Relationship of Asian Dust Events with Atmospheric Endotoxin and Protein Levels in Sasebo and Kyoto, Japan, in Spring

Mohammad Shahriar Khan, a Yuya Deguchi, b Takahiro Matsumoto, a Hiroaki Nagaoka, b Nobuyuki Yamagishi, a Keiji Wakabayashi a,d and Tetsushi Watanabe*, a,d

a Department of Public Health, Kyoto Pharmaceutical University; 1 Misasagi-Shichonocho, Yamashina-ku, Kyoto 607–8412, Japan; b Faculty of Pharmaceutical Sciences, Nagasaki International University; 2825–7 Huis Ten Boschcho, Sasebo, Nagasaki 859–3298, Japan; c Faculty of Pharmaceutical Sciences, Setsunan University; 45–1 Nagaotogecho, Hirakata, Osaka 573–0101, Japan; and d Graduate Division of Nutritional and Environmental Sciences, University of Shizuoka; 32–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan.

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Asian dust events are caused by dust storms originating from deserts in Mongolia and northern China, and these events are observed in Japan, mainly in spring. To explore the effect of Asian dust events on atmospheric endotoxin and protein levels, we collected the total suspended particles (TSP) in the spring months (March, April, and May) of 2015 in Sasebo and Kyoto, Japan, and assessed the levels of biological elements at both locations. At both locations, the daily concentrations of TSP, water-soluble Ca2+ (an indicator mineral of soil in dust), endotoxins, and proteins were found to be high during and after Asian dust events recorded by the Japan Meteorological Agency. The concentration of Ca2+ showed a strong positive correlation with the concentrations of TSP and endotoxin, while the concentration of protein was moderately positively correlated with Ca2+ in both Sasebo and Kyoto. There were large concentrations of endotoxins, and the fluctuation ranges were higher in Sasebo than in Kyoto. In contrast, protein concentrations showed low levels of fluctuation, and no major differences were found in the concentration at each location.

Key words bioaerosol; lipopolysaccharide; airborne particle; long-range transport

INTRODUCTION

An Asian dust event is a meteorological occurrence in which the dust from desert areas in Mongolia and northern China is transported east by prevailing westerly winds. Heavy Asian dust events mainly occur during the spring months (March, April, and May) in Japan.1,2 Epidemiological studies have suggested that Asian dust exposure leads to increased emergency department visits and hospitalization for asthma in Japan.3,4 Bacteria and fungi attach to dust particles in the atmosphere over the source regions and travel areas of Asian dust from Mongolia and China.4,5 Ichinose et al. detected endotoxin and β-glucan from soil samples collected at Asian dust source areas (Yanchin Shabianzi in the Maowusu Desert, and Shapotou in the Tengger Desert) and from samples of Asian dust particles collected from the atmosphere in Beijing, China.5 Endotoxin is the major element of the outer membrane of Gram-negative bacteria, and it is suggested that the symptoms of asthma may be exacerbated by increased endotoxin exposure.6–12 β-Glucan is a major component of fungal cell walls, and many allergenic proteins have been isolated from fungi.13 Pollens are the another source of protein which increase in spring on air,14 and allergenic protein from pollen acts as a risk factor for asthma.15 These findings suggest that the levels of endotoxins and proteins in the atmosphere may increase in spring when Asian dust arrives in Japan, and protein level may also be affected by local factors. It is important to understand the association between ambient endotoxin and protein levels and the arrival of Asian dust to prevent the exacerbation of respiratory diseases such as asthma. However, there are currently few reports investigating the atmospheric endotoxin and protein levels in Japan.

The purpose of this study was to clarify the daily fluctuations in the concentrations of atmospheric endotoxins and proteins during the Asian dust season, from March to May, in Japan, and to understand the relationship of those fluctuations with Asian dust events. We collected total suspended particles (TSP) at Sasebo in Nagasaki Prefecture, on the western coast of Japan and closest to mainland China, and in Kyoto in Kyoto Prefecture, located about 590 km east of Sasebo where Asian dust events are expected to have a lesser impact. We analyzed the concentrations of endotoxins, proteins, and water-soluble calcium ion (Ca2+), which is an indicator mineral of soil in dust,16 in TSP. We compared the data from these two locations to clarify the effect of Asian dust events.

MATERIALS AND METHODS

Materials and Reagents Quartz filters were purchased from Pall Life Science (Port Washington, NY, U.S.A.). Limulus Color KY Test Wako and oxalic acid were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The micro bicinchoninic acid (BCA) Protein Assay Kit was supplied by Thermo Fisher Scientific (Waltham, MA, U.S.A.). Standard solutions of Ca2+ were supplied by Kanto Chemical Co., Inc. (Tokyo, Japan).

Collection of TSP We collected TSP in Sasebo (129.79°E, 33.10°N) and Kyoto (135.81°E, 34.99°N) using high-volume air samplers (HV1000R; Shibata Scientific Technology, Soka, Japan) (Fig. 1). Quartz filters were used for the collection of TSP at a flow rate of 1 m3/min for about 24 h per filter. Filters were heated at 250°C for 2 h prior to use. TSP collection was
performed from March 2015 to May 2015. In total, 69 and 70 samples were collected from Sasebo and Kyoto, respectively. Filters were weighed both before and after the collection of airborne particles. Filters containing collected air samples were stored at $-80^\circ\text{C}$ until component analysis.

**Analysis of Endotoxin, Protein, and Water-Soluble $\text{Ca}^{2+}$**

Five percent of the sample filters (corresponding to 71.5 m$^3$ air) were extracted using 0.025% Tween 20 for 30 min by an ultrasonic device for analyzing endotoxin and protein. The extract was centrifuged, and a portion of the supernatant was utilized for endotoxin and protein analysis.

The kinetic chromogenic limulus amebocyte lysate (LAL) method (Limulus Color KY Test Wako kit; Wako Pure Chemical Industries, Ltd.) with a microplate reader (Sunrise Thermo RC-R; Tecan Austria GmbH) were used to analyze endotoxin concentrations according to the manufacturer's protocols, as described previously. The acceptable recovery rates according to the kit ranged from 50–200% for spiked samples.

To analyze protein concentration, a BCA assay (Micro BCA Protein Assay Kit; Thermo Fisher Scientific) and a microplate reader (Sunrise Thermo RC-R; Tecan Austria GmbH) were used as described previously. The water-soluble $\text{Ca}^{2+}$ concentration was examined by an ion chromatograph using a conductivity detector (CDD-10A, Shimadzu Co., Kyoto, Japan) and a Shim-pack IC-C4 column (Shimadzu Co.). In the mobile phase, oxalic acid solution (2.5 mM) was used at a flow rate of 1.0 mL/min.

**Information about Asian Dust Scattering**

The Japan Meteorological Agency (JMA) monitors Asian dust scattering...
at meteorological stations by visual inspection. We obtained the Asian dust data from the JMA in Nagasaki city, which is about 50 km from Sasebo, and in Kyoto city.  

**Statistical Analysis**  
Microsoft Office 2013 was used to calculate correlation coefficients and p-values (<0.05) by simple linear regression analysis.

**Backward Trajectory Analysis**  
The HYSPLIT model, provided by the National Oceanic and Atmospheric Admin-
istration (NOAA), U.S.A., was used for Backward trajectory analysis. Model vertical velocity was used to perform the analysis. The start time was set at 10:00 pm (JST). The height of analysis was set at 1500 m, and the duration was 72 h.

RESULTS

Atmospheric Concentrations of TSP and Water-Soluble Ca\(^{2+}\)
The atmospheric concentrations of TSP and water-soluble Ca\(^{2+}\) in Sasebo and Kyoto are shown in Fig. 2. The medians and ranges of the TSP concentrations in Sasebo and Kyoto were 29.79 and 9.70–141.34, and 29.23 and 2.80–105.68 µg/m\(^3\), respectively. For the Ca\(^{2+}\) concentrations, the median and ranges were as follows: Sasebo, 374.7 and 50.4–2735.8 ng/m\(^3\); Kyoto, 331.8 ng/m\(^3\) and 39.6–1442.1 ng/m\(^3\). High levels of TSP were found in both Sasebo and Kyoto on March 22, when JMA registered an Asian dust event in Nagasaki and Kyoto, and the levels of TSP and Ca\(^{2+}\) in Sasebo were 1.6- and 2.1-fold higher than those in Kyoto, respectively. Similarly, high levels of Ca\(^{2+}\) (>800 ng/m\(^3\)) were observed on March 21 and April 17 at both locations. In addition, the Ca\(^{2+}\) levels on March 10, 20, 23, 25, and 30; and April 15, 16, 22, and 23 were high in Sasebo, and those on April 18 and 27; and May 13 and 27 were high in Kyoto. These results indicate that the atmospheric concentration of soil increased in Sasebo and Kyoto on those days. The correlation coefficients for the concentration of airborne particles and Ca\(^{2+}\) in Sasebo and Kyoto were 0.827 \((p < 0.01)\) and 0.810 \((p < 0.01)\), respectively. These results indicated that the concentration of TSP was largely affected by the amount of atmospheric soil present during the Asian dust season.

Atmospheric Concentrations of Endotoxin and Protein
The atmospheric concentration of endotoxin in Sasebo and Kyoto is shown in Fig. 3. The medians and ranges of endotoxin concentrations in Sasebo and Kyoto were as follows: Sasebo, 0.0131 and 0.0014–0.266 EU/m\(^3\); Kyoto, 0.0065 and 0.0004–0.105 EU/m\(^3\). The endotoxin levels fluctuated greatly each day at each location, and the fluctuation range was larger in Sasebo than in Kyoto. High levels of endotoxin were found in both Sasebo and Kyoto on March 22, a registered Asian dust day in Nagasaki and Kyoto, and on April 17. Endotoxin levels in Sasebo on March 22 and April 17 were 2.6- and 1.8-fold higher than those in Kyoto on those days, respectively. High levels of endotoxin (>0.05 EU/m\(^3\)) were found on March 4, 10, 11, 12, 23, 24, and 25; and April 16 and 22 in Sasebo, and on March 21; April 18; and May 13, 17, and 27 in Kyoto. Increased levels of endotoxin were detected on March 4 and 23; April 30; and May 21 and 26 in Kyoto.

Figure 4 shows the atmospheric concentrations of protein in Sasebo and Kyoto. The medians and ranges of protein concentrations in Sasebo and Kyoto were as follows: Sasebo, 2.07 and 0.90–5.61 µg/m\(^3\); Kyoto, 2.44, 0.47–6.78 µg/m\(^3\). High levels of protein (>3.9 µg/m\(^3\)) were observed on March 21, 22 (a registered Asian dust day), and 30; and April 17 and 23 at both
locations. In addition, the protein levels on April 22 and May 28 were high (>3.9 µg/m³) in Sasebo, and those on March 15; April 18 and 24; and May 27 and 29 were high in Kyoto. The concentrations of protein were similar in Sasebo and Kyoto.

**Association of Endotoxin and Protein Levels with Ca²⁺ Level**

Figures 5 and 6 show the scatter plots of entire TSP collected in Sasebo and Kyoto for endotoxin-Ca²⁺ and protein-Ca²⁺, respectively. Endotoxin levels were strongly positively correlated with Ca²⁺ levels at both locations (Sasebo: $r = 0.679$, $p < 0.01$; Kyoto: $r = 0.707$, $p < 0.01$). Protein levels were moderately positively associated with Ca²⁺ levels in Sasebo ($r = 0.588$, $p < 0.01$) and Kyoto ($r = 0.541$, $p < 0.01$). These results indicated that endotoxin levels were more strongly correlated than protein levels with the amount of atmospheric soil in both Sasebo and Kyoto.

**Backward Trajectory Analysis**

The backward trajectories of the air masses are shown in Figs. 7 and 8, in which high concentrations of endotoxin were detected. The trajectories of air masses beginning in Sasebo on March 4, 10, 22, and 23; and April 17 and 22 suggested that the air masses moved over northern China, where desert areas (the Gobi Desert and Loess Plateau) are located (Fig. 7). The trajectories of air masses on March 24 and 25 in Sasebo were similar to that on March 23 (data not shown). The trajectories of air masses from Kyoto on March 22; April 17; and May 13 and 17 suggested that the air masses passed through the desert areas in northern China and southern Mongolia (Fig. 8). These results suggested that the air masses containing high levels of endotoxin may have traveled through desert areas in China and Mongolia to Sasebo and Kyoto.

**DISCUSSION**

In this study, we collected TSP during the Asian dust season (March, April, and May) in Sasebo and Kyoto, and analyzed the concentrations of water-soluble Ca²⁺, endotoxin, and protein to understand the relationship of Asian dust events with these biological substances. High levels of airborne particles, endotoxin, protein, and Ca²⁺ were found on a JMA-registered Asian dust day, March 22, at both locations. The levels of airborne particles, Ca²⁺, and endotoxin were observed to be more than 1.5-fold higher in Sasebo than in Kyoto. The endotoxin levels fluctuated greatly on each day at both locations, and higher levels of endotoxin were more frequently observed in Sasebo than in Kyoto. The endotoxin levels fluctuated greatly on each day at both locations, and higher levels of endotoxin were more frequently observed in Sasebo than in Kyoto during the study period. In contrast, the protein levels did not fluctuate greatly at either location, and the protein levels at both locations were similar. The Ca²⁺ level, an indicator mineral of soil,

16) was highly positively correlated with the concentrations of airborne particles and endotoxin at both locations. Although sea salt aerosol is a possible source of Ca²⁺ in airborne particles, the involvement of Ca²⁺ from sea salt aerosol was unclear in this study. Our results of backward trajectory analysis (Figs. 7, 8) suggest that air masses in which high levels of endotoxin were observed might be moved through the desert areas in China and Mongolia to Sasebo and Kyoto. These results suggest that ambient endotoxin levels were highly affected by the Asian dust event, although the Asian dust event had a little effect on protein...
levels.

Tang et al. previously analyzed microorganisms in bioaerosol samples collected in transport pathway areas of Asian dust in China (Erenhot, Zhangbei, and Jinan) from March to June 2016, and clarified that the bacterial count was remarkably increased during the dust event, as well as the diversity of bacterial communities.7) Furthermore, Guan et al. quantified airborne endotoxins in fine particulate matter (PM$_{2.5}$, <2.5 µm in aerodynamic diameter) collected in Beijing, which is located in the transport pathway of Asian dust.20) Their one-year study reported that endotoxin levels were very high in March ($n$: 27, geometric mean: 2.15 EU/m$^3$, range: 0.39–60.96 EU/m$^3$). On the other hand, previous studies on airborne bacterial communities in the atmosphere performed at several sites in Japan revealed that the concentration of bacterial cells and the structure of airborne bacterial communities in the atmosphere were affected by Asian dust events.21–24) These results were consistent with our findings in this study, indicating that the ambient endotoxin levels are affected by Asian dust events.

Regarding protein concentrations, Kang et al. analyzed proteins in PM$_{10}$ (<10 µm in aerodynamic diameter) collected in Hefei, China, from June 2008 to February 2009, and reported that protein concentrations ranged from 2.08 to 36.71 µg/m$^3$ (average: 11.42 µg/m$^3$).25) In addition, it was reported that protein concentration was significantly correlated with the air pollution index and mean visibility, suggesting the potential impact of anthropogenic and/or crustal sources.25) In a previous study, we investigated seasonal fluctuations of ambient protein concentrations in Sasebo and reported that the protein concentrations were positively correlated with NO$_3^-$ and SO$_4^{2-}$ concentrations, indicating that the combustion of organic materials may contribute to increases in atmospheric protein.17) In Japan, Japanese cedar (Cryptomeria japonica) and Japanese cypress (Chamaecyparis obtusa) pollens are the most common allergens, and their dispersal reaches a peak in the spring months.26) These results suggest that anthropogenic activities and natural products, including pollen, may be potential sources of the protein detected in Sasebo and Kyoto during this study.

In the present study, we found that Asian dust events greatly increased atmospheric endotoxin levels in both Sasebo and Kyoto in spring. Although a limited number of Asian dust day events were registered by the JMA during the study period, our results clearly indicate a strong correlation between Asian dust events and endotoxin concentrations. Further study is necessary to clarify the influences of atmospheric endotoxin on asthmatic patients, especially in terms of the exacerbation of asthma.

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REFERENCES

1) The Japan Meteorological Agency. “Data bank of global environment-Kosa.” ‹http://www.data.jma.go.jp/gmd/env/kosahp/kosa_data_index.html›, accessed January 2019.

2) Nakamura T, Hashizume M, Ueda K, Shimizu A, Takeuchi A, Kubo T, Hashimoto K, Moriuchi H, Odajima H, Kitajima T, Tashiro K, Tomimatsu K, Nishiwaki Y. Asian dust and pediatric emergency department visits due to bronchial asthma and respiratory diseases in Nagasaki. Japan. J. Epidemiol., 26, 593–601 (2016).

3) Kanatani KT, Ito J, Al-Delaany WK, Adachi Y, Mathews WC, Ramsdell JW. Desert dust exposure is associated with increased risk of asthma hospitalization in children. Am. J. Respir. Crit. Care Med., 182, 1475–1481 (2010).

4) Kakikawa M, Kobayashi F, Maki T, Yamada M, Higashi T, Chen B, Shi G, Tobo Y, Iwasaka Y. Dustborne microorganisms in the atmosphere over an Asian dust source region, Dunhuang. Air Qual. Atmos. Health, 1, 195–202 (2008).

5) Puspitasari F, Maki T, Shi G, Bin C, Kobayashi F, Hasegawa H, Iwasaka Y. Phylogenetic analysis of bacterial species compositions in sand dusts and dust aerosol in an Asian dust source area, the Taklimakan Desert. Air Qual. Atmos. Health, 9, 631–644 (2016).

6) Maki T, Kurosaki Y, Onishi K, Lee KC, Pointing SB, Jugder D, Yamada M, Kobayashi F, Hasegawa H, Shimoda M. Variations in the structure of airborne bacterial communities in Tsogt-Ovoo of Gobi desert area during dust events. Air Qual. Atmos. Health, 10, 249–260 (2017).

7) Tang K, Huang Z, Huang J, Maki T, Zhang S, Ma X, Shi J, Bi J, Zhou L, Wang G, Zhang L. Characterization of atmospheric bioaerosols along the transport pathway of Asian dust during the Dust-Biogeoerosol 2016 Campaign. Atmos. Chem. Phys., 18, 7131–7148 (2018).

8) Ichinose T, Nishikawa M, Tokano H, Sera N, Sagakane K, Mori I, Yamagusawa R, Oda T, Tamura H, Hiyoshi K, Quan H, Tomura S, Shihamoto T. Palmonary toxicity induced by intratracheal instillation of Asian yellow dust (Kosa) in mice. Environ. Toxicol. Pharmacol., 20, 48–56 (2005).

9) Michel O, Ducluzeau F, Sergysels R. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. J. Appl. Physiol., 66, 1059–1064 (1989).

10) Kitz R, Rose MA, Borgmann A, Schubert R, Zienl S. Systemic and bronchial inflammation following LPS inhalation in asthmatic and healthy subjects. J. Endotoxin Res., 12, 367–374 (2006).

11) Rubinovich N, Lio AH, Zhang L, Rodes CE, Foarde K, Dutton SJ, Murphy JR, Gelfand EW. Importance of the personal endotoxin cloud in school-age children with asthma. J. Allergy Clin. Immunol., 116, 1053–1057 (2005).

12) Khan MS, Coulibaly S, Matsumoto T, Yano Y, Miura M, Nagasaki Y, Shima M, Yamagishi N, Wakabayashi K, Watanabe T. Association of airborne particles, protein, and endotoxin with emergency department visits for asthma in Kyoto, Japan. Environ. Health Prev. Med., 23, 41 (2018).

13) Fukutomi Y, Taniguchi M. Sensitization to fungal allergens: Resolved and unresolved issues. Allergol. Int., 64, 321–331 (2015).

14) Yamamoto N, Matsuki Y, Yokoyama H, Matsuhi K. Relationships among indoor, outdoor, and personal airborne Japanese cedar pollen counts. PLOS ONE, 10, e0131710 (2015).

15) Gautier C, Charpin D. Environmental triggers and avoidance in the management of asthma. J. Asthma Allergy, 10, 47–56 (2017).

16) Takahashi Y, Kanai Y, Kamioka H, Ohta A, Maruyama H, Song Z, Shimizu H. Speciation of sulfate in size-fractionated aerosol particles using sulfur k-edge X-ray absorption near-edge structure. Environ. Sci. Technol., 40, 5052–5057 (2006).

17) Khan MS, Coulibaly S, Abe M, Furukawa N, Kubo Y, Nakaoy J, Kawase Y, Matsumoto T, Hasei T, Deguchi Y, Nagoaka H, Yamagishi N, Watanabe M, Honda N, Wakabayashi K, Watanabe T. Seasonal fluctuation of endotoxin and protein concentrations in outdoor air in Sasebo, Japan. Biol. Pharm. Bull., 41, 115–122 (2018).

18) Air resources Laboratory. “HYSSPLIT-Hybrid Single-Particle Lagrangian Integrated Trajectory Model.” ‹http://www.ready.noaa./HYSSPLIT_traj.php›, accessed January 2019.

19) Sultana CM, Collins DB, Prather KA. Effect of structural heterogeneity in chemical composition on online single-particle mass spectrometry analysis of sea spray aerosol particles. Environ. Sci. Technol., 51, 3660–3668 (2017).

20) Guan T, Yao M, Wang J, Fang Y, Hu S, Wang Y, Dutta A, Yang J, Wu Y, Hu M, Zhu T. Airborne endotoxin in fine particulate matter in Beijing. Atmos. Environ., 97, 35–42 (2014).

21) Yamaguchi N, Ichijo T, Sakotani A, Baba T, Nasu M. Global dispersion of bacterial cells on Asian dust. Sci. Rep., 2, 525 (2012).

22) Yamaguchi N, Park J, Kodama M, Ichijo T, Baba T, Nasu M. Changes in the airborne bacterial community in outdoor environments following Asian dust events. Microbes Environ., 29, 82–88 (2014).

23) Maki T, Puspitasari F, Haru K, Yamaida M, Kobayashi F, Hasegawa H, Iwasaka Y. Variations in the structure of airborne bacterial communities in a downwind area during an Asian dust (Kosa) event. Sci. Total Environ., 488–489, 75–84 (2014).

24) Maki T, Hara K, Kobayashi F, Kurokawa Y, Kikakawa M, Matsuki A, Chen B, Shi G, Hasegawa H, Iwasaka Y. Vertical distribution of airborne bacterial communities in an Asian dust downwind area, Noto Peninsula. Atmos. Environ., 119, 282–293 (2015).

25) Kang H, Xie Z, Hu Q. Ambient protein concentration in PM10 in Hefei, central china. Atmos. Environ., 54, 73–79 (2012).

26) Okamoto Y, Heriguchi S, Yamamoto H, Yonekura S, Hanazawa T. Present situation of cedar pollenosis in Japan and its immune responses. Allergol. Int., 58, 155–162 (2009).