ORIGINAL

Difference in interleukin-8 transcriptional activity induced in THP-G8 cells by particulate matter collected in winter and summer in western Japan

Masanari Watanabe[1], Jun Kurai[1], Sayaka Minato[2], Hisashi Noma[3], Hiroyuki Sano[4], Rumiko Saito[5], Setsuya Aiba[6], Mitsuo Oshimura[7], Keisuke Hatakeyama[2], Akira Yamasaki[1], and Eiji Shimizu[1]

[1]*Department of Respiratory Medicine and Rheumatology, Tottori University Faculty of Medicine, Yonago, Japan,* [2]*Tottori Prefectural Institute of Health and Environment, Yurihama, Japan,* [3]*Department of Data Science, Institute of Statistical Mathematics, Tokyo, Japan,* [4]*Department of Respiratory Medicine and Allergology, Kindai University Faculty of Medicine, Osakasayama, Japan,* [5]*Department of Integrative Genomics, Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan,* [6]*Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, Japan,* [7]*Chromosome Engineering Research Center, Tottori University, Yonago, Japan*

Abstract : ***Background :*** **Airborne particulate matter (PM) may stimulate production of pro-inflammatory cytokines, and thus exposure to PM affects pulmonary diseases. However, the effects of PM on pulmonary diseases have differed among studies.** ***Methods :*** **PM was collected from February 2 to 28 (winter) and June 1 to 17 (summer) in 2013 in Yurihama, Japan, using filters to separate particles with different aerodynamic diameters (1.1 to < 2.2 μm, 2.2 to <3.3 μm, 3.3 to <7.0 μm, and 7.0 to <10 μm). Interleukin (IL)-8 transcriptional activity in THP-G8 cells was examined in response to winter and summer PM with different diameters.** ***Results :*** **IL-8 transcriptional activity induced by winter PM was significantly higher than that with summer PM for each diameter. For winter PM, IL-8 activity increased with an increased diameter, whereas this activity did not differ between a solvent control and each summer PM diameter. This indicates that summer PM does not stimulate production of IL-8 in THP-G8 cells.** ***Conclusion :*** **The effects of PM on production of pro-inflammatory cytokines differ with the season and PM diameter. This suggests that the compositions of PM differ on season, and the evaluation of compositions of PM is important in understanding the association of health with short-term exposure to PM.**
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INTRODUCTION

Air pollution is now the third leading cause of disability-adjusted life years associated with chronic respiratory disease globally. (1) Particulate matter (PM) is a major source of air pollution, and the association between PM exposure and pulmonary disease is well established. Aerosols are transported from East Asia to Japan and the health problems caused by PM have become a major concern in Japan. (2) However, only a few studies have investigated the association between PM and the respiratory system in Japan. Several overseas epidemiologic studies have examined the association of PM with pulmonary function. (3) However, a European multicenter study failed to detect a consistent relationship between PM and short term health effects, despite the wide range of climatic conditions and pollutant mixes encountered across the sites, (4) and several other studies have also not been able to establish an association between PM and health. These differences may be caused by the complexity of dealing with individual variability in each study, and the disparity between air pollutants in the winter and summer. (5) In order to investigate associations with health and PM, almost of studies define the level of PM as the amount of exposure to PM, and do not evaluate compositions of PM.

Exposure to PM increases the concentration of interleukin (IL)-8 in bronchial lavage fluid and elevates IL-8 mRNA expression in bronchial biopsy tissue in healthy and asthmatic subjects. (6) Sierra-Vargas *et al.* showed that neutrophils migrate to the lungs during acute inflammation induced by exposure to air pollutants. (7) Patients with asthma had asymptomatic reduction of pulmonary function and an inflammatory response after walking along an urban road for only 2 hours, suggesting that the number of neutrophils in the airway was increased. (8) In animal models, PM can also induce other cytokines. (9) However, when humans are exposed to particulate matter, these findings suggest that IL-8 is the most important cytokine in airway inflammation.

The large scale and long-range transport of aerosols from East Asia to Japan, including in Asian dust storms (ADS), is more common from February to May. (10) Our hypothesis is that the effects of PM on health may differ with the season in Japan. In this study, we examined IL-8 transcriptional activity induced by PM collected in winter and summer, with the goal of determining how the season influences the health effects of short-term exposure to PM.

MATERIAL AND METHODS

Collection and preparation of airborne particles

Airborne particles were collected using a high-volume air sampler (HV-1000R ; Shibata Co., Ltd., Tokyo, Japan) in Yurihama, Tottori from February 2 to 28 and June 1 to 17 in 2013, with separation based on diameter. Yurihama is rural and has no source of air pollutants except for motor vehicles, and the observatory in Yurihama is not located close to populated areas. Airborne particles were separated according to their aerodynamic diameters (Andersen Sampler ; Shibata Co., Ltd., Tokyo, Japan) into 4 filtered

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Address correspondence and reprint requests to Masanari Watanabe, M.D., Ph.D. Department of Respiratory Medicine and Rheumatology, Tottori University Faculty of Medicine, 36-1 Nishichou, Yonago 683-8504, Japan and Fax : +81-859-38-6539.

categories (1.1 to < 2.2 μm, 2.2 to < 3.3 μm, 3.3 to < 7.0 μm, 7.0 to < 10 μm) and each filter was dried in a desiccator before and after sampling to be weighed. Collected airborne dust from February 2 to 28 was defined as winter PM, and that from June 1 to 17 as summer PM. This dust was sterilized at 121°C for 30 min in an autoclave (Tomy SX-300 ; Tomy Co., Tokyo, Japan) to prevent growth of bacteria and fungi, and dried at 80°C for 4 h with a drying sterilizer (SG600 ; Yamato Scientific Co., Ltd., Tokyo, Japan). To stimulate THP-G8 cells, the airborne particles were diluted to 400 μg/ml with distilled deionized water. The pH of the particles was measured with a pH meter (MP220 ; Mettler Toledo, Schwerzenbach, Switzerland).

IL-8 promoter-luciferase gene reporter assay

THP-G8 cells are THP-1-derived reporter cells 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. (11) THP-G8 cells (5×10^4 cells/100 μl/well) in 96-well black plates (Greiner Bio-One GmbH, Frickenhausen, Germany) were stimulated for 5 h with solvent (negative control), 100 ng/ml lipopolysaccharide (LPS) (Wako Pure Chemicals, Osaka, Japan), and airborne particles (400 μg/ml) of various diameter ranges. The maximum induction of IL-8 transcriptional activity of THP-G8 cells was evaluated after stimulation with 100 ng/ml LPS for 5 h. (11) 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 of treated cells divided by that of untreated cells. (11)

Statistical analysis

Differences of nSLO-LA of THP-G8 cells were analyzed by ANOVA using SPSS Statistics (Japanese ver. 21.0 for Windows ; IBM Japan, Tokyo, Japan). All quoted P values are two-sided and the significance level was set to 0.05.

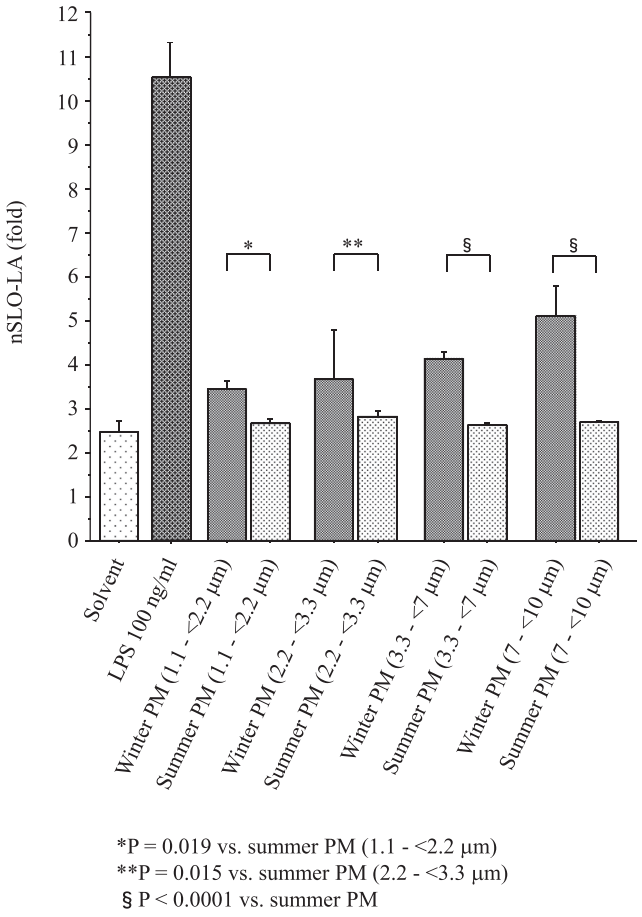

Figure 1. IL-8 transcriptional activity measured using an IL-8 luciferase assay in a stable THP-1-derived IL-8 reporter cell line. Winter particulate matter (PM) was collected from February 2 to 28, 2013 ; and summer PM was collected from June 1 to 17, 2013. Cells were treated with solvent only (n=6, negative control), LPS (n=6, 100 ng/ml, positive control), and with winter PM or summer PM with each indicated diameter range (all n=6, 400 μg/ml).

RESULTS

The collected PM ranged in pH from 7.6 to 7.9. The nSLO-LAs were 2.48±0.23 fold with vehicle (n=6) and 10.49±0.81 fold with LPS (n=6, 100 ng/ml) (Fig. 1). With winter PM (all n=6, 400 μg/ml), the nSLO-LAs were 3.45±0.18 fold for 1.1 to < 2.2 μm diameter, 3.66±1.12 fold for 2.2 to < 3.3 μm, 4.14±0.13 fold for 3.3 to < 7 μm, and 5.09±0.69 fold for 7 to < 10 μm. With summer PM (all n=6, 400 μg/ml), the nSLO-LAs were 2.65±0.09 fold for 1.1 to < 2.2 μm, 2.83±0.12 fold for 2.2 to < 3.3 μm, 2.62±0.03 fold for 3.3 to < 7 μm, and 2.69±0.04 fold for 7 to < 10 μm. The nSLO-LAs with winter PM were significantly higher than those for summer PM for every PM diameter. With winter PM, nSLO-LAs increased as the diameter increased, whereas there was no dependence on diameter for summer PM and no significant difference in nSLO-LA between the vehicle control and summer PM.

DISCUSSION

IL-8 is a key cytokine in air pollutant-induced inflammation, especially in airways. In this study, we compared induction of IL-8 transcriptional activity in THP-G8 cells by PM collected from February 2 to 28 (winter PM) with that by PM collected from June 1 to 17 (summer PM) in 2013. Our key findings were that winter PM elicited significantly greater IL-8 transcriptional activity than summer PM, and that the IL-8 activity induced by winter PM increased in a diameter-dependent manner. These results show that the effects of PM on production of pro-inflammatory cytokines differ with the season and the PM diameter.

Based on its morphological, chemical, physical, and thermodynamic properties, PM is formed from various substances through processes such as combustion and motor vehicle emission. (12) Numerous epidemiologic studies have shown that long-and short-term exposures to PM are associated with mortality, hospitalization, and cancer. Experimental data indicate that coarse PM is more injurious to cells than fine and ultrafine particulates. (13) In the current study, we also found that IL-8 transcriptional activity induced by PM increased with an increase in the PM diameter.

The composition of PM may play the main role in the effects of coarse PM on health. (13) Coarse PM includes a lot of crustal dust originating from geological materials containing elements such as silicon, barium, sodium, calcium and aluminum. (14, 15) There is considerable evidence that this dust can augment inflammation with an increase of pro-inflammatory cytokines. (6, 7, 16) Our previous study showed that coarse PM on ADS days in western Japan increased IL-8 secretion in THP-G8 cells, but that the original ADS soil from the China Loess Plateau did not do so. (17) In the current study, winter PM had significantly greater effects on IL-8 transcriptional activity than summer PM. This result may suggest that anthropogenic components in PM play a more important role

in induction of pro-inflammatory cytokines, compared to crustal dust originating from geological materials. Other anthropogenic components of coarse PM are also potentially more injurious compared to crustal dust. (7, 18, 19)

Chen and colleagues reported on the "Great Smog of 2013" in Beijing, China, when residents were exposed to the worst air quality on record. (20) The average concentration of PM smaller than 2.5 μm ($PM_{2.5}$) during this smog period was 231 mg/m^3, with a peak daily value of 443 mg/m^3, both substantially higher than the World Health Organization (WHO) safety levels of 25 mg/m^3 over a 24-hour period. There were significant increases in hospital visits for cardiovascular and respiratory diseases, but increased mortality was not specifically found. Long-range transport of aerosols from East Asia to Japan increases the levels of PM in Japan, (8, 21, 22) and this may be one of the causes of the difference in induction of IL-8 transcriptional activity between winter and summer PM. The increase of PM in China is a serious environmental problem (23, 24) and our results suggest that this change may also be contributing to the damaging effects of PM in Japan.

Many studies have indicated increased production of IL-8 induced by PM. (6-9) However, in this study, we found that summer PM did not induce production of IL-8. Alfaro-Morena and colleagues showed induction of IL-6 and inhibition of IL-8 secretion by PM in human airway Calu-3 cells, (25) and thus it is possible that summer PM in western Japan may augment production of other pro-inflammatory cytokines, such as IL-6. The results of this study simply indicate a difference in the effects of PM in western Japan between winter and summer. Many studies from Europe, North America and China have investigated the association between health and $PM_{2.5}$, but few such studies have been performed in Japan. The composition of $PM_{2.5}$ differs in each country and area, and the current findings suggest that induction of pro-inflammatory cytokines induced by $PM_{2.5}$ may also differ with the season. Thus, the study timing may be important in investigating the health effects of short-term exposure to $PM_{2.5}$ in Japan.

At present, it is extremely difficult to collect enough amount of particulate matter and separate it according to defined aerodynamic diameters. The smaller the particulate matter is, the more difficult it becomes to collect. An IL-8 promoter luciferase assay in THP-G8 cells, the so-called IL-8 Luc assay, was a suitable method because it has high sensitivity for evaluation of the IL-8 level using a small amount of material. Additionally, we had already investigated that IL-8 transcriptional activity by IL-8 Luc assay correlated significantly with secretion of IL-8. (26)

This study provides only preliminary evidence and has limitations. First, we could not analyze the composition of the PM because we were could not collect a sufficient amount. Therefore, this study was unable to investigate which components induce IL-8. Second, for the same reason, we did not consider other pro-inflammatory cytokines. Third, in order to collect the quantity of PM required for experiments, the collection periods differed in the winter and summer.

Within these limitations, the results of the study indicate that there are significant differences in induction of IL-8 transcriptional activity between winter PM and summer PM, and that the IL-8 activity induced by winter PM increases in a PM diameter-dependent manner. These findings suggest that the effects on health of short-term exposure to PM differ with the season. Further studies are needed to better define the mechanisms associated with the differences in production of pro-inflammatory cytokines induced by PM in each season.

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