Asian dust storm (ADS) contains airborne particles that can have negative effects on health in Asian countries. The objective of the study was to investigate the association of ADSs with asthma and airway inflammation in western Japan. We designed a telephone survey to assess the influence of an ADS on upper and lower respiratory systems in adult patients with asthma. The patients also recorded scores for daily upper and lower respiratory tract symptoms and measured morning peak expiratory flow (PEF). Production of interleukin-8 was assessed in THP-1-derived IL-8 reporter cell line (THP-G8) cells that were exposed to airborne particles collected during ADS days. Our results showed that 11–22% of patients with asthma had worsening of lower respiratory tract symptoms on ADS days. The ADS also had a significant negative association with pulmonary function of patients with asthma. However, emergency treatment for augmented symptoms caused by the ADS was not needed because exacerbation of asthma was mild in adult patients. Comorbid allergic rhinitis has been suggested to be an important determinant of worsening of lower respiratory symptoms during an ADS in patients with asthma. Airborne particles collected on ADS days induced interleukin-8 in THP-G8 cells, but this effect was not observed for the original soil sample of the ADS. Thus, exposure to an ADS aggravates upper and lower tract respiratory symptoms in patients with adult asthma and ADS airborne particles may increase airway inflammation through elevation of interleukin-8.

Key words: Asian dust storm, asthma, interleukin-8, respiratory tract systems

Introduction

Dust storms originating in the deserts of Mongolia, northern China, and Kazakhstan are referred to as Asian dust storms (ADSs). ADS events disperse dust over East Asia from spring until late autumn, and this dust contains chemicals, metals, microorganisms and ions (1–3) that cause health problems. Thus, recent studies have shown an association of ADSs with an increased risk of exacerbation of asthma (4–7). However, ADS events were not found to be significantly associated with the risk of hospitalization for asthma or the incidence of asthma attacks in Taipei (8,9). Similarly, studies performed in other Asian countries have found no significant association of ADSs with hospital attendance/admission or the incidence and mortality of cerebrovascular or pulmonary disease and conjunctivitis/rhinitis (10–17). This discrepancy may be due to differences in materials attached to particles during ADSs in each country. This is because the storms reach different countries after passing over different cities and seas, and thus the ADS particles include different chemicals, metals, microorganisms and ionic components from urban or industrial emissions (1–3,18).

The mechanism through which ADSs aggravate asthma remains unclear. Sierra-Vargas et al. showed that neutrophils migrate to the lung during acute inflammation induced by exposure to air pollutants (19). 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 (20). IL-8 is increased in the blood of asthma patients during exacerbation (21), and thus is thought to be a key cytokine in exacerbation of asthma.

The effects of ADSs on health may differ among countries. In this study, we investigated the relationship between ADSs and asthma in Japan, including comparison with our previous studies (22–25). We also investigated the effect of ADS particles on production of IL-8.

Materials and Methods

Patients: Patients with asthma aged > 18 years old were recruited into the study from 2006 to 2013. 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. Based on Global Initiative for Asthma (GINA) criteria (26), 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 hyperresponsiveness to methacholine; and (3) reversible airflow limitation (12% and 200 mL variability in FEV$_1$). The Research Ethics Committees of each participating institution approved the study and all patients gave written informed consent.

**Recording of daily upper and lower respiratory tract symptoms:** From February to May 2011, patients with asthma recorded their daily respiratory tract symptoms. Scores for upper respiratory tract symptoms such as stuffiness and sneezing, and for 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 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 $>37.5^\circ$C.

**Peak Expiratory flow (PEF) monitoring:** Patients with asthma recorded the best PEF value from 3 attempts within 30 min of waking up and before taking inhaled corticosteroids (ICS), $\beta_2$-agonists or oral drugs, using a peak flow meter (Mini-Wright, Harlow, England, American Thoracic Society scale).

**Telephone survey:** A survey was done by telephone within three days of the ADS event. We inquired whether patients had exacerbation of upper and lower respiratory, ocular, or cutaneous symptoms during the ADS. Questions were asked about the following items: (1) worsening of cough, sputum, wheezing, and dyspnea, use of short-acting $\beta_2$-agonists and hospital visits for lower respiratory symptoms; (2) tearing, itching, mucus, and pain among ocular symptoms; (3) stuffiness, sneezing, pharyngalgia and itching among upper respiratory symptoms; and (4) itching, redness, and pain among cutaneous symptoms. In each of the four categories, worsening was judged to have occurred if a patient mentioned that at least one symptom had worsened, excluding patients who had respiratory tract infection (as defined above) or suspected infection.

**Definition of ADS days and monitoring of air pollutants:** The Japan Meteorological Agency has observatories throughout Japan and defines an ADS day using a criterion of visibility $<10$ km due to dust arising from the deserts of East Asia, as determined by meteorological satellites monitoring each area. These data were used to define ADS days and also as a source for concentrations of suspended particulate matter (SPM), PM$_{2.5}$, sulfur dioxide (SO$_2$), nitrogen dioxide (NO$_2$), and photochemical oxidants (O$_3$), as monitored by the Japanese Ministry of the Environment.

**Airborne pollen:** Airborne pollen in Yonago City was measured with a Durham sampler (Asahi-rika, Chiba, Japan) on the roof of a building 10 m above ground with free air movement on all sides. Slides covered with glycerine jelly containing fuchsin were exposed to air for 24 h, and then pollen grains on slides in an $18 \times 18$ mm area were identified and counted after staining with Cabela’s solution (Muto Pure Chemicals, Tokyo, Japan). Total daily pollen counts are expressed as the number of pollen particles per cm$^2$ per day.

**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 is reference material certified by the National Institute for Environmental Studies and the National Research Center for Environmental Analysis and Measurement (Beijing, China). Dust particles were collected using a large acrylic basin with a collection area of 5,000 cm$^2$ and a depth of 30 cm (custom made by Denyo Inc., Tokyo, Japan). CJ-1 and collected dust were sterilized at 121$^\circ$C for 30 min in an autoclave (Tomy SX-300; Tomy Co., Tokyo, Japan) and stored in a freezer at $-20^\circ$C to prevent growth of bacteria and fungi. For stimulation of THP-1-derived IL-8 reporter cell line (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 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 (27). The THP-G8 cell line was kindly provided by the Department of Dermatology at Tohoku University Graduate School of Medicine (Sendai, Japan) and was cultured using a published method (28). To measure the changes of SLO and SLR luciferase activity after exposure to CJ-1 soil and ADS airborne particles, THP-G8 cells ($5 \times 10^4$ cells/100 $\mu$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. IL-8 transcriptional activity of THP-G8 cells was evaluated at maximum induction after stimulation by 100 ng/mL LPS for 5 h (27). 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 the 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 level of treated cells divided by that of untreated cells (27).

**Statistical analysis:** Results are shown as the mean ± 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 χ² 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 scores 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. Multiple regression analysis was performed to assess the relationship between PEF values and levels of air pollutants or weather conditions. Significance was defined as p<0.05 in all analyses.

**Results**

**Results of the telephone survey:** Worsening of scores for upper and lower respiratory tract symptoms on ADS days is shown for a typical patient with asthma and comorbid allergic rhinitis in Fig. 1. In the 2007 survey, we enrolled a total of 112 asthma patients and conducted telephone surveys twice, on April 3–5 and May 28–29 (22). Ninety-eight patients participated in the first telephone survey, while 12 patients could not be contacted; and two patients met the exclusion criteria (Fig. 2). Table 1 shows a summary of the 98 patients.

Worsening of lower respiratory symptoms was found in 22 patients, including five who also had worsening of upper respiratory symptoms, four with worsening of ocular symptoms, and five with worsening of upper respiratory and ocular symptoms. In contrast, of the 98 patients without worsened lower respiratory symptoms, only eight had worsening of upper respiratory, ocular, and/or cutaneous symptoms.

The 22 patients with worsening of lower respiratory symptoms in the first survey were reviewed in the second survey, and 11 patients still had worsening of these symptoms. Of the patients without worsening of lower respiratory symptoms, four had worsening of upper respiratory symptoms only, one had worsening of ocular symptom only, and one had worsening of both upper respiratory and ocular symptoms.

Lower respiratory symptoms that worsened during ADS events included coughing in 14 patients (63.0%), sputum in 12 (55.6%), dyspnea in six (29.6%), and wheezing in one (7.4%) (Fig. 3). Eight patients used a short-acting β₂-agonist for symptoms and noted improvement. None required emergency treatment for exacerbation, but two patients with wheezing had to increase their inhaled corticosteroid dose. There was a significant difference in the prevalence of allergic disease, allergic rhinitis, and atopy between patients with and without worsening of lower respiratory symptoms during ADS events.

**Influence of ADS event on PEF:** The influence of ADS events on PEF of patients with asthma in the 2007 survey (22) is shown in Fig. 3. To assess PEF data, we defined a one-week “dust dispersion” period as the day of the ADS plus the six days after the event. The “control” period was the seven days preceding the event. The lowest PEF during the dust dispersion period was expressed as a percentage of the highest PEF to calculate Min%Max PEF (28). The lowest PEF during the one-week period before the event was expressed as a percentage of the highest PEF. The mean morning PEF was compared to Min%Max for the dust dispersion and control periods. “Control days” were defined as the period from February 1 to May 31, except for ADS days, to compare mean morning PEF/personal best ratio between control and ADS days.
Fig. 2. Consort diagram showing the flow of patients through the 2007 survey (22).

Table 1. Characteristics of subjects in 2007 survey

<table>
<thead>
<tr>
<th>Number</th>
<th>98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>57 ± 17.2</td>
</tr>
<tr>
<td>Gender, Male/Female</td>
<td>42/56</td>
</tr>
<tr>
<td>Asthma duration (month)</td>
<td>109.3 ± 102.4</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>77 (78.6%)</td>
</tr>
<tr>
<td>Former</td>
<td>20 (20.4%)</td>
</tr>
<tr>
<td>Current</td>
<td>1 (1.0%)</td>
</tr>
<tr>
<td>Severity of asthma</td>
<td></td>
</tr>
<tr>
<td>Mild persistent</td>
<td>39 (39.8%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>59 (60.2%)</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.D.

In patients with worsening of lower respiratory symptoms during ADS events, the mean morning PEF/personal best ratio did not differ significantly between ADS days and control days (87.2 ± 6.7% vs. 89.2 ± 5.6%; Fig. 4A), but Min%Max was significantly lower during dust dispersion compared to that during the control period (88.7 ± 6.6% vs. 92.0 ± 5.3%, p < 0.05; Fig. 4B). There were no significant differences in mean morning PEF/personal best ratio and Min%Max between ADS days and control days in patients without worsening of upper respiratory or ocular symptoms (n=68).

Table 2. Partial correlation coefficient for daily morning PEF/Personal best value associated with air pollutants by Watanabe et al. (23)

<table>
<thead>
<tr>
<th>All patients</th>
<th>Partial correlation coefficient</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average temperature (°C)</td>
<td>0.203</td>
<td>-0.015, 0.103</td>
<td>N.S.</td>
</tr>
<tr>
<td>Average atmospheric pressure (hPa)</td>
<td>0.184</td>
<td>-0.011, 0.055</td>
<td>N.S.</td>
</tr>
<tr>
<td>SPM</td>
<td>-0.082</td>
<td>-0.012, 0.006</td>
<td>N.S.</td>
</tr>
<tr>
<td>SO2 (ppb)</td>
<td>0.069</td>
<td>-0.044, 0.072</td>
<td>N.S.</td>
</tr>
<tr>
<td>NO2 (ppb)</td>
<td>-0.006</td>
<td>-0.007, 0.007</td>
<td>N.S.</td>
</tr>
<tr>
<td>Pollen (/cm²/day)</td>
<td>-0.134</td>
<td>-0.002, 0.001</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients with worsening of upper and/or lower respiratory tract symptoms</th>
<th>Partial correlation coefficient</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average temperature (°C)</td>
<td>0.334</td>
<td>0.035, 0.292</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Average atmospheric pressure (hPa)</td>
<td>0.054</td>
<td>-0.057, 0.085</td>
<td>N.S.</td>
</tr>
<tr>
<td>SPM</td>
<td>-0.367</td>
<td>-0.048, -0.008</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>SO2 (ppb)</td>
<td>0.194</td>
<td>-0.037, 0.216</td>
<td>N.S.</td>
</tr>
<tr>
<td>NO2 (ppb)</td>
<td>-0.232</td>
<td>-0.027, 0.002</td>
<td>N.S.</td>
</tr>
<tr>
<td>Pollen (/cm²/day)</td>
<td>-0.135</td>
<td>-0.004, 0.001</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

CI; confidence intervals, N.S.; not significant.
Lower respiratory symptoms were exacerbated during ADS events (22). The frequency was higher for cough and sputum compared with other lower respiratory symptoms. One patient with wheezing and dyspnea had an asthma attack. SABA; short acting β2-agonists.

Results of monitoring PEF in patients with worsening of lower respiratory symptoms during ADS events (22). (A) The mean morning PEF/personal best ratio was 87.2 ± 6.7% on ADS days vs. 89.2 ± 5.6% on control days. (B) Min%Max was 88.7 ± 6.6% in the dust dispersion period vs. 92.0 ± 5.3% in the control period. A significant difference between the dust dispersion and control periods was detected (p = 0.04).

Influences of Asian Dust Storm on Asthma

Association with PEF and air pollutants: Associations among the daily morning PEF/personal best ratio, air pollutants, and weather conditions were investigated by multiple regression analysis in our 2009 survey (23). In the 2009 survey, we enrolled 145 outpatients aged >18 years old with moderate asthma who were managed at Tottori University Hospital and answered the telephone survey, as well as completing the 2007 survey. Patients measured their morning PEF value daily from January to May 2009.

In all patients, there was no significant association between the daily morning PEF/personal best ratio and the levels of SPM, pollen, SO2, and NO2, or the average temperature and atmospheric pressure (Table 2). In patients with worsening of upper and/or lower respiratory tract symptoms, the daily morning PEF/personal best ratio was significantly associated with SPM and temperature, but not with the levels of pollen, SO2 or NO2, or with atmospheric pressure. An increase of SPM led to a decrease of morning PEF.

Recording of daily upper and lower respiratory tract symptoms: From February to May 2011, 112 patients with asthma recorded their daily upper and lower respiratory tract symptoms, and 31 of the 112 patients were also diagnosed with allergic rhinitis and/or chronic sinusitis (24). 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; Fig. 4A). Similarly, patients with comorbid allergic rhinitis and/or chronic sinusitis had significantly increased scores for upper
Fig. 5. Scores for upper (A) and lower (B) respiratory tract symptoms in periods without (April 10 to 30) and with (May 1 to 3) an Asian Dust Storm (ADS) in all patients, and in patients with and without comorbid allergic rhinitis (AR) and/or chronic sinusitis (CS) (24). 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 tract symptoms on ADS days compared to non-ADS days (Fig. 5A). 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, \( p < 0.005 \); Fig. 5B). 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 (Fig. 5B). 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.

IL-8 transcriptional activity in THP-G8 cells: 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 (24). Exposure to pH-adjusted CJ-1 soil (\( n = 6 \)) did not increase nSLO-LA in THP-G8 cells (Fig. 6). In contrast, nSLO-LA increased in a dose-dependent manner after exposure of cells to ADS airborne particles (\( n = 6 \)) (Fig. 6). Stimulation of THP-G8 cells with supernatant of ADS airborne particles caused nSLO-LA to increase to 1.3 ± 0.1 at 100 \( \mu \)g/mL and 3.1 ± 0.2 at 400 \( \mu \)g/mL (Fig. 6). However, the increase of nSLO-LA was smaller with the supernatant compared with the ADS airborne particles.
Fig. 6. 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).

Discussion
Several epidemiological studies have shown an association of an ADSs with health, but only a few studies have examined this association in Japan. Our results suggest that an ADS has an influence on Japanese adult patients with asthma, with 11–22% of our patients noting worsened lower respiratory tract symptoms during ADS events. An ADS can also aggravate the pulmonary function of adult patients with asthma and may exacerbate upper respiratory tract symptoms in asthma patients with comorbid allergic rhinitis and/or chronic sinusitis.

In our previous studies, emergency treatment for exacerbated symptoms caused by ADS events was not needed because the worsening of symptoms was mild in adult asthma patients. In contrast, Kanatani et al. found an increased risk of hospitalization caused by an ADS in Japanese children with asthma (4). Thus, children may be more susceptible to an ADS compared to adults. A larger study is required to examine differences in the effects of an ADS on adult and childhood asthma.

Allergic rhinitis may be an important determinant of worsening of lower respiratory symptoms during ADS events. Shturman-Ellstein et al. suggested that the nasopharynx and oropharynx play important roles in the phenomenon of exercise-induced bronchoconstriction (29). Dry air and particulate matter can directly reach the lower airways when patients are unable to breathe through the nose. Thus, asthma patients with allergic rhinitis may be more susceptible to worsening of lower respiratory symptoms due to inflammatory factors in an ADS that affects the nose, compared to those without allergic rhinitis.

IL-8 plays an important role in airway inflammation induced by air pollutants (20,21) and Honda et al. found that substances attached to ADS particles can increase release of IL-6 and IL-8 from airway epithelial cells (30). Airborne particles in an ADS in Japan contain chemicals, metals, microorganisms and ions (2,3). To examine exacerbation of respiratory function by airborne ADS particles, we investigated production of IL-8 by THP-G8 cells exposed to ADS airborne particles and the original soil sample of the ADS. The airborne particles produced significantly more IL-8 compared to the original soil sample. 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). The supernatant containing watersoluble ADS airborne particles also increased production of IL-8 in THP-G8 cells. Thus, we suspect that materials attached to or contained in the particles caused IL-8 elevation. Thus, ADS particles may exacerbate aggravation of asthma when chemicals, metals and microorganisms adhere to these particles.

Conclusion
We found that exposure to an ADS induced exacerbation of asthma. 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 ADS may exacerbate asthma by elevation of IL-8. Further studies are needed to better define the association between asthma and ADS, and to investigate the mechanism through which an ADS aggravates asthma.

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References
2. Mori I, Nishikawa M, Tanimura T, Quan H. Change in