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Original article

A panel study of airborne particulate matter composition versus concentration: Potential for inflammatory response and impaired pulmonary function in children

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CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; IQR, interquartile range; NO₂, nitrogen dioxide; PEF, peak expiratory flow; PM, particulate matter; PM₁₀, particulate matter smaller than 10 µm; PM_{2.5}, particulate matter smaller than 2.5 µm; SO₂, sulfur dioxide; SPM, suspended particle matter

ABSTRACT

Background: The relationship between airborne particulate matter (PM) and pulmonary function in children has not been consistent among studies, potentially owing to differences in the inflammatory response to PM, based on PM types and sources. The objective of this study was to investigate the effect of airborne PM on pulmonary function in schoolchildren and its potential for an inflammatory response. **Methods:** Daily morning peak expiratory flow (PEF) was measured in 339 schoolchildren in February 2015. Interleukin (IL)-8 production was assessed in THP1 cells stimulated by airborne PM collected every day during the study period, and these IL-8 concentrations are described as the daily IL-8 levels. A linear mixed model was used to estimate the association between PEF values and the daily levels of suspended PM (SPM), PM diameters smaller than 2.5 µm (PM_{2.5}), and IL-8.

Results: The daily IL-8 levels were significantly associated with those of SPM and PM_{2.5}. A 0.83 µg/mL increase in IL-8 levels was significantly associated with a −1.07 L/min (95% confidence interval, −2.05 to −0.08) decrease in PEF. A 12.0 µg/m³ increase in SPM and a 10.0 µg/m³ increase in PM_{2.5} were associated with a −1.36 L/min (−2.93 to 0.22) and −1.72 L/min (−3.82 to 0.36) decreases in PEF, respectively. There were no significant relationships between PEF, SPM, and PM_{2.5}.

Conclusions: These findings suggest that the effects of airborne PM on pulmonary function in schoolchildren might depend more on the pro-inflammatory response than the mass concentration of the PM.

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Introduction

Although numerous epidemiological studies have documented significant associations between airborne particulate matter (PM) and pulmonary function,^{1–3} not all studies have found a

relationship.^{4–6} In particular, a European multicenter study was unable to consistently detect an association between airborne PM and pulmonary function, despite the wide range of climatic conditions and pollutant mixes encountered across the sites.⁶ Thus, the effects of PM on pulmonary function and respiratory symptoms are not the same across studies.

Airborne PM is usually categorized based on particle size, as PM₁₀ or PM_{2.5}, which represents median aerodynamic diameters of smaller than 10 µm or 2.5 µm, respectively.^{7,8} Although PM is a mixture of solids and liquid droplets that form from various substances and float in the air,⁹ previous studies have primarily focused on mass concentrations of PM₁₀ or PM_{2.5} to estimate the

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relationship between airborne PM and pulmonary function. However, various components of PM can influence the subsequent pro-inflammatory cytokine response,¹⁰ and the inflammatory potential of PM is heterogeneous regarding city and season.^{11–14} The discordant results between studies investigating the association between airborne PM and pulmonary function might be attributable to the disparity of inflammatory responses to different compositions of PM.

Exposure to airborne PM increases the interleukin (IL)-8 concentration in bronchial lavage fluid and elevates IL-8 mRNA expression in bronchial biopsy tissue in healthy and asthmatic subjects.¹⁵ Additionally, IL-8 is one of the most important neutrophil chemotaxins present within the lower respiratory tract.¹⁶ Neutrophils mainly migrate to the lungs in patients with asthma during the acute inflammatory phase after exposure to PM.¹⁷ Patients with asthma experienced an asymptomatic reduction in pulmonary function and an inflammatory response after walking along an urban road for only 2 h in a previous study, suggesting an increase in the number of neutrophils in the airway.¹⁸ Therefore, exposure to airborne PM may augment neutrophilic airway inflammation depending on IL-8 production.

Nevertheless, for the association between airborne PM and health, including respiratory disorders, the quantity of PM is the most important factor. However, the effects of airborne PM on pulmonary function may be related to the production of pro-inflammatory cytokines, especially IL-8, depending on the PM types and sources. This study aimed to investigate the association between pulmonary function in schoolchildren and the daily levels of IL-8 production in the presence of PM.

Methods

Study design

In this panel study, the peak expiratory flow (PEF) of schoolchildren was monitored daily in the morning in February 2015 in Matsue, the capital city of Shimane Prefecture, southwest Japan. This city has a population of approximately 200,000 and covers an area of 530.2 km². Students aged 10–12 years in four of the 35 elementary schools in Matsue City in 2015 were enrolled. These four elementary schools were within 10 km of each other, and all subjects lived within a 1-km radius from the schools. All children went to school on foot and were potentially exposed to air pollutants.

Sex, height, weight, and the presence of asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis, and food allergies were recorded in February 2015. Because the influence of airborne PM exposure on pulmonary function may differ depending on the presence of allergic diseases, asthma and allergic diseases were collected. The subjects were considered to have asthma if they met any of the following criteria in the previous 12 months: diagnosis of asthma by a pediatrician, presence of wheezing, use of asthma medication, or a visit to a hospital for asthma. The subjects were considered to have allergic rhinitis, allergic conjunctivitis, atopic dermatitis, and/or food allergy if they met any of the following criteria in the previous 12 months: diagnosis of any of these conditions by a pediatrician, use of medication for any of these conditions, or a visit to a hospital for any of these conditions. The study was approved by the institutional ethics committee (Ethics Committee of the Faculty of Medicine, Tottori University, Approval Number 2473), the Matsue City Board of Education, and the Parent Teacher Association of each elementary school. The children and their parents were informed of the study by the teachers and provided written consent.

Recording of daily morning peak expiratory flow

The children and teachers were taught how to measure PEF before study initiation. From February 2, 2015 to February 27, 2015, all children measured their PEF daily in the morning using a peak flow meter (Mini-Wright, Harlow, England, American Thoracic Society scale). The children recorded their best PEF value from three attempts between 8 AM and 9 AM.

Measurement of air pollutant levels

Suspended PM (SPM) is defined under the National Air Quality Standard as any particle with a diameter <10 µm with a 100% cut-off.¹⁹ In Japan, the Japanese Ministry of the Environment monitors SPM levels instead of PM₁₀. The concentrations of SPM, PM_{2.5}, sulfur dioxide (SO₂), nitrogen dioxide (NO₂), and ozone in Matsue City were obtained from the Japanese Ministry of the Environment, which monitors these values. Meteorological variables such as daily temperature, humidity, and atmospheric pressure were obtained from the Japan Meteorological Agency. These data were used to examine the associations between changes in PEF and air pollutant levels. Daily average levels of air pollutants (SPM, PM_{2.5}, SO₂, NO₂, and ozone) were calculated from 6 AM of one day to 5 AM of the next day.

Preparation of airborne particles and endotoxin concentrations

In one of the four schools, airborne PM was collected from February 1, 2015, to February 28, 2015, on a 20 × 25-cm quartz filter (2500QAT-UP; Tokyo Dylec, Tokyo, Japan) at a flow rate of 1000 L/min by using a high-volume air sampler (HV-1000R; Shibata, Tokyo, Japan) for 24 h from 6 AM to 6 AM the following day. Before sampling, to remove endotoxins from the filters, the filters were sterilized using dry heat at 240 °C for 30 min. To extract the collected PM from the filter, 40 mL endotoxin-free distilled deionized water (Sterile water endotoxin free; Wako Pure Chemicals, Osaka, Japan) was used with an ultrasonic apparatus (BRANSONIC2800; Emerson Japan, Atsugi, Japan) for 60 min.²⁰ The extraction liquids were filtered through 10 µm filters (pluriStrainer 10 µm; pluriSelect, Leipzig, Germany) to remove PM >10 µm in diameter. PM <10 µm in diameter in the dissolving solution was sterilized at 121 °C for 30 min in an autoclave (Tomy SX-300; Tomy, Tokyo, Japan) and stored in a freezer at –70 °C to prevent growth of bacteria and fungi. The concentrations of endotoxin in these extraction liquids collected between February 1, 2015 and February 28, 2015 were calculated with the Limulus color KY test (Wako Pure Chemicals) according to the manufacturer's protocols with endotoxin-free 96-well plates (Toxipet plateLP; Seikagaku, Tokyo, Japan). Samples were run in triplicate and read using an automated ELISA reader (Model 680, Bio-Rad, Philadelphia, USA). The concentrations of endotoxin are described as daily endotoxin levels.

Cell culture and measurement of IL-8 production

THP1 (ATCC® TIB-202™) human monocyte cell lines were cultured in Roswell Park Memorial Institute medium 1640 containing 10% (v/v) fetal bovine serum, 0.05 mM 2-mercaptoethanol, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.5 µg/mL amphotericin B at 37 °C and 5% CO₂ and in a humidified cell culture incubator. IL-8 release stimulated by collected PM liquid was calculated using the calculations in previous studies.^{10,21,22} THP1 cells (1 × 10⁵ cells/450 µL/tube) in an endotoxin-free tube (pirotube; Seikagaku, Tokyo, Japan) were exposed for 24 h at 37 °C with solvent only (negative control) or 50 µL of each PM <10 µm dissolving solution collected between February 1, 2015 and February

28, 2015. After exposure, the viability of the THP1 cells exceeded 95% in all samples, as assessed using a trypan blue-exclusion test. After exposure, supernatants were removed and centrifuged first at $250\times g$ to remove floating cells and then at $2500\times g$ to remove remaining particles. The final supernatants were stored at -70°C . The IL-8 concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit for IL-8 (R&D Systems, Minneapolis, USA), according to the manufacturer's protocols, with endotoxin-free 96-well plates (Toxipet plateLP; Seikagaku). Samples were run in triplicate and read using an automated ELISA reader (Model 680, Bio-Rad). The IL-8 concentrations induced by each dissolving solution are described as daily IL-8 levels.

Statistical analysis

Associations between the daily PEF values and SPM, $\text{PM}_{2.5}$, IL-8, and endotoxin levels were assessed using linear regression analysis with SPSS software (Japanese version 20.0 for Windows; IBM SPSS Japan Inc., Tokyo, Japan). Linear mixed models that accounted for associations among repeated measurements within a subject were used to estimate the associations between the daily PEF values and daily SPM, $\text{PM}_{2.5}$, IL-8, and endotoxin levels.^{23,24} The linear mixed models included a random intercept for the subjects; individual characteristics (sex, height, weight, and presence of asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis, and food allergy); and meteorological variables such as daily temperature, humidity, and atmospheric pressure. Estimates are given as the absolute difference in PEF values per interquartile range (IQR) change in SPM, $\text{PM}_{2.5}$, IL-8, and endotoxins, with 95% confidence intervals. For the treatment of missing data, the multiple imputation method was used, which adequately addresses uncertainty of the prediction of missing values based on multiple imputation values.²⁵ Linear mixed model analyses were performed using R ver. 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria). All quoted *P*-values are two-sided, with a significance level of 0.05.

Results

Subject characteristics

Of the 345 children who were recruited, 6 were excluded because they failed to maintain a daily record of PEF. The characteristics of the remaining 339 children are shown in Table 1. Data were missing for sex ($n = 2$), age ($n = 3$), height ($n = 6$), and body weight ($n = 8$).

Table 1
Characteristics of the 339 children included in this study.

Boy/girl	170/167	
Age (years)		
10	42	(12.4)
11	293	(86.4)
12	1	(0.3)
Height (cm)	144.6	± 7.07
Weight (kg)	36.4	± 7.14
Allergic disease		
Asthma	36	(10.6)
Allergic rhinitis	54	(15.9)
Allergic conjunctivitis	8	(2.4)
Atopic dermatitis	26	(7.7)
Food allergies	16	(4.7)

Data are shown as mean $\pm$ standard deviation or *n* (%).
Data were missing for sex ($n = 2$), age ($n = 3$), height ($n = 6$), and body weight ($n = 8$).

SPM and $\text{PM}_{2.5}$ levels and relationships with SPM, $\text{PM}_{2.5}$, IL-8, and endotoxins

Figure 1 presents the daily levels of SPM, $\text{PM}_{2.5}$, IL-8, and endotoxins from February 1, 2015 to February 28, 2015. Figure 2 shows the associations between the daily IL-8 and endotoxin levels and the daily averages of SPM and $\text{PM}_{2.5}$ from February 1, 2015 to February 28, 2015. There was a significant relationship between daily IL-8 levels and daily averages of SPM. However, $\text{PM}_{2.5}$ was not associated with IL-8 levels. Both SPM and $\text{PM}_{2.5}$ were significantly associated with daily endotoxin levels. There was a significant correlation between the daily IL-8 levels and daily endotoxin levels (Fig. 3).

Peak expiratory flow

The changes in PEF values for an IQR increase in the SPM, $\text{PM}_{2.5}$, IL-8, and endotoxin levels are presented in Table 2. A $0.83\text{ }\mu\text{g/mL}$ increase in the IL-8 level was significantly associated with a -1.07 L/min decrease in PEF. Increases in SPM ($12.0\text{ }\mu\text{g/m}^3$), $\text{PM}_{2.5}$ ($10.0\text{ }\mu\text{g/m}^3$), and endotoxin (35.9 EU/mL) levels were associated with changes in the PEF value of -1.36 L/min , -1.72 L/min , and -0.61 L/min , respectively. There were no significant associations between PEF values and SPM, $\text{PM}_{2.5}$, and endotoxin levels. To evaluate the between-school differences in the effects, additional analyses were conducted using interaction models; there were no significant differences across schools. Also, Figure 4 shows the relationship between the daily average PEF values and daily IL-8 levels for descriptive purpose. Although there was nearly a null correlation between the aggregated summary statistics, it might be considered as the aggregate bias (ecological bias).

Discussion

To estimate the relationship between pulmonary function in schoolchildren and airborne PM, the present study calculated the amount of endotoxin and IL-8 production in PM and explored the association between pulmonary function and daily endotoxin and IL-8 levels. While the daily levels of SPM, $\text{PM}_{2.5}$, and endotoxins were not associated with PEF values, the daily IL-8 levels had a significant negative association with PEF.

Although several reviews and meta-analyses have demonstrated the effects of airborne PM on pulmonary function in children, especially those with asthma, the results were considerably heterogeneous.^{1,4,26,27} Our previous studies also found variations in the effect of airborne PM on pulmonary function in schoolchildren.^{28,29} Additionally, the effect of airborne PM on pulmonary function depended on the ability of PM to produce IL-8 in our previous studies,^{28–30} leading to our hypothesis that the effects of airborne PM on pulmonary function might relate more with IL-8 production that is dependent on the PM types and sources rather than the mass concentration of airborne PM. Because we were not able to measure the daily IL-8 concentration induced by PM in our previous studies, we aimed to test our hypothesis in the present study. Several studies recently found that the effects of airborne PM on cardiovascular diseases differed with the PM source.^{31,32} Other studies showed that PM has a different inflammatory potential by city and season.^{11–14} Airborne PM usually consists of various components from several different sources such as crustal material, traffic, biomass combustion, waste incineration, industrial processes, transported air pollution, road abrasion and resuspension, car brake debris, and bacteria and fungi dust.³³ The results of the present study might support that the inflammatory potential relies more upon PM components than mass concentration.

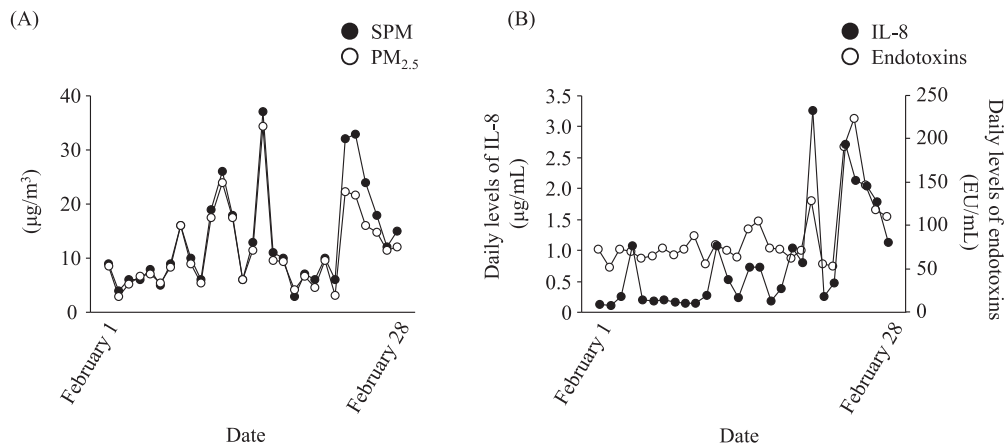


Fig. 1. Daily average levels of (A) suspended particulate matter (SPM) (closed circles) and particulate matter smaller than 2.5 μm in diameter ($\text{PM}_{2.5}$) (open circles) and (B) interleukin (IL-8) (closed circles) and endotoxins (open circles) from February 1, 2015 to February 28, 2015.

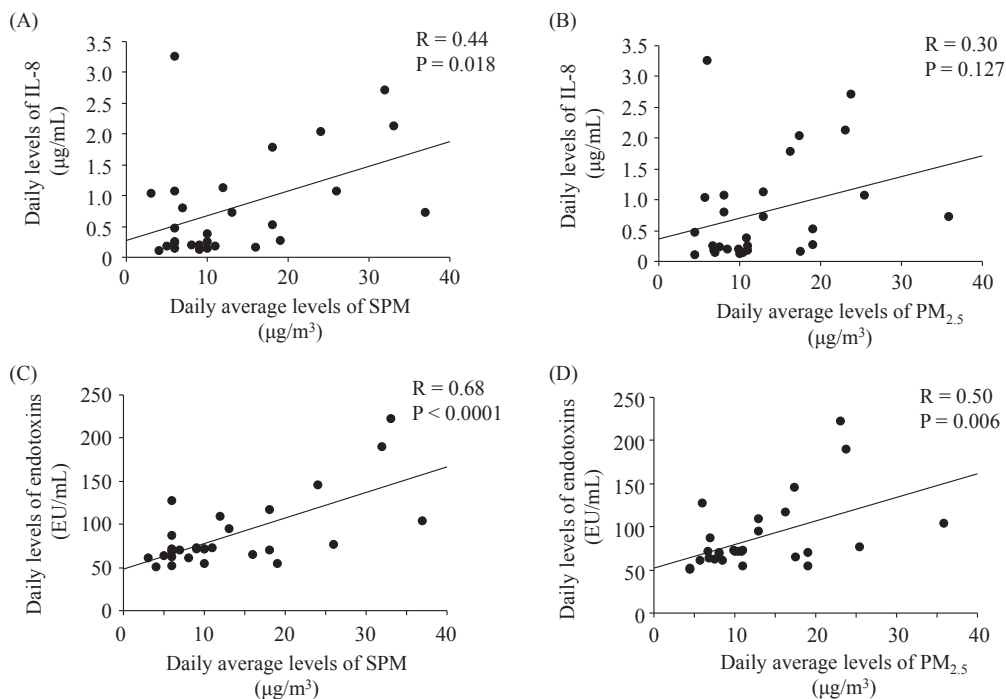


Fig. 2. Associations between daily levels of interleukin (IL-8) and (A) suspended particulate matter (SPM) and (B) particulate matter less than 2.5 μm ($\text{PM}_{2.5}$) levels and between daily levels of endotoxins and (C) SPM and (D) $\text{PM}_{2.5}$ levels.

PM can induce various pro-inflammatory cytokines, and the majority of studies assessed IL-6 and IL-8 *in vitro*.^{10,34} Among 22 types of pro-inflammatory cytokines *in vitro*, IL-8 is reportedly the pro-inflammatory cytokine that is produced most by various PM components.¹⁰ The characteristic inflammatory responses to PM exposure in humans are increased IL-8 levels and number of neutrophils in the bronchus, lung and blood.^{15,17,18,35} IL-8 has been implicated in the recruitment of neutrophils to sites of acute and chronic tissue inflammation.¹⁶ Therefore, aggravated neutrophilic airway inflammation is an important mechanism for airborne PM-related deterioration in pulmonary function, which is potentially supported by the results of the present study. Further study is needed to investigate the association with the deterioration of pulmonary function induced by exposure to airborne PM and neutrophilic airway inflammation.

Several studies have demonstrated that airborne endotoxins can decrease pulmonary function and augment an inflammatory reaction *in vivo*.^{36–38} Endotoxins have a strong potency for producing IL-8 from human cells and increasing neutrophils.³⁹ Therefore, the present study also measured the daily endotoxin levels in the collected PM and evaluated their association with pulmonary function; however, pulmonary function and the daily endotoxin levels were not significantly associated. In contrast, daily endotoxin levels were significantly associated with those of IL-8. These findings suggest that airborne endotoxins are important for IL-8 production; however, these findings are not sufficiently able to explain all of the variations in IL-8 production induced by the collected airborne PM. Airborne PM consists of various components such as metals, proteins, and saccharides; these components are known to induce the production of pro-inflammatory cytokines.^{40–43}

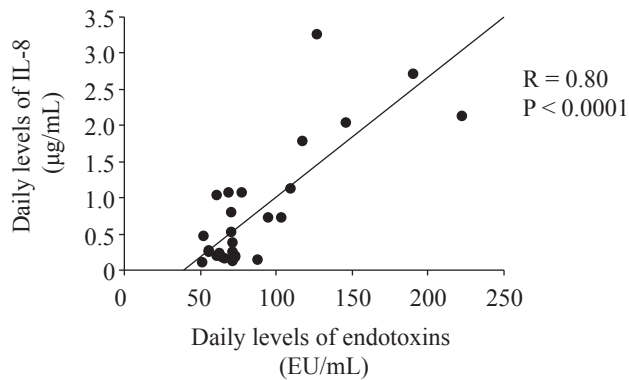


Fig. 3. Associations between daily levels of interleukin (IL)-8 and endotoxins.

Therefore, other components may also play an important role in IL-8 production.

It is important to determine the constituents and components of PM that are most responsible for decreased pulmonary function and increased IL-8 production. Although, a number of studies have evaluated the epidemiological and toxicological evidence for the role of specific components in the cause or contribution to the PM-associated health effects, the specific components that contribute most significantly to the inflammatory response have not been determined.^{44,45} Although we previously estimated the association between the metal components in PM and IL-8 production, we were unable to document the specific metal responsible for IL-8 production.²⁸ PM forms from various substances, most of which have a potential for pro-inflammatory cytokines.^{9–15,44,45} It is likely that PM components have an additive and synergistic effect on the pro-inflammatory response, making it difficult to determine the specific component(s) for IL-8 production.

In a preliminary experiment, we calculated the endotoxin and IL-8 levels per unit quantity of collected airborne PM. Before using dry heat to remove the endotoxins from the filter, the filters were humidified at 5% and 20 °C,²⁰ following which the filter weights were measured. After sampling, the filters were also humidified at 5% and 20 °C, then weighed. However, the daily amount of airborne PM was too small compared with the filter weight, and the quantity of daily collected airborne PM lacked precision. Therefore, we concluded that the endotoxin and IL-8 levels per unit quantity of collected airborne PM were not suitable for this study. In the present study, after the collected airborne PM was dissolved with 4 mL distilled deionized water, we measured the endotoxin and IL-8 concentrations in these extraction liquids, with THP1 cells stimulated by airborne PM. Then, the endotoxin and IL-8 concentrations were described as the daily endotoxin and IL-8 levels. These methods seemed appropriate to investigate the relationship between pulmonary function and airborne PM-related IL-8 production because the amount of inhaled airborne PM particles is related with SPM and PM_{2.5} levels.

Table 2

Multivariate analysis using linear mixed models of the association between peak expiratory flow (PEF) and suspended particulate matter (SPM), particulate matter <2.5 µm in diameter (PM_{2.5}), interleukin (IL)-8, and endotoxin levels, by inter-quartile range (IQR).

Exposure metric	IQR	Change in PEF (L/min)	95% CI	P value
SPM	12.0 µg/m ³	−1.36	−2.93 to 0.22	0.090
PM _{2.5}	10.0 µg/m ³	−1.72	−3.82 to 0.36	0.106
IL-8	0.83 µg/mL	−1.07	−2.05 to −0.08	0.035
Endotoxin	35.9 EU/mL	−0.61	−1.40 to 0.14	0.105

CI, confidence interval.

SPM did not exceed the ambient air quality standard for SPM, as defined by the Japanese Ministry of the Environment (daily average level ≤100 µg/m³ and hourly level ≤200 µg/m³), on any of the days during the study period. The highest daily average SPM was 37 µg/m³ (Fig. 1); therefore, exposure to airborne PM was low during the study period. However, the daily IL-8 levels were significantly associated with those of SPM. Despite the significant association between pulmonary function and daily IL-8 levels induced by airborne PM in the present study, the absolute changes in the PEF values were not high when compared with the average PEF value. Therefore, greater amounts of airborne PM, which has a high potential for pro-inflammatory cytokine production, are associated with greater effects on pulmonary dysfunction. This indicates that the quality, not the quantity, of airborne PM is important. We should endeavor to monitor the detrimental health effects of airborne PM.

Nasopharyngeal dysfunction caused by allergic rhinitis is one cause of bronchoconstriction.⁴⁶ In children, atopy is associated with airway hyperresponsiveness, which can increase airway sensitivity.⁴⁷ Therefore, the present study adjusted the analyses for allergic diseases.

There are several limitations in this study. We filtered the extraction liquids of collected airborne PM through 10 µm filters to remove PM >10 µm. Water-soluble components attached to PM >10 µm were included in the extraction liquid for measurement. Therefore, the IL-8 and endotoxin levels in this study might have been collected with PM >10 µm. However, PM >10 µm is also inhaled into the lower respiratory tract by mouth breathing.⁴⁸ This limitation did not affect the associations between PEF values and the daily IL-8 and endotoxin levels. As described, to avoid the effects of other contaminants, which can increase the IL-8 and endotoxin concentrations, the filters and fixture tools were sterilized using dry heat at 240 °C for 30 min. We also used endotoxin-free materials as far as possible when we measured IL-8 and endotoxin concentrations. However, some contaminants might still have affected the IL-8 and endotoxin levels. Missing PEF values due to absence from school were excluded from the data analysis. However, these intermittent missing data were statistically independent and did not cause any serious bias in the results. Finally,

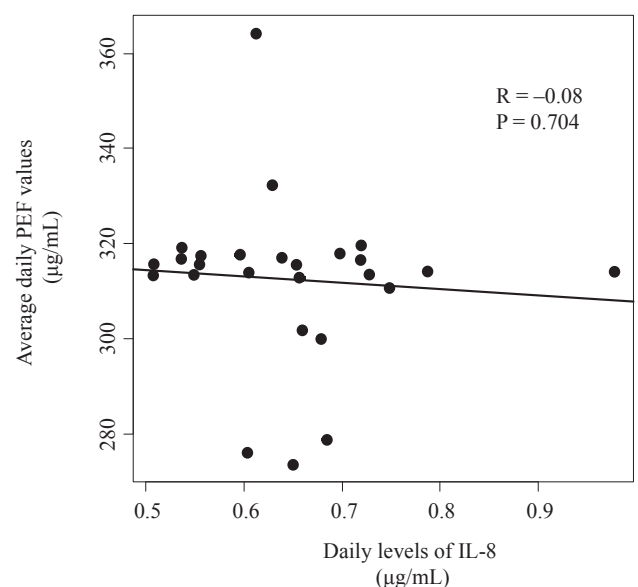


Fig. 4. Associations between daily average peak expiratory flow (PEF) values and daily interleukin (IL)-8 levels.

we were unable to measure the individual amount of exposure to airborne PM.

In conclusion, we found a significant association between pulmonary function in schoolchildren and daily levels of IL-8 induced by the collected airborne PM. However, the SPM and PM_{2.5} levels were not significantly associated with pulmonary function. These findings might suggest that the effects of airborne PM exposure on pulmonary function in schoolchildren differ with the types and sources of PM and might depend more on the pro-inflammatory response to the PM composition than the PM mass concentration.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

MW and JK conceived the study. MW, HN, JK, HS, MU, HK, and ES designed the study. MW and HN wrote the manuscript. MW, JK, and DH contributed to data collection. MW, JK, and DH performed the laboratory work. MW and HN performed the statistical analysis and interpretation of the results. MW, HN, and JK contributed to critical revision of important intellectual content. All authors read and approved the final manuscript.

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