The Association of Ambient Air Pollution With Airway Inflammation in Schoolchildren

Bing-Yu Chen, Chang-Chuan Chan, Chung-Te Lee, Tsun-Jen Cheng, Wen-Chuan Huang, Ji-Ci Jhou, Yueh-Ying Han, Chu-Chih Chen, and Yue Leon Guo*

* Correspondence to Dr. Yue Leon Guo, Department of Environmental and Occupational Medicine, National Taiwan University (NTU) and NTU Hospital, 17, Syujhou Road, Taipei 100, Taiwan, Republic of China (e-mail: leonguo@ntu.edu.tw).

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The biologic mechanisms involved in airway inflammatory response to air pollution are not clearly understood. The authors conducted a longitudinal study to investigate whether exposure to ambient air pollutants affected inflammatory cells and mediators from nasal lavage in schoolchildren. Study participants were 100 elementary and middle-school students in New Taipei City, Taiwan. A structured respiratory health questionnaire was administered in September 2007, followed by monthly measurement of nasal inflammation from October 2007 to November 2009. During the study period, daily concentrations of air pollutants were obtained from the Environmental Protection Administration monitoring station and the Aerosol Supersite. Mixed-effects models were applied to examine the association between air pollution and nasal inflammatory cells and mediators, including percentages of neutrophils, eosinophils, and monocytes in lavaged cells and interleukin-8. A total of 824 measurements were obtained from 100 participants over a period of 10 months. The level of particulate matter with an aerodynamic diameter of 2.5 μm or less (PM2.5) was found to be associated with percentage of neutrophils (β = 3.45%, 95% confidence interval: 0.89, 6.01) and interleukin-8 level (β = 29.98 pg/mL, 95% confidence interval: 3.26, 56.69) in the nasal lavage on the day of exposure. In this longitudinal cohort study of schoolchildren, results indicated that exposure to PM2.5 might induce nasal inflammation.

Epidemiologic studies have shown that ambient air pollution is associated with increased respiratory symptoms (1, 2) and decreased lung function (3–6) in children. Little is known about the mechanisms by which air pollution increases respiratory morbidity, but airway inflammation is likely to be involved (7). Numerous in vitro (8, 9), experimental animal (10, 11), and experimental human (12–17) studies have demonstrated that air pollutants act as adjuvants in the immune system and might lead to inflammation of the respiratory system. Furthermore, an association between decreased lung function and elevated airway inflammation has been reported (3). Therefore, increased airway inflammatory response might mediate the association between air pollution and respiratory health in schoolchildren.

In Taiwan, the annual average levels of ambient air pollutants during the years 2007–2010 were 30.3 parts per billion (ppb) for ozone (8-hour standard in Taiwan (18): 60 ppb), 0.50 parts per million (ppm) for carbon monoxide (8-hour standard: 9 ppm), 17.1 ppb for nitrogen dioxide (annual standard: 50 ppb), 4.4 ppb for sulfur dioxide (annual standard: 30 ppb), 58.4 μg/m³ for particulate matter with an aerodynamic diameter of 10 μm or less (PM10) (annual standard: 65 μg/m³), and 33.6 μg/m³ for particulate matter with an aerodynamic diameter of 2.5 μm or less (PM2.5) (annual standard not under regulation in Taiwan by the end of 2010; annual standard in the United States (19): 15 μg/m³), respectively. Under the current exposure levels and air pollution criteria, it is important to know whether airway inflammatory...
response can be detected in children. However, only a few epidemiologic studies have been conducted, and the results have been inconclusive (3, 5, 20).

Using bronchoalveolar lavage might provide an answer to the question of whether there is an association between air pollution and airway inflammation, but use of this invasive procedure is not feasible in an epidemiologic setting. Noninvasive procedures such as nasal lavage could be a useful means of studying the association between air pollution and upper airway inflammation. In addition, an experimental human study demonstrated that a qualitative correlation in the changes of polymorphonuclear granulocytes existed between nasal and bronchoalveolar lavage after air pollutant exposure, suggesting that inflammatory biomarkers in nasal lavage might serve as surrogates for the events occurring in lower airways (21). Therefore, we conducted a longitudinal study to investigate whether exposure to ambient air pollutants might affect inflammatory cells and mediators from nasal lavage in schoolchildren.

MATERIALS AND METHODS

Study design

A follow-up study of healthy children and children with asthma or allergic rhinitis was conducted between September 2007 and November 2009. The children were enrolled in September 2007 after completion of an initial questionnaire. Measurements of nasal inflammation were performed monthly between October 2007 and June 2008, excluding January 2008 because of the schools’ winter break. Two additional follow-ups were conducted in June and November of 2009. The study was approved by the institutional review board of the National Taiwan University Medical Center.

Study population

The study was conducted in 3 elementary schools and 2 middle schools located within a 2.5-km radius of the Taiwan Environmental Protection Administration (EPA) monitoring station and the Aerosol Supersite in Sinjuang.
District, New Taipei City, Taiwan (Figure 1). The latter was established to continuously monitor environmental particulate matter. The students brought home a modified and validated (22) Chinese version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, which was answered by their parents. Out of 5,664 candidates, questionnaires were completed for 4,221 children (75%).

The questionnaire requested information on respiratory health history. Children were defined as currently having asthma if their parents answered “yes” to all of the following questions: 1) “Has this child ever been diagnosed by a physician as having asthma?”; 2) “Has this child ever experienced dyspnea with wheezing in the chest?”; and 3) “Has this child ever experienced the above symptoms in the past 12 months?” A “yes” answer to all 3 of the following questions was used to designate current allergic rhinitis: 4) “Has this child ever been diagnosed by a physician as having allergic rhinitis?”; 5) “Has this child ever experienced problems with sneezing or a runny or blocked nose without having a cold or the flu?”; and 6) “Has this child ever experienced the above symptoms in the past 12 months?” Children whose parents answered “no” to questions 1, 2, 4, and 5 were placed in the healthy control group. A total of 133, 1,059, and 2,745 children were grouped as having current asthma, as having current allergic rhinitis, or atopic eczema in either the father or the mother. Parental atopy was defined as a history of asthma, allergic rhinitis, or atopic eczema in either the father or the mother. Information on the presence of cockroaches, water damage, walls with visible mold, and exposure to secondhand tobacco smoke at home was collected.

### Outcome measurements

**Nasal lavage.** Trained interviewers visited the children’s schools and conducted nasal lavage. The measurements were mostly taken on the last week of every month during the follow-up period, and the number of measurements made per month ranged from 3 to 4. Nasal lavage was performed using a method adapted from Koren et al. (23). Briefly, 10 mL of phosphate-buffered saline was applied to the nostrils while the child was seated. With a syringe, 1–2 mL of warm (37°C) phosphate-buffered saline was instilled into each nostril at a time. After 10 seconds, the fluid was collected by having the child blow the nasal contents gently into a container. The average recovery of fluid from nasal lavage was approximately 70%. Lavage fluid was centrifuged at 1,000 \( g \) for 10 minutes at 4°C. The supernatants were aliquoted and stored in 1.5-mL Eppendorf tubes (Axygen, Inc., Union City, California) at -20°C. The cell pellet was resuspended in 200 \( \mu L \) of phosphate-buffered saline, and 2 cytospin slides were prepared.
Figure 2. Distribution of nasal inflammatory cells as a percentage of all nasal lavage cells and mediators, New Taipei City, Taiwan, October 2007–November 2009. A) Children without upper respiratory infection (586 events); B) children with upper respiratory infection (238 events). ○, leukocytes; ●, neutrophils; ◦, eosinophils; □, monocytes; △, interleukin-8. Measurements of nasal inflammation were performed between October 2007 and June 2008, excluding January 2008 because of the schools’ winter break. Two additional follow-ups were conducted in June and November of 2009.

from each sample using a cytocentrifuge (Cytospin4; Thermo Shandon Ltd., Cheshire, United Kingdom), with centrifugation at 800 revolutions per minute for 3 minutes.

**Nasal cytology.** Cytospin slides were stained with Liu’s stain and were analyzed with a light microscope. The differential cell count of epithelial cells, neutrophils, eosinophils, and monocytes was performed on 100 cells on each of the 2 separate cytopsins, and the average number of cells was calculated.

**Cytokine and chemokine assessment.** Levels of interleukin-4, interleukin-5, interleukin-8, interleukin-13, chemokine ligand 11, and interleukin-12, chemokine ligand 3, and interleukin-13, chemokine ligand 5, and interleukin-13, chemokine ligand 10, and interleukin-13, chemokine ligand 9 were measured using an enzyme-linked immunosorbent assay (R&D Systems Inc., Minneapolis, Minnesota) according to the manufacturer’s instructions. Results were calculated as the average of duplicates. When the values were under detection limits, one-third of the detection limits were used for data analysis.

**Exposure assessment**

**Particulate matter.** The monitoring data for PM10 and PM2.5 were obtained from the Aerosol Supersite. PM10 and PM2.5 were measured using a tapered element oscillating microbalance as previously detailed (24). Levels of PM2.5 were derived by subtracting the PM2.5 level from the PM10 level.

**Criteria air pollutants.** Data for ozone, carbon monoxide, nitrogen dioxide, and sulfur dioxide were obtained from the EPA monitoring station. Ozone was measured by ultraviolet absorption, carbon monoxide by nondispersive infrared absorption, nitrogen dioxide by chemiluminescence, and sulfur dioxide by ultraviolet fluorescence.

The concentrations of air pollutants were measured continuously and reported hourly. The 8-hour moving average was calculated for ozone. For all other air pollutants, the 24-hour mean was calculated. Personal air pollutant exposure was assumed to follow the daily data as described above.

**Potential confounders**

The child’s age, height, and weight and the presence or absence of upper respiratory infection were recorded during each visit. The presence of upper respiratory infection was defined as having typical upper respiratory symptoms, and among children with asthma and/or allergic rhinitis, such symptoms could not have been accounted for by their usual attacks of asthma or allergic rhinitis. A daily card for recording of symptomatic asthma/allergic rhinitis attacks and use of asthmatic/allergic rhinitis medicine was dispatched to children with current asthma or allergic rhinitis and was answered by their parents during the follow-up period. Ambient temperature and relative humidity were measured at the schools during the nasal lavage measurements.

**Statistical analysis**

A mixed-effects model with repeated measurements to account for between-subject and within-subject variation was used to analyze the association between nasal inflammatory response and exposure to each of the air pollutants. The model was fitted using restricted maximum likelihood estimation. An unstructured correlation structure was chosen, given that it allows the most flexible assumption of correlations among repeated observations. The first-order autoregressive correlation structure was also chosen because correlations between observations are expected to decrease with sampling time. The exposure measurements were obtained on the day of the lung-function measurements and 1, 2, and 3 days before the lung-function measurements (i.e., using 0-, 1-, 2-, and 3-day-lag assumptions). Results from the models were adjusted for potential confounders, including age, body mass index, the presence of upper respiratory infection, symptomatic asthma/allergic rhinitis attacks, use of asthmatic/allergic rhinitis medicine, ambient temperature, ambient relative humidity, and the day of the week, for each sampling time. Factors that remained constant during the follow-up period, such as gender, school, parental education, parental atopy, and secondhand smoke exposure at home, were also included.

As previously documented, levels of air pollutants were not independent of each other. Therefore, we used 2-pollutant models to delineate whether the observed association of a single pollutant was related to the copollutants. Two criteria were used to determine whether the original coefficients were not nullified by the copollutant: 1) the coefficient remained statistically significant and 2) the coefficients obtained before and after addition of the copollutant were not statistically different by 2-sample t test. In addition, we added an interaction term of 2 pollutants (multiplicative scale) to the model to examine whether effect modification (or interaction) existed. Analyses were conducted using SAS, version 9.2 (SAS Institute Inc., Cary, North Carolina).

**RESULTS**

The characteristics of the study population are shown in Table 1. The distributions of demographic characteristics and indoor environmental factors were similar between the participants and nonparticipants.

A total of 824 observations were available for analysis. The number of observations (repeats) per subject ranged from 5 to 10. Missing data from the measurements resulted from refusals, transfers, or absence due to participant illness. Figure 2 shows the percentages of leukocytes, neutrophils, eosinophils, and monocytes in lavaged cells and interleukin-8 concentrations in nasal lavage. Levels of interleukin-4, interleukin-5, interleukin-13, chemokine ligand 11, and...
Figure 3. Daily ambient levels of air pollutants for each sampling time in Sinhuang District, New Taipei City, Taiwan, October 2007–November 2009. A) Particulate matter (△, particulate matter with an aerodynamic diameter of 10 µm or less; ●, particulate matter with an aerodynamic diameter of 2.5 µm or less); B) criteria air pollutants (○, carbon monoxide (10 ppb); ●, ozone; △, nitrogen dioxide; □, sulfur dioxide). Monitoring of ambient air pollutants was performed between October 2007 and June 2008, excluding January 2008 because of the schools' winter break. Two additional follow-ups were conducted in June and November of 2009. Data are presented as 24-hour mean values. For ozone, data are presented as an 8-hour moving average.

interferon-gamma were below the detection limit for all participants; thus, only the interleukin-8 results are reported. In children without upper respiratory infections (238 events), the average levels of leukocytes, neutrophils, eosinophils, monocytes, and interleukin-8 were 28.2%, 22.9%, 5.1%, 0.2%, and 179.5 pg/mL, respectively. In children with upper respiratory infections (586 events), the average levels of nasal inflammatory cells and mediators were 38.7%, 34.2%, 4.3%, 0.2%, and 264.2 pg/mL, respectively. In children without upper respiratory infections, interleukin-8 was found to be correlated with leukocytes and neutrophils.

There were 35 sampling times in the 10 months of follow-up. The distributions of ambient air pollutants in each sampling time are shown in Figure 3. The average concentrations of PM_{10}, PM_{2.5}, ozone, carbon monoxide, nitrogen dioxide, and sulfur dioxide were 39.0 μg/m³, 25.1 μg/m³, 31.8 ppb, 0.58 ppm, 21.7 ppb, and 6.1 ppb, respectively. Higher daily concentrations of PM_{10} (76.6 μg/m³), PM_{2.5} (50.5 μg/m³), and ozone (53.3 ppb) occurred on February 27, 2008, February 27, 2008, and June 5, 2008, respectively. The Pearson correlation coefficients for the air pollutants showed that PM_{2.5} was correlated with each of the other pollutants and that ozone was correlated with PM_{2.5} and PM_{2.5–10} (Table 3).

Table 4 presents changes in nasal inflammatory cells and mediators in relation to interquartile-range increases in concentrations of exposure in a single-pollutant model. Fitting an unstructured correlation structure required 55 empirical variance estimators for the 10 repeated measurements, and the sample size of this study was not adequate to support this. Thus, the first-order autoregressive correlation structure was used instead. There were no significant relations between nasal inflammatory markers and exposure to air pollutants under 1-, 2-, and 3-day-lag assumptions. Therefore, we focused our data analysis by using the 0-day-lag assumption and continued the data analysis using the 0-day-lag assumption. After adjustment for potential confounders, an interquartile-range increase in 0-day-lag PM_{2.5} level (11.5 μg/m³) was associated with a 3.51% increase (95% confidence interval (CI): 0.78, 6.23) in leukocytes, a 3.45% increase (95% CI: 0.89, 6.01) in neutrophils, and a 29.98-pg/mL increase (95% CI: 3.26, 56.69) in interleukin-8. Ozone was associated with increases in the percentages of leukocytes and neutrophils. Exposure to PM_{2.5–10}, carbon monoxide, nitrogen dioxide, and sulfur dioxide was not found to affect nasal inflammatory response.

We used 2-pollutant models to adjust for the potentially confounding effects of copollutants (Table 5). For PM_{2.5}, the 2-pollutant model yielded coefficients and statistical significance that were essentially unchanged from those in the single-pollutant model. The associations of ozone with leukocytes and neutrophils became nonsignificant when PM_{2.5} was included in the models. Furthermore, we found that the term for interaction between any 2 pollutants was statistically nonsignificant.

The significant associations between PM_{2.5} and nasal inflammation were further expressed as scatterplots of nasal

### Table 3. Pearson Correlation Coefficients for Air Pollutants in Sinjhuang District, New Taipei City, Taiwan, October 2007–November 2009

<table>
<thead>
<tr>
<th>Variable</th>
<th>PM_{2.5–10}</th>
<th>Ozone</th>
<th>Carbon Monoxide</th>
<th>Nitrogen Dioxide</th>
<th>Sulfur Dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM_{2.5}</td>
<td>0.41*</td>
<td>0.35*</td>
<td>0.67*</td>
<td>0.61*</td>
<td>0.42*</td>
</tr>
<tr>
<td>PM_{2.5–10}</td>
<td>0.32*</td>
<td>0.06</td>
<td>0.02</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Ozone</td>
<td>0.00</td>
<td>-0.01</td>
<td>0.00</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>0.89*</td>
<td>0.38*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td></td>
<td>0.48*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05.

Abbreviations: PM_{2.5}, particulate matter with an aerodynamic diameter of 2.5 μm or less; PM_{2.5–10}, particulate matter with an aerodynamic diameter of 2.5–10 μm.

### Table 4. Association of 0-Day-Lagged Ambient Air Pollutant Concentrations With Childhood Nasal Inflammation in Single-Pollutant Models, New Taipei City, Taiwan, October 2007–November 2009

<table>
<thead>
<tr>
<th>Variable</th>
<th>PM_{2.5}, μg/m³</th>
<th>PM_{2.5–10}, μg/m³</th>
<th>Ozone, ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>β</td>
<td>95% CI</td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td>Leukocytes, %</td>
<td>3.51 0.78, 6.23</td>
<td>0.76</td>
<td>2.39</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>-0.11 -1.18, 0.97</td>
<td>-0.19</td>
<td>0.29</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>3.45 0.89, 6.01</td>
<td>0.44</td>
<td>2.17</td>
</tr>
<tr>
<td>Monocytes, %</td>
<td>0.23 -0.08, 0.55</td>
<td>0.15</td>
<td>-0.03</td>
</tr>
<tr>
<td>Interleukin-8, pg/mL</td>
<td>29.98 3.26, 56.69</td>
<td>10.28</td>
<td>-9.83</td>
</tr>
</tbody>
</table>

* Abbreviations: CI, confidence interval; PM_{2.5}, particulate matter with an aerodynamic diameter of 2.5 μm or less; PM_{2.5–10}, particulate matter with an aerodynamic diameter of 2.5–10 μm.

* The coefficient was calculated for an interquartile-range increase in the level of each pollutant: 11.5 μg/m³ for PM_{2.5}, 7.9 μg/m³ for PM_{2.5–10}, and 12.3 ppb for ozone. Results were adjusted for age, body mass index, the presence of upper respiratory infection, symptomatic asthma/allergic rhinitis attacks, use of asthmatic/allergic rhinitis medicine, ambient temperature, ambient relative humidity, day of the week, gender, school, parental education, parental atopy, and secondhand exposure to tobacco smoke in the home. The ambient temperature and relative humidity were measured at the schools during nasal lavage sampling.

leukocytes, neutrophils, and interleukin-8 associated with 0-day-lag PM$_{2.5}$ (Figure 4). Results for each nasal inflammatory biomarker were adjusted for potential confounders as mentioned above, using mixed-effects models.

**DISCUSSION**

In this study, we used a longitudinal follow-up approach to examine the association between air pollutants and childhood upper airway inflammation. An interquartile-range change of 11.5 µg/m$^3$ (from 17.3 µg/m$^3$ to 28.8 µg/m$^3$) in PM$_{2.5}$ had observed associations with nasal inflammatory cells and mediators, including an increase of neutrophils and interleukin-8 in the nasal lavage on the day of exposure. Such associations were not altered when copollutants were included in the models.

Inflammation is a physiologic response to a variety of stimuli, such as tissue injury and infections. Inflammation leads to vasodilation and increased vascular permeability, allowing phagocytes to migrate to the inflammation site by extravasation and chemotaxis. Chemokines act as chemoattractants and activating molecules during extravasation of leukocytes (25). Interleukin-8 represents the prototypical chemokine and bears the principal responsibility for recruitment of neutrophils, the signature cells of the acute inflammatory response (26). Particulate matter can directly generate reactive oxygen species through the presence of free radicals and oxidants on the particle surface (27). The production of reactive oxygen species has been suggested to play an important role in subsequent oxidative stress and the inflammatory response (28). Human exposure to particulate matter may result in adverse airway cellular effects through mechanisms such as secretion of cytokines and chemokines, which enhance neutrophil adherence to respiratory cells. In vitro and experimental animal studies have indicated that particulate matter at levels ranging from 250 µg/m$^3$ to 2,500 µg/m$^3$ might cause the formation of excessive amounts of leukocytes, neutrophils, and interleukin-8 associated with 0-day-lag PM$_{2.5}$ (Figure 4). Results for each nasal inflammatory biomarker were adjusted for potential confounders as mentioned above, using mixed-effects models.

**Table 5.** Association of 0-Day-Lagged Ambient Air Pollutant Concentrations With Childhood Nasal Inflammation in 2-Pollutant Models, New Taipei City, Taiwan, October 2007–November 2009$^{a,b}$

<table>
<thead>
<tr>
<th>Variable</th>
<th>PM$_{2.5}$ µg/m$^3$ β</th>
<th>95% CI</th>
<th>PM$_{2.5}$–10 µg/m$^3$ β</th>
<th>95% CI</th>
<th>Ozone, ppb β</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, % Adjusted for PM$_{2.5}$</td>
<td>-3.77</td>
<td>-9.72, 2.18</td>
<td>1.81</td>
<td>-0.87, 4.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for PM$_{2.5}$–10</td>
<td>4.60</td>
<td>1.37, 7.82</td>
<td>2.41</td>
<td>0.24, 4.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for ozone</td>
<td>3.53</td>
<td>0.78, 6.28</td>
<td>1.97</td>
<td>-3.54, 7.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils, % Adjusted for PM$_{2.5}$</td>
<td>-0.12</td>
<td>-2.51, 2.28</td>
<td>0.31</td>
<td>-0.76, 1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for PM$_{2.5}$–10</td>
<td>-0.07</td>
<td>-1.36, 1.22</td>
<td>0.28</td>
<td>-0.78, 1.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for ozone</td>
<td>-0.11</td>
<td>-1.18, 0.97</td>
<td>0.24</td>
<td>-1.92, 2.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils, % Adjusted for PM$_{2.5}$</td>
<td>-4.04</td>
<td>-9.58, 1.51</td>
<td>1.57</td>
<td>-0.94, 4.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for PM$_{2.5}$–10</td>
<td>4.61</td>
<td>1.60, 7.62</td>
<td>2.19</td>
<td>0.29, 4.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for ozone</td>
<td>3.49</td>
<td>0.92, 6.07</td>
<td>1.47</td>
<td>-3.65, 6.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes, % Adjusted for PM$_{2.5}$</td>
<td>-0.08</td>
<td>-0.73, 0.57</td>
<td>-0.10</td>
<td>-0.40, 0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for PM$_{2.5}$–10</td>
<td>0.26</td>
<td>-0.10, 0.62</td>
<td>-0.03</td>
<td>-0.33, 0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for ozone</td>
<td>0.24</td>
<td>-0.08, 0.56</td>
<td>0.13</td>
<td>-0.49, 0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-8, pg/mL Adjusted for PM$_{2.5}$</td>
<td>-26.17</td>
<td>-84.79, 32.45</td>
<td>-16.97</td>
<td>-43.69, 9.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for PM$_{2.5}$–10</td>
<td>37.38</td>
<td>5.88, 68.88</td>
<td>-9.69</td>
<td>-35.91, 16.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for ozone</td>
<td>30.90</td>
<td>5.32, 56.48</td>
<td>-6.41</td>
<td>-58.74, 45.93</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; PM$_{2.5}$, particulate matter with an aerodynamic diameter of 2.5 µm or less; PM$_{2.5}$–10, particulate matter with an aerodynamic diameter of 2.5–10 µm.

$^a$ The coefficient was calculated for an interquartile-range increase in the level of each pollutant: 11.5 µg/m$^3$ for PM$_{2.5}$, 7.9 µg/m$^3$ for PM$_{2.5}$–10, and 12.3 ppb for ozone. Results were adjusted for age, body mass index, the presence of upper respiratory infection, symptomatic asthma/allergic rhinitis attacks, use of asthmatic/allergic rhinitis medicine, ambient temperature, ambient relative humidity, day of the week, gender, school, parental education, parental atopy, secondhand exposure to tobacco smoke in the home, and a term for interaction between the 2 pollutants. The ambient temperature and relative humidity were measured at the schools during nasal lavage sampling.

$^b$ Examination of carbon monoxide, nitrogen dioxide, and sulfur dioxide effects of nasal inflammation by adding these pollutants to the 2-pollutant models did not change the positive associations of PM$_{2.5}$, PM$_{2.5}$–10, and ozone.
of reactive oxygen species and lead to tissue inflammation and cell death (9, 11). Controlled-chamber exposure studies of healthy volunteers exposed to 100–300 µg/m³ of diesel exhaust demonstrated increased levels of neutrophils and interleukin-8 in airway lavage and bronchial epithelium biopsies (14, 16, 17). In a longitudinal study by Barraza-Villarreal et al. (3), exposure to ambient PM₂.₅ (average concentration: 28.9 µg/m³) was associated with an increase in interleukin-8 levels in nasal lavage among schoolchildren. It is believed that the smaller the size of particulate matter, the greater the toxicity from mechanisms of oxidative stress and inflammation (29, 30). This may explain our findings, in which increases in levels of neutrophils and interleukin-8 were related to PM₂.₅ but not to PM₂.₅₋₁₀.

Ozone reacts slowly with water to yield reactive hydroxyl radicals, which oxidize a wide range of biomolecules. Ozone can diffuse freely into cells and lead to sulfhydryl oxidation and inflammation (31). Release of inflammatory mediators and leukocytes and increased epithelial damage were seen in cultured human bronchial epithelial cells (8) and in mice (10) during exposure to 0.8–2.0 ppm of ozone. Changes in neutrophil and interleukin-8 levels in airway lavage and sputum induced by controlled-ozone exposure ranging from 0.2 ppm to 0.4 ppm have been found to reflect the respiratory inflammatory response of both asthmatics and healthy volunteers (12, 13, 15). Studies in schoolchildren have found associations between ambient ozone levels (average concentration: 31.6–42.3 ppb) and increases in nasal interleukin-8, leukocytes, and eosinophil cationic proteins (3, 20). In the present study, we found similar associations between nasal inflammatory biomarkers and ambient ozone (average concentration: 31.8 ppb) in single-pollutant models. However, the associations disappeared after we adjusted for PM₂.₅ using 2-pollutant models. It is possible that there were some associations between ozone and nasal markers, but not as strong as those of PM₂.₅. Since ozone and PM₂.₅ were correlated, adding PM₂.₅ to the model made the ozone coefficient insignificant. Because of low water solubility, it is likely that the effect of ozone on the lower airway is more protracted than the effect on the nasal cavities (6, 32, 33).

The data presented here were based on 100 children from the original 3,937 eligible children selected through stratified random sampling. It is unlikely that selection bias was introduced, given that the demographic characteristics of participants and nonparticipants were similar. Moreover, subject selection was not associated with the magnitude of respiratory health responses to air pollution. Although children with different atopic backgrounds were recruited, this study was not designed to answer the question of whether air pollutants affected nasal inflammation differently among these groups. The sample size did not provide us with sufficient statistical power to analyze data according to atopic conditions.

This study was designed to examine the association between respiratory health and the temporal variation in air pollutants but not spatial variation. An assumption of this study was that the participating schools and their students were exposed to similar levels of air pollutants. Although the variable “school” was added to the analytical model to avoid potential confounding caused by unforeseen differences, we do not believe that major differences in air pollutants existed among the schools, because of their geographic
proximity. Results showed that school did not affect nasal inflammation, nor did it interact with levels of air pollutants. The effect of spatial variation in ambient air pollution levels should have been minimal. We limited potentially confounding effects from socioeconomic status by recruiting our subjects from the same public school system as a proxy for socioeconomic status and by adjusting for parental education. The ethnicities of our subjects were homogeneous (99% Taiwanese).

Some study limitations need to be considered. First, the exposure assessment used district-scale air pollutant levels as surrogates for personal exposure. Using a criterion with the correlation coefficient set at 0.9, the Taiwan EPA reported that the coverage of the air monitoring station had a radius of 2.1–3.3 km for air pollutants (34). In our study, 96% of children attended schools within 1 km of their homes. Monitoring stations located near the schools (within a 2.5-km radius) were likely to be near the children’s homes. Therefore, the monitoring stations provided reasonably good assessments of exposure both at school and at home. Second, information on individual time spent outdoors, indoors, or exercising, which might have modified the children’s exposure to air pollutants, was not available. We assumed that the amounts of time spent in each activity among schoolchildren were similar because of their compulsory school timetable. The children were directly exposed to outdoor air at school because all classrooms in the participating schools had open environments without air-conditioning facilities. Exposure to air pollutants was probably lower in the indoor environment at home. Nondifferential exposure misclassification would be caused if the proportion of time spent indoors/outdoors was unrelated to the air pollution level. A bias toward observing less association with air pollutants would also be caused if the children chose to stay indoors when the air pollution levels were higher. In any case, it was unlikely that the observed associations were overestimated. Third, misclassification from upper respiratory infection being reported at times when the child was actually suffering from a more pronounced upper respiratory response to air pollutants could not be totally ruled out. However, since upper respiratory infection was included in the mixed-effects models, such misclassification would have slanted the observed association between air pollutants and respiratory inflammation towards the null hypothesis. Fourth, pollen levels were not measured in this study, and respiratory inflammation has been reported to be eosinophilic instead of neutrophilic (35). Although we could not completely rule out the confounding effect of pollen in this study, it should have been minimal. Finally, the illness-related absences of participants in each sampling time could have resulted from respiratory health conditions, which would have caused underestimation of the association between air pollutants and respiratory health.

The results from our study provide evidence that exposure to daily ambient PM$_{2.5}$ is associated with observable nasal inflammatory changes among schoolchildren. These associations were observed in a longitudinal setting—more specifically, in a cohort of schoolchildren that included healthy children. Increased airway inflammatory response might have mediated the associations between air pollution and respiratory health in these schoolchildren. Our results emphasize the continued need to examine the existing standards for ambient air pollutants by documenting potential adverse effects on human health.

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Author affiliations: Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University, Taipei, Taiwan (Bing-Yu Chen, Chang-Chuan Chan, Tsun-Jen Cheng, Yue Leon Guo); Graduate Institute of Environmental Engineering, National Central University, Jhongli, Taoyuan, Taiwan (Chung-Te Lee); Department of Environmental and Occupational Medicine, National Taiwan University (NTU) College of Medicine and NTU Hospital, Taipei, Taiwan (Wen-Chuan Huang, Ji-Ci Jhou, Yue Leon Guo); School of Health Sciences and Practice, New York Medical College, New York, New York (Yueh-Ying Han); and Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Miaoli, Taiwan (Chu-Chih Chen).

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