Effect of relocating to areas of reduced atmospheric particulate matter levels on the human circulating leukocyte count

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Submitted 9 January 2004; accepted in final form 25 June 2004

EPIDEMIOLOGICAL STUDIES HAVE established that high levels of atmospheric particulate matter (PM), especially particles with an aerodynamic diameter of <10 μm, are associated with increased cardiopulmonary morbidity and mortality (3, 7). Residents of communities exposed to high levels of PM have been shown to require more hospital admissions for chronic obstructive lung disease, pneumonia, and cardiovascular disease (28, 31), and they have had lower values for several indicators of pulmonary function than residents from areas with lower levels of PM (1, 27, 29, 41). However, the biological mechanisms underlying the effects of atmospheric PM level or health are largely unknown. Acute PM exposure derived from biomass burning induces an elevation in circulating polymorphonuclear leukocyte (PMN) counts (38). Alveolar macrophages, the only macrophages in the body exposed to air, possess high phagocytic potential and represent the first line of defense against inhaled PM in the lower airway (17). Stimulated alveolar macrophages produce inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor, and granulocyte colony-stimulating factor (G-CSF) (8, 11, 15, 16, 39). These cytokines are reported to increase in the circulation during exposure to PM (22), induce systemic inflammatory responses (17, 22), and stimulate the bone marrow to release PMNs into the circulation (23, 34, 40). An increase in circulating leukocyte counts is a known predictor of total and cardiovascular mortality (43). Recent animal studies suggest that exposure to PM induces progression of atherosclerosis and increased vulnerability to plaque rupture that might cause the increased mortality (36). Some years ago, Muchmore and others (10, 21) showed that human circulating total leukocytes decreased in Antarctica and increased toward normal levels after individuals returned to the United States. A mechanism was suggested but was not addressed by the data provided. The observation that the PM level in Antarctica is low (12, 20) suggests that low levels of atmospheric PM cause decreases in the production of cytokines in the lungs and in marrow output. The present study took advantage of the 41st Japanese Antarctic Research Expedition (JARE-41) to test this hypothesis. We determined the atmospheric PM concentration, circulating leukocyte counts, serum levels of G-CSF and IL-6, and pulmonary function on the ship on the voyage to Antarctica, throughout the stay there, on the journey home, and after arrival back in Japan.

METHODS

Subjects. The 39 members of JARE-41 who participated in the study followed similar daily work routines that included outdoor activities (3–8 h per day). Nineteen of the subjects were support staff (engineers, carpenters, chefs, communications engineers, aircraft engineers, pilots, waste disposal managers) who were exposed to occupational pollutants, and 20 others were researchers. All subjects had the same diet from departure to arrival back in Japan. Written, informed consent was obtained from each subject, and the protocol was approved by the Institutional Review Board of The Ministry of Education, Culture, Sports, Science, and Technology, Japan.
Setting. The expedition team left Tokyo, Japan by diesel-powered ship on November 14, 1999, lived at the Showa Station in Antarctica from February 1, 2000, remained in Antarctica in complete isolation for 366 days until January 31, 2001, boarded the ship and left Antarctica on February 1, 2001, and arrived back in Japan on March 28, 2001. Showa Station is located on Ongul island (69°00′22″S, 39°35′24″E) in eastern Antarctica ~4,000 km from the nearest country. The station consists of 47 buildings, and the total floor space is 5,578 m². A floor-heating system using hot water was used to keep the air clean, and the air was continuously taken into the rooms directly from outdoors. The smoking area at the station and on the ship was completely separated to prevent exposure to secondhand smoke.

Atmospheric PM concentration. Atmospheric PM concentration was evaluated by direct measurement of the number density of PM. The data were collected every minute with a laser particle counter (type TD100; Rion, Tokyo, Japan). The JARE-41 Atmosphere and Glaciology Group was in charge of this measurement. The PM concentration was measured at Showa Station in Antarctica, at the prow of the ship during the voyages, and in the city center of Saga, Kyusyu, Japan. The PM number densities were measured for the aerodynamic diameter ranges of PM0.3–2.0 (0.3 to 2.0 μm in aerodynamic diameter), PM2.0–5.0 (2.0 to 5.0 μm), and PM5.0 (5.0 to 10 μm). The daily value was obtained by averaging the data collected each minute. We did not measure the mass density of PM because the concentration of most elements as well as of organic compounds in Antarctica is in an extremely low range, normally at the picograms per gram and parts per trillion volume (10⁻¹² vol/vol) levels or even below (13).

Blood sampling. Blood was obtained from the median cubital vein of participants on day 1 (November 14, 1999) on the ship right after departure, day 109 (March 1, 2000), day 210 (June 10, 2000), day 271 (August 10, 2000), and day 354 (November 1, 2000) in Antarctica, day 447 (February 2, 2001) on the ship 2 days after leaving the Showa Station, and day 516 (April 12, 2001) after arrival in Japan. To avoid cold exposure, all subjects were rested at room temperature (from +15 to +23°C) at least 12 h before blood sampling and we collected blood samples at room temperature. Blood samples for the circulating leukocyte count and the differential leukocyte count were collected into vacutainer tubes containing potassium ethylene diaminetetraacetic acid (Terumo, Japan). Blood samples for G-CSF and IL-6 measurements were collected into standard plastic tubes and centrifuged at 3,000 rpm for 15 min. The supernatant was stored at −85°C until assayed.

Blood cell counts. Total leukocyte counts were determined with a blood counter (type K-4500; Sysmex, Tokyo, Japan). Differential counts were done by two experienced, independent observers by counting 200 leukocytes in randomly selected fields of view of Wright’s-stained blood smears.

G-CSF and IL-6 measurements. G-CSF was measured by KAINOS Laboratories (Tokyo, Japan) with their high-sensitivity ELISA kits (Cyclizer G-CSF). IL-6 was measured with high-sensitivity ELISA kits (AN’ALYZA IL-6 HS; Genzyme Tech, Minneapolis, MN) according to the manufacturer’s instructions.

Lung function tests. Forced vital capacity, forced expiratory volume in 1 s, forced expiratory flow rate, peak expiratory flow, and maximum voluntary ventilation (MVV) were measured with a portable, automated spirometer (type AS-505; Minato Medical Science, Tokyo, Japan) at room temperature on the days of blood sampling.

Statistical analysis. PM levels recorded every month were visualized in figures and compared among mainly four locations (outward and homeward voyages, Antarctica, and Japan) by one-way analysis of variance (ANOVA). Differences in the circulating leukocyte counts, serum G-CSF and IL-6 levels, and pulmonary function between locations and between individuals were tested by using repeated-measure ANOVA with Bonferroni-type multiple comparison. Differences between smokers and nonsmokers were examined by two-way ANOVA. We evaluated standardized regression coefficients to clarify the contribution of the total PM level to other inhaled factors among other possible background causes, which were smoking history (packs per day times years) and occupational pollutant exposure (support staff vs. researchers). All values were expressed as the means ± SE, and P < 0.05 was adopted as statistically significant. All statistical analyses were conducted by use of SPSS 11.5J (SPSS, Chicago, IL).

RESULTS

Characteristics of subjects. The subjects were all men aged 36.1 ± 4.7 (range 24–57) yr old and included 16 smokers (41.0%) and 23 (59.0%) nonsmokers. They had an average height of 170.1 ± 5.7 cm and an average weight of 71.0 ± 8.4 kg. There was no significant difference in characteristics between smokers and nonsmokers. Average cigarette smoking history was 20.3 ± 2.3 cigarettes per day for 14.2 ± 1.8 yr. Smoking habits remained the same throughout the study. None of the subjects had clinical symptoms of respiratory or infectious disease during the study period.

Atmospheric conditions. During the stay in Antarctica, the outdoor temperatures ranged from −15.4°C in January to −33.3°C in July. The barometric pressure ranged from 980.8 to 995.0 hPa. The relative humidity was 61–75%. The indoor temperature at Showa Station was maintained between +15 and +23°C. The altitude of Showa Station is 28 m above sea level.

PM concentration. The number densities of PM during the study are shown in Fig. 1. The level of each size of PM was lower in Antarctica than the corresponding level aboard the ship and in Japan. The PM2.0–5.0 and PM5.0 levels were <1% of those in Japan (P < 0.001). The PM2.0–5.0 and PM5.0 levels were below the detection limits of the laser counter for 55 (15.0%) and 162 (44.3%) of the 366 days of the study, and there was no significant seasonal change in levels. There was no significant difference between outside and inside PM levels at Showa Station.

Circulating leukocytes. Total leukocyte counts decreased from 6.31 ± 2.45 × 10³/mm³ on day 1 to 5.23 ± 1.70 × 10³/mm³ on day 271 (P < 0.05, Fig. 2A). Segmented PMN counts decreased from 2.94 ± 1.76 × 10³/mm³ on day 1 to 2.08 ± 1.08 × 10³/mm³ on day 271 (P < 0.05, Fig. 2B). Band-forming PMN counts decreased from 2.37 ± 0.23 × 10³/mm³ on day 1 to 1.18 ± 0.13 × 10³/mm³ on day 271 (P < 0.05, Fig. 2C). Monocyte counts decreased from 3.04 ± 0.17 × 10³/mm³ on day 1 to 1.46 ± 0.11 × 10³/mm³ on day 354 (P < 0.05, Fig. 2D). Monocyte counts decreased more slowly than PMN counts decreased. Lymphocyte counts did not change significantly from 25.84 ± 11.35 × 10³/mm³ on day 1 to 28.96 ± 11.22 × 10³/mm³ on day 210. Total leukocyte and segmented PMN counts returned to baseline during the homeward voyage to Japan. Band-formed PMN and monocyte counts increased toward baseline after arrival in Japan, and the band-formed PMN count was actually higher than the baseline count. Total leukocyte, segmented PMN, band-formed PMN, and monocyte counts in Antarctica were significantly lower than those in other locations, the outbound and homeward voyages, and Japan. Location factors were more significant compared with individual factors (repeated-measure ANOVA, P < 0.001). Three inhaled factors that may affect circulating
leukocyte counts and cytokine levels are summarized in Table 1. Multiple-regression test showed that PM levels had more significant effects on segmented PMN, band-formed PMN, and monocyte counts than cigarette smoking and type of work. At the beginning of our study, circulating segmented PMN, band-formed PMN, and monocyte counts in smokers were significantly higher than the corresponding counts in nonsmokers. This difference in cell counts between smokers and nonsmokers disappeared during the stay in Antarctica and remained similar on return to Japan.

G-CSF and IL-6. Serum G-CSF levels did not decrease significantly from 10.42 ± 0.64 pg/ml on day 1 to 9.07 ± 0.57 pg/ml on day 271 (Fig. 3A). However, G-CSF levels in total and in nonsmokers rose above any previously determined levels immediately after departure from Antarctica and remained maximal throughout the return journey to Japan (P < 0.05), although those in smokers did not change. There was no significant difference between smokers and nonsmokers at each experimental point. Serum IL-6 levels decreased significantly from 1.45 ± 0.30 pg/ml on day 1 to 0.67 ± 0.07 pg/ml on day 271 (P < 0.05, Fig. 3B) and then increased to 1.25 ± 0.28 pg/ml on day 516 after arrival in Japan (P < 0.05). Although IL-6 levels were not different between smokers and nonsmokers at the beginning and at early time points of the Antarctic stay, some IL-6 levels in smokers were higher than those in nonsmokers at the late time points of the Antarctic stay and on the homeward voyage. G-CSF levels were affected by the type of work and cigarette smoking but not total PM, whereas IL-6 levels were affected by total PM and cigarette smoking (Table 1).

Lung function tests. Forced vital capacity, forced expiratory volume in 1 s, forced expiratory flow rate, and peak expiratory flow did not change significantly (Table 2). MVV increased from 133.7 ± 4.7 l/min on day 1 to 169.5 ± 6.1 l/min on day 271 (P < 0.05) and then decreased to 143.9 ± 3.6 l/min on day 446 (P < 0.05).

DISCUSSION

Exposure to atmospheric PM causes bone marrow stimulation and the release of leukocytes (22, 23, 38, 40). Our data suggest that exposure to low levels of atmospheric PM decreases circulating leukocytes, dominantly segmented PMNs, band-formed PMNs, and monocytes. Serum IL-6 levels decreased significantly. These findings suggested that low level of PM result in the reduced stimulation of the bone marrow and the decrease of bone marrow release of leukocytes.

Particulates of <10 μm in diameter are inspired deep into the lung. The distribution of PM deposition on the lung surface depends on the aerodynamic diameter of PM (30). The number density of atmospheric PM in Antarctica was extremely low compared with that in Saga, a midsized city in Japan. PM levels inside and outside the living facilities on the ship and in Antarctica were lower than in Saga. Although composition analyses of PM were not done, the presence of air pollutant sources in Antarctica was assumed to be low because of the absence of industrial activity and the low fossil fuel combustion. Secondhand exposure to cigarette smoke was also absent because the smoking area was strictly separated. Support staff might have inhaled more PM and gaseous pollutants than...
researchers. Multiple regression tests showed that support work had some effects, but not as strong as PM levels, on the circulating leukocyte counts. Therefore, the effect of the type of work might have been minimum and the amount of PM that support staff inhaled is considered have been extremely low in this study.

Although a composition analysis of gaseous pollutants was not done, it is considered to be possible that a change in gaseous pollutants such ozone or SO₂ affected the results. However, a previous study showed that the gaseous pollutants were not consistently associated with the circulating leukocyte counts (32). Microorganisms are also potential atmospheric contaminants from human activities. PM measured in this study ranged from 0.3 to 10 μm and included organic and inorganic substances, which include most microorganisms. The majority of microorganisms in Antarctica are competitively disadvantaged because of environmental extremes such as low temperature, water activity, and high ultraviolet radiation (2). Therefore, it is also possible that the change in the amount and types of microorganisms, which were measured as a part of PM, might have affected the results in this study.

During the Antarctic stay, segmented PMN and monocyte counts decreased gradually compared with the immediate reduction in band-formed PMNs, suggesting that there was an immediate decrease in active bone marrow release of PMNs, as band-formed PMN represents active release of PMN from the bone marrow (38, 42). On the homeward voyage, the increase in total leukocyte counts was due to the increase in PMN and monocyte counts. Segmented PMN counts returned to baseline immediately, whereas band-formed PMN and monocyte counts increased gradually and reached baseline after arrival in Japan. Human bone marrow stores mature segmented PMNs in the postmitotic pool (18, 19), and the magnitude of stimulation of the bone marrow by PM exposure is related to the quantity of PM phagocytosed by alveolar macrophages (22). We suspect

Table 1. Multiple regression analysis between inhaled factors in Antarctica

<table>
<thead>
<tr>
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<th>Cigarette Smoking</th>
<th>Support Staff</th>
<th>Total PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocyte†</td>
<td>0.211* (&lt;0.001)</td>
<td>0.139* (0.024)</td>
<td>0.168* (0.004)</td>
</tr>
<tr>
<td>Segmented PMN†</td>
<td>0.015 (0.805)</td>
<td>0.097 (0.119)</td>
<td>0.272* (&lt;0.001)</td>
</tr>
<tr>
<td>Band-formed PMN†</td>
<td>0.035 (0.543)</td>
<td>0.010 (0.864)</td>
<td>0.470* (&lt;0.001)</td>
</tr>
<tr>
<td>Monocyte†</td>
<td>0.081 (0.187)</td>
<td>0.019 (0.759)</td>
<td>0.328* (&lt;0.001)</td>
</tr>
<tr>
<td>G-CSF†</td>
<td>0.131* (0.038)</td>
<td>0.176* (0.085)</td>
<td>0.078 (0.186)</td>
</tr>
<tr>
<td>IL-6†</td>
<td>0.182* (0.004)</td>
<td>0.076 (0.228)</td>
<td>0.158* (0.008)</td>
</tr>
</tbody>
</table>

The values in parentheses are P value of testing that the coefficient is equal to 0. PM, particulate matter; PMN, polymorphonuclear leukocytes; G-CSF, granulocyte colony-stimulating factor.*Statistically significant P value.
†Global hypothesis of the model was significantly rejected at 0.05 level.
that the increase in PM induced the release of segmented PMNs from the postmitotic pool. The release of band-formed PMNs occurs late, which suggests that significant bone marrow stimulation to release band-formed PMN takes longer to develop or that stronger stimulus is necessary.

Although serum G-CSF levels did not change significantly except for a few points after leaving Antarctica, IL-6 changes were parallel with band-formed PMN counts. A recent Japanese study in Antarctica has also showed decreases in IL-1a, IL-1ß, IL-6, and TNF-α during an Antarctic stay (33). Both G-CSF and IL-6 are representative cytokines that stimulate granulocytopsis, but each works at a different level within the hemopoietic hierarchy (24). G-CSF stimulates the proliferation of progenitor cells committed to myeloid differentiation and releases the mature PMNs into the circulation (25). IL-6 stimulates multipotential cells, releases less mature PMNs (26, 34, 35), and accelerates monocyte differentiation (4, 14). The association between band-formed PMN, monocyte counts, and IL-6 levels is consistent with previous reports (4, 14, 26, 34, 35). G-CSF levels in our study were not associated with PM levels and leukocyte counts. Although other factors such as granulocyte-macrophage colony-stimulating factor, TNF-α, and IL-1, which regulate the circulating leukocyte counts, were not measured in this study, they also might have affected the changes in the leukocyte counts.

Leukocyte counts in smokers were significantly higher than those in nonsmokers at baseline. This fact is consistent with previous reports (6, 42). It is interesting that this difference between the two groups almost disappeared during the Antarctic stay and reappeared on the voyage home. This suggests that atmospheric PM provide a stronger stimulus to the bone marrow than cigarette smoking or that there may be an interaction between PM and cigarette smoking.

None of the indicators of pulmonary function changed during the study except for MVV. This observation contrasts with a previous study on the south polar plateau that showed increases in pulmonary function (10). The south polar plateau is 2,800 m above sea level, whereas Showa Station is 28 m above sea level. This difference in altitude might explain the discrepancy between the two studies, because pulmonary function is known to improve at high altitude (9).

Other Antarctic environmental factors such as dietary changes, cold exposure, and circadian rhythm might have affected the circulating leukocyte counts. As for dietary factors, all subjects had the same diet at the station and on the ship. Acute cold exposure, heat exposure, and the combination of both exposures induced leukocytosis, granulocytosis, monocytosis, and an increase in serum IL-6 levels (5). On the other hand, chronic cold or heat exposure did not alter leukocyte counts (5). We precluded acute cold exposure by taking samples after stays inside the station of more than 12 h. If acute or chronic cold exposure affected the circulating leukocyte counts in this study, it would have increased or maintained the circulating leukocyte counts. Daily circadian rhythms of several hormones affect the circulating leukocyte counts (5), whereas longitudinal effects such as the light-dark cycle are not well known. A previous Antarctic study (37) showed that serum cortisol levels, one of the major hormones affecting circulating leukocyte counts, had maintained a normal range during Antarctic stay. Therefore, it is unlikely that the changes in diet or circadian rhythms or cold or heat exposure affected the circulating leukocyte counts in this study, although the possibility cannot be denied.

The results in this study show that a low level of atmospheric PM is associated with a decrease in bone marrow stimulation, which results in decreased circulating segmented PMN, band-formed PMN, and monocyte counts. It is considered that the atmospheric PM level is one of the important factors affecting circulating leukocyte counts and basal inflammatory status, which are thought to be involved in the pathogenesis of cardiopulmonary disease.
ACKNOWLEDGMENTS

We are indebted to Masaru Ayukawa, Ph.D. (chief of JARE-41), Kentaro Watanabe, Ph.D. (chief of the wintering team), Makoto Wada, Ph.D. (chief of the Department of Atmosphere and Glaciology), and all members of the 41st Japanese Antarctic Research Expedition for general management of the study, all medical staff of the Japanese Maritime Self-Defense Force for assistance in the study, and Dr. James C. Hogg and Dr. Stephan F. van Eeden for reviewing the manuscript.

GRANTS

This work was funded by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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