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Inflammatory and degranulation effects of yellow sand on RBL-2H3 cells in relation to chemical and biological constituents

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Recent studies pointed out that allergic diseases have increased during the Asian dust storm event (ADSE) in Japan. Daily observations and the atmospheric concentrations of yellow sand (YS) aerosol have been increasing. In this study, YS samples collected from three sites of Japan during ADSE in 2009-2010 were used. The particles were analyzed by X-ray photoelectron spectroscopy (XPS) and X-ray fluorescence - energy dispersive spectrometer (XRF-EDS). We investigate ability of YS extract on enhancing the chemical mediator release and cytokine production from rat basophilic leukemia (RBL-2H3) cells. The dust particles at Fukuoka and Tsukuba were abundant in aluminum (Al), iron (Fe), potassium (K) and titan (Ti) than those at Naha. Concentration of the trace endotoxin and Cryptomeria japonica pollen allergen (Cry j 1) were measured in YS extract. After exposure of RBL-2H3 cells to YS extract, the β-hexosaminidase (β-hex) release, tumor necrosis factor-alpha (TNF-α) production were enhanced in RBL-2H3 cells. This process depends on endotoxin, Cry j 1 and other allergen present in the YS extract. YS water extract also show a strong cytotoxic effect on the cells. This data suggest that low levels of endotoxin and Cry j 1 in YS may cause allergy during the ADSE.

**Keywords**

Allergens, Asian dust storm, β-hexosaminidase, RBL-2H3, Yellow sand

**Abbreviations**

ADSE, Asian dust storm event; β-hex, β-hexosaminidase; Cry j 1, Cryptomeria japonica pollen
allergen; Fuku., Fukuoka; LPS, Lipopolysaccharide; MTT, 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide; PM, Particulate matter; RBL-2H3, Rat basophilic leukemia cells; TNF-α, Tumor necrosis factor-α; Tsu., Tsukuba; YS, Yellow sand.
1. Introduction

The prevalence and morbidity of asthma and other allergic diseases have increased dramatically during the last 30 years, particularly in industrial countries (Narita et al., 2007). A lot of studies have demonstrated that allergens commonly associated with dust storms include fungal spores, plant and grass pollens, anthropogenic emissions, and organic detritus (Griffin 2007). Recently, it was clarified that dust event enables atmospheric long distance transport of bacteria, fungi, viruses, pollen etc. (Kellogg and Griffin 2006; Griffin 2010). Risk of hospitalization was increased during the Sahara dust event in European cities (Middleton et al., 2008). There was an association between the increase in paediatric asthma admissions and the increased Saharan dust event in Caribbean island (Gyan et al., 2005). Recently, daily observations and the atmospheric concentrations of YS aerosol have been increasing steadily in the eastern Asia region, including Japan (Goto et al., 2010). YS increase the risk of mortality and hospitalization in cardiovascular and respiratory patients during the ADSE in China, Korea, and Japan (Kwon et al., 2002; Meng and Lu 2007). Total fungi and particulate matter (PM) had significantly higher concentrations during the period affected by ADSE in Taiwan (Ho et al., 2005). In Japan, it was pointed out that fine YS particles make people’s allergy more severe from February to May. Hua et al. (2007) have detected some bacteria, such as Bacillus subtilis, B. licheniformis in YS collected in Hiroshima, Japan during ADSE, and provided evidence that ADSE can transport these microorganisms in Northeast Asia.

Endotoxin, the lipopolysaccharide (LPS) components of the outer membrane of Gram-negative bacteria, has been associated with an increase in asthma symptoms, asthma
medications, and reductions in lung function in patients with atopic or asthma. Airborne endotoxin appears to be a risk factor for clinically symptomatic respiratory illnesses (Dales et al., 2006) and allergic inflammation (Imrich et al., 2000; Gon et al., 2005), and induces or aggravates a variety of respiratory diseases (Tulic et al., 2000). On the other hand, in recent decades in Japan, the number of Japanese cedar (Cryptomeria Japonica, JC) hay fever patients has increased and an increase in JC pollen is a probable principal cause (Sakurai et al., 2002). The pollen grains of JC pollen (Cry j 1) usually exist as coarse particles about 30 μm. However, the conglomeration of dust-storm particles with sea salt forms larger hygroscopic particles during the long distance transport (Ma et al., 2004). Suspended PM (SPM) is present in the aqueous phase and induces the release of allergen particles (Wang et al., 2012). It was supposed that the major allergen Cry j 1 could be release to the atmosphere as respirable-sized particles and modified by some air pollutants during airborne transportation (Wang et al., 2009a).

An increase in ambient antigen is one of the most probable reasons for the increase in allergic disorders such as allergic rhinitis, bronchial asthma, and atopic dermatitis. Allergic diseases are immunologic disorders, traditionally referred to as immediate or type I hypersensitivity reactions with IgE playing an important role. Crosslinking of the FceRI (high-affinity IgE receptor) induced by complex formation of IgE with an antigenic protein is an essential event in the IgE-mediated allergic reaction (Beaven and Metzger 1993). The interaction of IgE with allergen on mast cells or basophils leads to allergic reactions causing the release of an array of inflammatory mediators resulting in the inflammation of airway mucus membrane leading to clinical symptoms in the target organ (Novak et al. 2001). Mast cells and
basophils play essential roles in the pathogenesis of allergic reactions and in protection against Gram-negative bacteria. RBL-2H3 cells, a tumor analog of mast cells, display characteristics of mucosal-type mast cells and express several hundred thousand FcεRI on the membrane surface. After sensitization with IgE, the cells respond to the antigen and release histamine. β-Hex, which is stored in the secretory granules of mast cells is released concomitantly with histamine when mast cells are immunologically activated (Ortega et al. 1988; Schroeder et al. 1995). Thus, β-hex activity in the medium is used as a marker of mast cell degranulation (Yamada et al., 2010). Therefore, RBL-2H3 cells are considered as a good tool for studying the effect of environmental pollutant on chemical mediator release activity.

Recent studies regarding the impact of microbiological factors contained in YS on the air passage by Yanagisawa et al. (2007) and Ichinose et al. (2008) have indicated that YS has the potential of exacerbating symptoms in the human respiratory apparatus, eyes, and nose. Even though there are few reports concerning the biological activity of particular dust on macrophage and rat lung (Becker et al., 1996; Kim et al., 2003; Meng and Zhang 2007), and pulmonary toxicity and inflammatory allergy induced by administered of YS in mice and guinea pigs (Ichinose et al., 2005; 2009), there is no experimental evidence available regarding YS extract-mediated chemical mediator release by immediate type allergic model cells collected during ADSE in Japan. In this study, YS was collected from Naha, Fukuoka, and Tsukuba during ADSE in 2009 and 2010 of Japan, the chemical composition of YS was analyzed, and the endotoxin and Cry j 1 concentrations in the YS extracts were investigated. Furthermore, degranulation, cytokine production activity, and the cytotoxicity of YS extract on RBL-2H3 cells were examined.
2. Materials and methods

2.1. Chemicals and cells

LPS was obtained from Alexis Biochemical (Enzo Life Sciences, Inc., San Diego, CA). Fetal bovine serum (FBS) was obtained from Gibco BRL, Paisley, Scotland. The enzyme-linked immunosorbent assays (ELISA) for analysis of rat TNF-α and IL-13 were obtained from Invitrogen Co., CA. USA. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) was obtained from Dojindo, Japan. RBL-2H3 cells were purchased from JCRB Cell Bank, Japan. The cells were maintained in MEM supplemented with 10%FBS and 2 mM L-glutamine, and incubated at 37°C in a 5% CO₂.

2.2. Sampling and sample location

YS samples were obtained using a high volume sampler (HV-1000F, Shibata Science, Tokyo, Japan) set on the open top floor of building (15-20 m) during March to June, 2009-2010 based on the information on the YS storm movement reported by the Japan Meteorological Agency. Wind with aerosols including sands was sucked into sampler at a rate of 1000 L/min and the aerosols that adhered onto the filter (Quartz Fiber Filters, Adventec QR-100, Toyo Roshi Kaisha, Ltd.), which was set in the sampler were obtained. Three samples collected in 2009 and three samples collected in 2010, from Naha city (127°40'44"E, 26°12'44"N), Okinawa...
prefecture (named Naha09 and Naha10, collected during April 27th to June 10th in 2009 and March 10th to April 14th in 2010), Fukuoka city (130°24'06"E, 33°35'24"N), Fukuoka prefecture (samples Fuku.09 and Fuku.10, collected during March 16th to 19th in 2009 and March 20th to 24th in 2010) and Tsukuba city (140°10'37"E, 36°14'00"N), Ibaraki prefecture (samples Tsu.09 and Tsu.10, collected during March 16th to 20th in 2009 and March 19th to 23rd in 2010), Japan (Fig. 1), during the ADSE (MEGJ, 2010a) were used in this study. The sampling period, particle concentration, and particle density are shown in Table 1. The first sampling site in the city of Tsukuba (approximately 0.2 million inhabitants a population density of 284.07 inhabitants/km²) is located in an industrialized area near Tokyo, and the samples from Fukuoka City, Fukuoka prefecture (approximately 1.45 million inhabitants, 341.32 inhabitants/km²) situated in an industrialized area near the Japanese sea, whereas the samples from Naha City, Okinawa prefecture (approximately 0.3 million inhabitants, 39.24 inhabitants/km²) represented a rural site. The geographical distances between Naha and Fukuoka sampling sites is about 860 km, while Fukuoka and Tsukuba sampling site about 940 km.

2.3. Analysis of sand components

The collected samples were separated carefully from the filters and subjected to component analysis. First, the chemical components on the upper most 5 nm surface of the sands were analyzed by XPS with ESCA-300 (VG Scietna AB, Uppsala, Sweden). Then this surface layer was separated from bulk sands. The components of bulk sands were analyzed by XRF-EDS with JSX-3220 (JEOL Ltd., Tokyo, Japan).
2.4. Extraction of LPS from YS sample

The filters used to collect the dust samples were cut out and transferred into 15-ml Costar plastic tubes with 2 mL distilled MilliQ water. The tubes were then placed in an ultrasonic bath for 30 min. Then centrifugation was carried out at 1000 × g for 20 min at room temperature, and the supernatant (called water extract) were used for the determination of the endotoxin concentration, β-hex release, cytokine assay, and MTT assay.

2.5. Extraction of Cry j 1 from YS sample

The filters used to collect dust samples were cut out and transferred into 15-mL Costar plastic tubes with 2 mL Cry j 1 extract buffer of TAC® Cry j 1 ELISA Kit (AKCJ1-010, Shibayagi, Gunma, Japan). After standing the solution in 4 °C for 2 h, shaking was carried out at room temperature for 30 min. Centrifugation was then carried out at 1200 × g for 30 min at room temperature, and the supernatant (called Cry j 1 extract) was used as the samples for the pollen allergen Cry j 1 determination and the β-hex release assay after filter-sterilization (0.45 µm Millipore filter).

2.6. LPS determination

Endotoxin in the water extract was measured by a quantitative kinetic chromogenic
Limulus Amoebocyte Lysate (LAL) method using Endospecy ES-50M (Seikagaku Co., Tokyo, Japan) at 37°C. Analyses were performed with an automated microtitre plate reader (PowerScan HT; Dainippon Pharmaceutical Co., Ltd.). Standard Endotoxin CSE-Kit (Seikagaku Co., Tokyo, Japan) was used to determine the amount of endotoxin present in the sample. The detection limit was 0.002 EU/ml. The endotoxin concentration in each sample was calculated using a standard curve, and expressed as units per milliliters of sample (EU/mL). Endotoxin concentrations were reported as endotoxin units (EU) per mL of extract where 10 EU is equivalent to 1.25 ng of the reference standard endotoxin.

2.7. Cry j 1 determination

Cry j 1 concentration in the sample was quantified by an ELISA method using TAC® Cry j 1 ELISA Kit according to the manufacturer’s instructions. Finally, the absorbance of each solution obtained by ELISA was converted into Cry j 1 concentration (pg/mL) of the Cry j 1 extract.

2.8. Degranulation assay

The β-hex release assay using RBL-2H3 cells was performed as previously described (Yamada et al., 2010). RBL-2H3 cells were seeded onto 96-well plates at 5.0×10^4 cells/well in 100 μL of medium. Cells were incubated for 24 h at 37°C, then washed twice with PBS (-) to eliminate medium, and then the cells were exposed to various concentrations of water extract (0,
1/125, 1/100, 1/75, 1/50 dilution in medium) for 16 h at 37°C, 5%CO₂ incubator. As positive and negative controls, 5.0 ng/mL LPS and PBS (-) were used respectively. For Cry j 1 extract-treated cells, cells were sensitized with 0.3 μg/mL anti-DNP-IgE antibody before incubation for 24 h at 37°C, then washed twice with PBS (-) to eliminate the free IgE, and then the cells were exposed to various concentration of Cry j 1 extract (0, 1/100, 1/50, 1/25, 1/10, 1.0 dilution in medium) for 1 h. As positive and negative controls, 0.3 μg/mL DNP-BSA and PBS (-) were used respectively. The β-hex in the supernatant was measured as described in the previous study (Yamada et al., 2010).

2.9. Cytokine assay

To test the amount of TNF-α and IL-13, RBL-2H3 cells were seeded onto 24-well plates at 2.5 × 10⁵ cells/well in 500 μL of medium. Cells were incubated for 16 h in the presence of Naha 09 and Tsu.09 water extract at a final dilution of 1/100 in growth medium. For positive and negative controls, 5.0 ng/mL LPS and PBS (-) were used, respectively. Levels of TNF-α and IL-13 were measured in RBL-2H3 cells’ supernatant using ELISA kits (Inivitrogen Co., UAS, CA) according to the manufacturer’s instructions. The study was performed in triplicate using cells from three different passages.

2.10. MTT assay

The MTT reduction assay is the most frequently used method for quantitative cell viability
RBL-2H3 cells were seeded on 96-well plates at $5.0 \times 10^4$ cells/well in 100 µL of medium. After an overnight incubation, the growth medium was removed followed by addition 100 µL of the water extract (dilution 0, 1/1000, 1/100, 1/10, 1/2, 1.0 with medium). For the highest concentrations, additional controls (1, 5 and 10% PBS (-), data not shown) were considered as references in order to consider the sample effect exclusively on the survival and proliferation of RBL-2H3 cells. The cells were incubated for 24 h, and cell viability was measured as described in the previous study (Yamada et al., 2010).

2.11. Statistical analysis

Results are expressed as the mean ± standard deviation of at least three independent experiments. Experiments were realized in triplicates. Comparisons with the control were performed by analysis of variance (ANOVA), followed by the Newman-Keuls post hoc test. $p$ values of less than 0.05-0.01 were considered significant.

3. Results

3.1. Chemical compositions of YS

To confirm chemical composition of collected sample during ADSE, the results of component analysis of YS by XPS and XRF-EDS collected in 2009 were shown in Table 2. The upper most surface mainly consists of carbon (C) and oxygen (O). In all 3 samples, there
are much calcium (Ca) and sulfur (S) contents. The dust particles frequently contained Al and Fe besides silicon (Si) as indicators of dust particles (Nishikawa et al., 2000), and the percentage were higher in Fuku.09 and Tsu.09 compared with Naha09. Our observation was correlated with ASDE information of Japan Meteorological Agency (MEGI, 2010a). Other minerals components such as K, and magnesium (Mg) were also detected. A few amount nickel (Ni) and zinc (Zn) were detected in Naha and Fuku.09. The percentage of Ti was higher in Tsu.09 compared with Naha09 and Fuku.09.

3.2. Concentration of LPS in water extract of YS

To examine the endotoxin concentration in the collected sample during ADSE, we measured the endotoxin concentrations of each water extract (Table 3). The results showed that all water extracts contained LPS, Tsu.10 (9.49 ± 1.82 EU/mL), Fuku.10 (16.25 ± 2.34 EU/mL), and Naha10 (22.62 ± 1.96 EU/mL) contained the highest level of LPS, followed by Tsu.09 (8.39 ± 1.81 EU/mL), Fuku.09 (7.20 ± 0.36 EU/mL), and Naha09 (3.4 ± 0.26 EU/mL). The LPS level of the sample collected in 2010 was higher than the sample collected in 2009 (Table 3).

3.3. Concentrations of Cry j 1 in Cry j 1 extract of YS

To examine the Cry j 1 concentration in the collected sample during ADSE, the Cry j 1 concentration of each Cry j 1 extract was measured (Table 3). The results showed that all Cry j
1 extract contained Cry j 1. Fuku.10 (19.7 ± 2.1 pg/m$^3$) contained the highest level of Cry j 1, followed by Tsu.10 (13.4 ± 2.3 pg/m$^3$) and Naha10 (1.7 ± 0.3 pg/m$^3$). Tsu.09 also contained the a high level of Cry j 1 (17.2 ± 2.3 pg/m$^3$) (Table 3).

3.4. YS extract induces β-hex release from RBL-2H3 cells

We performed a series of experiments to screen for the effects of water extract on RBL-2H3 cells degranulation over a range of noncytotoxic concentrations (dilution rate 1/125-1/50). The β-hex release from RBL-2H3 cells as caused by treatment with the water extract is shown in Fig. 2. The water extract collected in 2009 and 2010 from each sampling site induced the β-hex release from RBL-2H3 cells after 16 h of exposure, the effect being significant at 1/100 treatment (except Fuku.10 sample, Fig. 2B) ($p<0.05$ - $p<0.01$ vs. the negative control of PBS (-)), and only Fuku.10 sample showed significant effect at 1/125 treatment (Fig. 2B). For comparison, a LPS (Escherichia coli 0111:B4) induced approximately 150% release of β-hex at 5 ng/mL concentration (Fig. 2D).

To confirm the effect of Cry j 1 extract on β-hex release, we performed a series of experiments to screen for the effects of various concentrations (dilution rate 1/100-1) of Cry j 1 extract on RBL-2H3 cells degranulation, and the result is shown in Fig. 3. For comparison, BSA induced approximately 193% β-hex release at 0.3 μg/mL treatment. The Cry j 1 extract collected in 2009 and 2010 from each sampling site induced the β-hex release from RBL-2H3 cells, the effect being significant at all treatment ranges with Fuku.10 sample (Fig. 3B), 1/10-1
treatment with Naha10 sample (Fig. 3A), and treated with Tsu.09 sample (Fig. 3C) ($p<0.05 -
\text{vs. the negative control of PBS (-)}$).

### 3.5. Water extract induces cytokine production in RBL-2H3 cells

The production of TNF-α and IL-13 following 16 h incubation with water extracts by both
Naha09 and Tsu.09 analyzed using ELISA assay are shown in Fig. 4. TNF-α production was
increased RBL-2H3 cells treated with noncytotoxic concentrations of water extract ($1/100$
dilution rate). The TNF-α production was $7.76 \pm 1.24$ pg/mL after stimulation with Tsu.09 for
16 h, and Naha09 was $7.66 \pm 0.62$ pg/mL, the effect being significantly ($p<0.05 -p<0.01$)
different from the control ($3.06 \pm 0.53$ pg/mL). Furthermore, the TNF-α production was $9.28$
$\pm 0.15$ pg/mL after stimulation with 5 ng/mL LPS, and the effect being significant ($p<0.01$),
while there was no significant different in the IL-13 production after stimulation with water
extract (data not shown).

### 3.6. Water extract exhibits cytotoxic effects on basophil cells

To characterize YS-induced cytotoxicity, cell viability was determined in water extract- and
Cry j 1 extract-stimulated RBL-2H3 cells using the MTT assay. The results showed that
exposure to water extract at different concentration rate for 24 h reduced the cell viability in a
concentration-dependent manner. The dilution rate of 1/10-1 can decrease the cell viability to
Naha09 was $69.2\% \pm 0.9\%$ (Fig. 5A), Fuku.09 was $82.0\% \pm 1.7\%$ (Fig. 5B), Tsu.09 was $79.8\%$
± 1.1% (Fig. 5C), Naha10 was 64.2% ± 1.0% (Fig. 5A), Fuku.10 was 73.6% ± 1.2% (Fig. 5B), and Tsu.10 was 67.6% ± 1.4% (Fig. 5C), the effect being significant compared with the control ($p<0.05 – p<0.01$ vs. the negative control of PBS (-)). Among the samples, those collected in 2010 showed the strongest toxic effect on RBL-2H3 cells compared with the 2009 samples (Fig. 5). However, Cry j 1 extract did not cause significant reduction in cell viability (data not shown).

4. Discussion

4.1. Allergenic

The presence of water-soluble material around dust-storm particles over Japan has been reported by previous research reports (Iwasaka et al., 2004; Iwasaka et al., 2008). Most of the dust particles are coated with water-soluble materials after their long-range transport from China to Japan, and that the dust storm particles were modified by sea-salt and/or anthropogenic pollutants and cloud process (Zhou et al., 1996). Our results showed that, YS extracts caused degranulation of RBL-2H3 cells. Biological species, such as pollen allergens and endotoxin, as well as transition metals in acidic environments are water soluble (Monn and Becker 1999). In concordance with several other studies (Becker et al., 1996; Dong et al., 1996; Long et al. 2001), our data suggest the possible involvement of endotoxin in promoting allergy after long exposure to low level of LPS (Fig. 2D). The endotoxin levels of water extract of samples collected from 2010 was higher than those of the 2009 samples from all sampling pointe (Table
and the differences in LPS content were weakly associated with the increase in filter density \((R^2 = 0.5888, \text{Fig. 6A})\), and only the Fukuoka and the Tsukuba samples were strongly correlated \((R^2 = 0.9592, \text{Fig. 6B})\). However, LPS content were weakly associated with the β-hex release rate \((R^2 = 0.461, \text{Fig. 6C})\). As shown in Fig. 2D, the effect of low level LPS on β-hex release was not dose-dependent, there is an optimal concentration. In addition, long-range transported pollutants such as nitrogen oxides (NOx) and sulfur oxides (SOx) were likely to interact with aeroallergens (Ichinose et al. 2005) that induce degranulation. Furthermore, metal elements have inhibitory effect (Tanaka et al., 1991) or induce and enhance (Walczak-Drzewiecka et al., 2003) action on chemical mediator release by mast cells. Therefore, YS water extract may have several different ways of inducing chemical mediator release from RBL-2H3 cells, suggesting the complexity of its action, although most of the mechanisms remain to be studied.

It is well known that JC pollinosis patients who start their medication several weeks before the first day of the pollen season can spend the pollen season without severe symptoms. Takahashi et al. (2007) found that the total pollen counts and the total Cry j 1 amounts during the pollen season (Jan. – May. in 2003 – 2006) collected from Yamagata prefecture of Japan did not correlate with pollen precisely, and the Cry j 1 levels reached 1 pg/m\(^3\) 2-3 weeks before the first day of the pollen season (5-25 ng/m\(^3\)). We hypothesize that the higher concentration of Cry j 1 collected before pollen season was probably transported from other sites to the sampling sites by ADSE. This study has shown that there was no correlation between the Cry j 1 contents (Table 3) and the increase in filter particle density (Table 1) \((R^2=0.0236, \text{Fig. 6D})\). Comparing samples from Naha and Fukuoka, the Cry j 1 concentration were increased when the filter particle density increased, but this result do not correlate with the pollen count information.
On the other hand, the results of this study have shown that Cry j 1 extract exhibited an effect on the β-hex release in both 2009 and 2010 (Fig. 3), and the differences in β-hex release rate were weakly associated with the increase in the Cry j 1 content ($R^2 = 0.5168$, Fig. 6E), but higher correlation for the Naha and Fukuoka samples were observed ($R^2 = 0.9455$, Fig. 6F). This result was consistent with Wang et al. (2009a) observation. This suggests that ADSE can transport, absorb or concentrate Cry j 1 allergen, and these have the possibility of contributing to the cause of the allergic reaction (Mori et al., 2003; Takahashi et al., 2007).

Sandstorm dust is a prolific source of potential triggers of allergic and non-allergic respiratory ailments (Kwaasi et al., 1998). Many of the cellular signals activated by Gram-negative bacteria are attributed to TLR4-mediated recognition of LPS (Gon et al., 2005). LPS is an agonist of TLR4, although TLR4 receptors are expressed on the RBL-2H3 cell surface (Passante et al., 2009). In this study, the role of endotoxin as a stimulant of cytokine production was confirmed (Becker et al., 1996; Dong et al., 1996; Long et al., 2001). The results have shown the possibility that low level LPS can induce TNF-α production in RBL-2H3 cells. Cytokine release showed a similar pattern only for TNF-α after stimulation with Tsu.09 and Naha09 samples (Fig. 4), while there was no effect on IL-13 expression. Mitogen activated protein kinase (MAPK) JNK was involved in LPS-induced IL-13, but not in TNF-α synthesis (Gon et al., 2005). This difference may be contributed by the proinflammatory activity of water extract. However, the endotoxin levels of water extract from Tsu.09 was 2-fold higher than Naha09 (Table 3). These results indicate that the cytokine-inducing capacity of Naha09 and Tsu.09 are not only due to the low amount of LPS present in these water extract. It was hypothesized that there are other proinflammatory...
components, ambient particles (Imrich et al., 2000) or other mechanism (Shoenfelt et al., 2009), and there may be greater synergism between endotoxin and components of ambient particles (Long et al., 2001). Dong et al. (1996) demonstrated that there was no cytokine induction by diesel particles. This suggests that there are other proinflammatory components in the water extract that may be responsible for the effects observed in cells treated with YS.

4.2. Cytotoxicity

Our results suggest that YS water extract exhibited cytotoxicity, with the cell treated with 2010 samples exhibiting higher cell viability than the 2009 samples (Fig. 5A, B, C). The differences in the cell viability were weakly associated with the increase in filter particle density ($R^2 = 0.6711$, Fig. 5D), but higher correlation for the Naha and Fukuoka samples was observed ($R^2 = 0.9253$, Fig. 5E). These results agree with the results of the study of Meng and Zhang (2007) which showed that the effect of PM on health is greater during dust storms because airborne PM’s mass is high. Ichinose et al. (2005) found that inflammatory lung injury was induced by microbiological materials, such as β-glucan, and by chemical materials such as $\text{SO}_4^{2-}$. Therefore, these materials adsorbed onto dust particles, are implicated in the pathogenesis of human respiratory disorders during a dust event. YS exhibits a cytotoxic effect on pneumocytes *in vitro*, and reactive oxygen species (ROS), fenton activity, reactive nitrogen species (RNS), and titania ($\text{TiO}_2$) are involved in this toxicity (Kim et al., 2003). Becker et al. (1996) also found that $\text{TiO}_2$ is toxic to both human and rat alveolar macrophage (AM). Monn and Becker (1999) reported that LPS is cytotoxic on human monocytes and rat AM. Toxicity
in macrophages has also been associated with metals, and higher metal concentrations are usually found in the PM (Monn and Becker 1999; Schins et al., 2004). Some studies also suggest that metals, either water-soluble of PM can catalyze reactions involved in oxidative stress and DNA damage (Meng and Zhang 2007). Nicel (Ni) has slight cytotoxic effect on CHO cells (Fredj et al., 2010), and significant positive dose response for LDH, a marker of cell death (Cho et al., 2010). In this study, composition analysis results showed that YS surface contained Al, C, Fe, nitrogen (N), Ni, S, Ti, and Zn (Table 2). Previous studies showed that transition metals such as Fe and Zn, components that might be bound to the fine particles, endotoxins, other organic compounds, and polycyclic aromatic hydrocarbon content of particles participated in the production of ROS and was correlated with oxidative stress in macrophages (Ortiz et al., 2006). Effect of diesel particulate matters on ROS production in human alveolar epithelial cells was observed (Patel et al., 2011). YS contain ultrafine particles from combustion sources, such as diesel and gasoline engines, and coal fired power plants, and concentrated during the long distance transport (Zhou et al., 1996; Mori et al., 2003; Ichinose et al., 2005). This was evident in the cytotoxic effect of the water extract which could have been induced by their constituents and enhanced by ADSE.

5. Conclusion

In conclusion, YS can rapidly enhance preformed chemical mediator release in basophilic cells. This process may depend on endotoxin, Cry j 1, and other airborne allergen content of YS. These findings may help explain the increase in the incidence of allergic diseases during
ADSE in East Asia. The detailed mechanism behind the enhancement of allergic affect of YS will be the subject of our future study. It is recommended that patients suffering from pollinosis, and physicians be made aware of the pollen dispersal that may be cause by YS during the ADSE.

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Characteristics of dust-storm particles and their long-range transport from China to Japan  
Figure captions

Fig. 1. A meteorological map showing areas in Japan, Korea and China impacted by YS. The sampling sites, Tsukuba, Fukuoka and Naha are indicated.

Fig. 2. Effect of water extract on β-hex release from RBL-2H3 cells. The cells (5.0 x 10^4 cells/well in 100 μg/mL) were preincubated with each extract at 37°C for 16 h. Results represent one trial (n = 6). Three additional trials show similar results. *Significantly different from the negative control (PBS (-)) (\(^*\): \(p<0.05\), \(^**\): \(p<0.01\)).

Fig. 3. Effect of Cry j 1 extract on β-hex release from RBL-2H3 cells. The cells (5.0 x 10^4 cell/well in 100 μg/mL) were incubated with each extract at 37°C for 1 h after IgE sensitization. Results represent one trial (n = 6). Three additional trials show similar results. *Significantly different from the negative control (PBS (-)) (\(^*\): \(p<0.05\), \(^**\): \(p<0.01\)).

Fig. 4. Effect of water extract on TNF-α production in RBL-2H3 cells. After exposed with extract (1/100 dilution) and LPS (5 ng/mL) for 16 h, conditioned media were collected for TNF-α ELISA Kit. Results represent one trial (n = 3). Three additional trials show similar results. Tsu.09: collected from Tsukuba city during ADSE in 2009; Naha09: collected from Naha city during ADSE in 2009. *Significantly different from the negative control (PBS (-)) (\(^*\): \(p<0.05\), \(^**\): \(p<0.01\)).

Fig. 5. Effect of water extract at different concentration on the cell viability of RBL-2H3 cells. After 24 h incubation, cell viability was determined using the MTT assay. The percent cell viability was calculated relative to the untreated control. The cells (5.0 x 10^4 cells/well) were incubated with yellow sand extract at 37°C for 24 h in 5%CO₂. Results represent one trial (n = 6). Three additional trials show similar results. *Significantly different from the negative control (PBS (-)) (\(^*\): \(p<0.05\)). A, B, and C: Cell viability of water extract; D and E: Correlation between cell viability and filter particle density.
Fig. 6. A and B: Correlation between LPS concentration of water extract and filter particle density; C: Correlation between β-hex release at 1/100 dilution and LPS concentration of water extract; D: Correlation between Cry j1 concentration of Cry j1 extract and filter particle density; E and F: Correlation between β-hex release at 1/100 dilution and Cry j1 concentration of Cry j1 extract.
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.

A

Cell viability (% of control)

0 1/1000 1/100 1/10 1/2 1.0

Sample treatment (dilution rate)

- Naha09
- Naha10

B

Cell viability (% of control)

0 1/1000 1/100 1/10 1/2 1.0

Sample treatment (dilution rate)

- Fuku09
- Fuku10

C

Cell viability (% of control)

0 1/1000 1/100 1/10 1/2 1.0

Sample treatment (dilution rate)

- Tsu.09
- Tsu.10

D

Cell viability (% of control)

0.0 0.5 1.0

Particle density (mg/cm²)

Fuku09  Tsu.09  Fuku.10  Tsu.10  Naha09  Naha10

R² = 0.6711

E

Cell viability (% of control)

0.0 0.5 1.0

Particle density (mg/cm²)

Fuku09  Fuku.10  Naha09  Naha10

R² = 0.9253
Fig. 6.

A. LPS concentration (EU/mL) vs. Particle density (mg/cm²)
   - $R^2 = 0.5888$
   - Points: Naha09, Fuku10, Tsu10, Tsu09, Fuku09

B. LPS concentration (EU/mL) vs. Particle density (mg/cm²)
   - $R^2 = 0.9592$
   - Points: Naha09, Fuku10, Tsu10, Tsu09, Fuku09

C. LPS concentration (EU/mL) vs. β-hexos release (% of control)
   - $R^2 = 0.4611$
   - Points: Naha09, Fuku09, Tsu09, Fuku10, Naha10

D. LPS concentration (EU/mL) vs. Particle density (mg/cm²)
   - $R^2 = 0.0236$
   - Points: Naha09, Fuku10, Tsu09, Fuku09, Naha10

E. LPS concentration (EU/mL) vs. β-hexos release (% of control)
   - $R^2 = 0.5168$
   - Points: Naha09, Fuku09, Tsu09, Fuku10, Naha10

F. LPS concentration (EU/mL) vs. Particle density (mg/cm²)
   - $R^2 = 0.9455$
   - Points: Naha09, Fuku10, Tsu09, Fuku09, Naha10
### Table 1

Sampling site and collection days during the ADSE.

<table>
<thead>
<tr>
<th>Year</th>
<th>Sample Name</th>
<th>Site</th>
<th>Days</th>
<th>Periods (days)</th>
<th>Particle conc. (μg/m$^3$)</th>
<th>Particle density (mg/cm$^2$)</th>
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</thead>
<tbody>
<tr>
<td>2009</td>
<td>Naha09</td>
<td>Naha</td>
<td>4/27 - 6/10</td>
<td>45</td>
<td>4.6 ± 0.1</td>
<td>0.58 ± 0.01</td>
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<tr>
<td></td>
<td>Fuku09</td>
<td>Fukuoka</td>
<td>3/16 - 3/19</td>
<td>3</td>
<td>59.7 ± 3.6</td>
<td>0.50 ± 0.03</td>
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<tr>
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<td>Tsu.09</td>
<td>Tsukuba</td>
<td>3/16 - 3/20</td>
<td>4</td>
<td>46.6 ± 1.8</td>
<td>0.52 ± 0.02</td>
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<tr>
<td>2010</td>
<td>Naha10</td>
<td>Naha</td>
<td>3/10 - 4/14</td>
<td>35</td>
<td>7.1 ± 0.4</td>
<td>0.69 ± 0.04</td>
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<tr>
<td></td>
<td>Fuku.10</td>
<td>Fukuoka</td>
<td>3/20 - 3/24</td>
<td>4</td>
<td>52.0 ± 1.8</td>
<td>0.58 ± 0.02</td>
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<tr>
<td></td>
<td>Tsu.10</td>
<td>Tsukuba</td>
<td>3/19 - 3/23</td>
<td>4</td>
<td>47.5 ± 0.9</td>
<td>0.53 ± 0.01</td>
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### Table 2

Components analysis of YS by XPS and XRF-EDS collected in 2009.

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<th></th>
<th></th>
<th></th>
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<tr>
<td>Na</td>
<td>13.91</td>
<td>7.58</td>
<td>7.75</td>
<td>C</td>
<td>40.57</td>
<td>38.31</td>
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<td>Mg</td>
<td>2.83</td>
<td>5.66</td>
<td>5.45</td>
<td>N</td>
<td>3.65</td>
<td>3.29</td>
<td>2.34</td>
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<tr>
<td>Al</td>
<td>9.45</td>
<td>13.42</td>
<td>17.87</td>
<td>O</td>
<td>38.84</td>
<td>34.81</td>
<td>33.58</td>
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<tr>
<td>P</td>
<td>1.12</td>
<td>1.96</td>
<td>2.05</td>
<td>Na</td>
<td>4.32</td>
<td>1.42</td>
<td>1.38</td>
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<td>S</td>
<td>35.78</td>
<td>24.90</td>
<td>30.80</td>
<td>Mg</td>
<td>0.76</td>
<td>3.68</td>
<td>3.06</td>
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<tr>
<td>Cl</td>
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<td>13.89</td>
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<td>Al</td>
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<td>2.20</td>
<td>2.92</td>
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<tr>
<td>K</td>
<td>6.76</td>
<td>7.89</td>
<td>9.23</td>
<td>Si</td>
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<tr>
<td>Ca</td>
<td>13.90</td>
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<td>18.73</td>
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<td>Ti</td>
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<td>Cl</td>
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<tr>
<td>Cr</td>
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<td>0.08</td>
<td>0.09</td>
<td>K</td>
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<td>0.77</td>
<td>0.62</td>
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<tr>
<td>Mn</td>
<td>0.09</td>
<td>0.14</td>
<td>0.18</td>
<td>Ca</td>
<td>1.05</td>
<td>1.84</td>
<td>0.88</td>
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<tr>
<td>Fe</td>
<td>3.59</td>
<td>4.37</td>
<td>6.58</td>
<td>Fe</td>
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<td>0.77</td>
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<tr>
<td>Ni</td>
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<td>0.01</td>
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<td></td>
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<tr>
<td>Zn</td>
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<td>0.07</td>
<td>0.13</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Total** | 100.00 | 100.00 | 100.00 | **Total** | 100.00 | 100.00 | 100.00

(atm%)

*─*: No detectable.
Table 3

LPS and Cry j 1 content of YS extract.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>LPS (EU/mL)</th>
<th>Cry j 1 (pg/m³)</th>
<th>Cry j 1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naha09</td>
<td>3.40 ± 0.26</td>
<td>1.0 ± 0.2</td>
<td>301.1 ± 68.1</td>
</tr>
<tr>
<td>Fuku.09</td>
<td>7.20 ± 0.36</td>
<td>16.2 ± 2.8</td>
<td>342.6 ± 58.9</td>
</tr>
<tr>
<td>Tsu.09</td>
<td>8.39 ± 1.81</td>
<td>17.2 ± 2.3</td>
<td>484.4 ± 65.3</td>
</tr>
<tr>
<td>Naha10</td>
<td>22.62 ± 1.96</td>
<td>1.7 ± 0.3</td>
<td>428.7 ± 74.5</td>
</tr>
<tr>
<td>Fuku.10</td>
<td>16.25 ± 2.34</td>
<td>19.7 ± 2.1</td>
<td>556.8 ± 60.0</td>
</tr>
<tr>
<td>Tsu.10</td>
<td>9.49 ± 1.82</td>
<td>13.4 ± 2.3</td>
<td>378.8 ± 64.9</td>
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</tbody>
</table>