and a classification of the particles into four size ranges.

Conclusion

We found that upper and lower respiratory tract symptoms in adult asthma patients worsened during a period of exposure to an ADS. Airborne particles collected on ADS days in western Japan, but not the origin soil of the ADS, increased IL-8 secretion in THP-G8 cells. This finding suggests that

exposure to an ADS may exacerbate asthma by enhancement of IL-8 transcriptional activity. Further studies are needed to better define the association between asthma and ADS.

Acknowledgements

The authors thank Dr. Toshiyuki Tatsukawa, Dr. Hirofumi Nakazaki, Dr. Kazuhiro Kato, Dr. Hiroyuki Yamamoto, Dr. Kenichi Takeda, Dr. Masaki Nakamoto, Dr. Yasuyuki Hasegawa, and Dr. Hiroki Chikumi for enrollment of subjects, and Dr. Yutaka Kimura for providing THP-G8 cells.

Declaration of interest

None of the authors have a conflict of interest regarding the work in this study. The authors alone are responsible for the content and writing of this article. This study was supported by the Environmental Research and Technology Development Fund (C-1154) of the Japanese Ministry of the Environment.

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