The Human Bone Marrow Response to Acute Air Pollution Caused by Forest Fires

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Atmospheric pollution increases cardiopulmonary morbidity and mortality by unexplained mechanisms. Phagocytosis of fine particles (PM_{10}) by rabbit alveolar macrophages elevates white blood cells (WBC) by releasing precursors from the bone marrow and this could contribute to the pathogenesis of cardiopulmonary disease. The present study examined the association between acute air pollution caused by biomass burning and peripheral WBC counts in humans. Serial measurements of the WBC count made during the 1997 Southeast Asian Smoke-haze (Sep 29, Oct 27) were compared with a period after the haze cleared (Nov 21, Dec 5) using peripheral blood PMN band cells to monitor marrow release. The results showed that indices of atmospheric pollution were significantly associated with elevated band neutrophil counts expressed as a percentage of total polymorphonuclear leukocytes (PMN), with maximal association on zero and 1 lag day for PM_{10} and 3, and 4 lag days for SO_2 (p value $<$ 0.000). We conclude that atmospheric pollution caused by biomass burning is associated with elevated circulating band cell counts in humans because of the increased release of PMN precursors from the marrow. We speculate that this response contributes to the pathogenesis of the cardiorespiratory morbidity associated with acute air pollution.

An elevated particulate concentration in the atmosphere is associated with increased cardiorespiratory morbidity and mortality by mechanisms that are unexplained (1–4). Animal studies have established that the deposition of fine particles on the alveolar surface and their phagocytosis by alveolar macrophages results in the production of mediators that enter the bloodstream and stimulate the bone marrow to shorten the time PMN precursors spend in the marrow pools and increase the rate of release of WBC precursors into the bloodstream (5, 6). This response is important in producing lung injury (7, 8) and it could conceivably initiate the vascular events associated with atmospheric pollution (9–12). In the present study, we took advantage of an acute episode of air pollution in Southeast Asia to test the hypothesis that atmospheric pollution elevates the circulating WBC and increases the release of immature PMN precursors from human bone marrow.

The widespread forest fires in the Indonesian islands of Southern Kalimantan, Sumatra, and Java in 1997 were worsened by prolonged drought linked to the El Niño weather phenomenon (13, 14). The prevailing winds brought a biomass-generated smoke-haze containing high levels of gases (NO_2, O_3, SO_2) and ultrafine particles capable of passing through the filters of most air-conditioned buildings and penetrating deep into the lung. This haze covered Singapore from the last week of August 1997, peaked in October, and was dispersed abruptly in early November with the change in the wind direction and the onset of the wet monsoons. We report here prospective measurements of air pollution, peripheral blood leukocyte counts, and lung function made during and after the haze in Singapore to test the hypothesis that air pollution is associated with an increased release of PMN precursors into the peripheral blood.

METHODS

Subjects

We took advantage of an unusually high pollution episode in Southeast Asia in 1997 to perform an opportunistic study on healthy volunteers where every attempt was made to control for variation in subject activities during the period of the study in the following ways.

The volunteers were national service men who had a defined program of identical daily outdoor activities, that remained for a period of 6 mo beginning from June 1997 to December 1997, which spanned the whole period of the study. We selected subjects who were categorized as fit for normal physical activities; spent most of the day outdoors, and whose work duties remained unchanged during the period of the study.

The 30 subjects who participated came from two camps (Dieppe and Kranji) situated about 18 kilometers (km) apart in the northern part of the island of Singapore, a total area of 640 square km. They ranged from 19 to 24 yr of age.

The work program was similar for all subjects, with a weekly schedule that consisted of daily activities outdoors (5 to 8 h per day) such as walking, marching, jogging, swimming, and obstacle training, as well as some indoor classroom studies.

The daily ambient temperature during the months of August to January in tropical Singapore (about 1 degree latitude north of the equator) ranges from 28° to 32° C.

Protocol

The study was initiated in September 1997, after the haze had begun, and continued for 2 mo after the subsidence of the haze and the return of the pollution indices to significantly lower values. Spirometry and venous blood sampling were performed at weekly intervals, with five sets of measurements being obtained during the haze and three sets of measurements obtained in the post-haze period in Singapore, which allowed haze post-haze comparison for individual subjects. The subjects were questioned for symptoms of cough, fever, rhinorrhea, and sore throat before each set of measurements.

The air quality consisting of 24-h average ambient (outdoor) concentrations of particulate material (PM_{10} in $\mu g/m^3$), sulfur dioxide (SO_2, $\mu g/m^3$), ozone (O_3, $\mu g/m^3$), nitrogen dioxide (NO_2, $\mu g/m^3$), and carbon monoxide (CO, mg/m^3) were obtained from the Research Department of the Ministry of the Environment of Singapore. Informed consent was obtained from each subject.

Measurements

Air pollution data. Ambient air quality data were collected from the Ministry of the Environment's 15 air-monitoring stations located throughout the island. PM_{10} levels were measured directly and in real-

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time by the Rupprecht and Patashnick Co. Inc. TEOM Series 1400 Ambient Particulate Monitor (Albany, NY), whereas the other pollutants were measured using the Thermo Environmental Instruments Inc. Sulfur Dioxide Analyzer Model K50206, Nitrogen Oxides Analyzer Model K50235, Carbon Monoxide Analyzer Model K50109, and Ozone Analyzer Model K50110 (Franklin, MA), all according to the methods approved by the U.S. Environmental Protection Agency (USEPA) (15).

Daily measurements for each of the five pollutants consisted of averages over the 24-h period, which ran from 4:00 P.M. to 4:00 P.M. the following day.

Blood cell counts. A total of eight blood samples were collected from each of the 30 subjects in Singapore during the period of pollution (Sept 29, Oct 6, 13, 20, 27) and after the pollution cleared (Nov 21, 28, Dec 5). Three milliliters of venous blood were collected into a standard Vacutainer tube containing potassium ethylene diaminetetraacetic acid (Becton Dickinson Vacutainer Systems Eur, Meylan Cedex, France). Blood cell counts and differential white cell counts were determined using the Bayer Technicon H*3 cell counter (Bayer, Tarrytown, NY). Band neutrophils were counted in Giemsa-stained blood films by two experienced independent observers who were naïve of the nature of the samples. Band neutrophils were identified using morphologic criteria recommended by the College of American Pathologists Hematology Survey Subcommittee (16, 17).

Lung Function

Forced expiratory maneuvers were performed to standards that met American Thoracic Society criteria (18) using a portable automated spirometer (Minato, Tokyo, Japan). Five sets of spirometric measurements were performed by each subject during the period of pollution (Sept 29, Oct 6, 13, 20, 27) and three in the period after the pollution cleared (Nov 21, 28, Dec 5).

Statistical Analysis

All of the available data for PM_{10} ($\mu g/m^3$), SO_2 ($\mu g/m^3$), O_3 ($\mu g/m^3$), NO_2 ($\mu g/m^3$), and CO (mg/m^3) from January 1, 1997 through January 31, 1998 were daily 24-h average measurements. Differences in symptom frequency between the haze and post-haze periods were compared using chi-square analysis. The effect of the haze in the Singapore subjects was examined in detail by evaluating the serial changes in the peripheral blood counts and spirometry using generalized linear modeling techniques for analysis of variance for repeated measures (19). Bonferonni adjustment of p values was made for multiple comparisons.

Regression techniques were used to examine the correlations of the peripheral blood counts and spirometry with the level of PM_{10} and other pollutants for different lag days.

RESULTS

The subjects (n = 30) were male 21.6 ± 1.7 (mean ± SD) yr of age and consisted of seven (23%) smokers and 23 (77%) never-smokers. They had an average height of 173 ± 5.3 cm and average weight of 64.6 ± 10.0 kg.

None of the subjects had clinical symptoms or signs indicating acute viral or bacterial infections during the period of the study. The frequency of mild throat discomfort admitted to by the subjects at blood sampling sessions was 13/150; 8% (haze period) compared with 7/90; 7.2% (post-haze period) ($p > 0.05$).

Air Pollution Data

The box plots of the mean daily 24-h PM_{10} level for the months from January 1997 to January 1998 for Singapore are shown in Figure 1. The mean daily PM_{10} levels were mostly below 50 $\mu g/m^3$ except for the months of August to October 1997. In these months, both the mean and median PM_{10} values were above the accepted safe level of 50 $\mu g/m^3$ (Figure 1). During the haze period of the study from September 23 to October 29, 1997, the mean daily concentration of PM_{10} was 125.4 ± 44.9 $\mu g/m^3$, with a range of 47 to 216 $\mu g/m^3$. These values were greater than those in the post-haze period (Table 1). This table shows that the levels of SO_2, NO_2, CO, and O_3 were also elevated during the period of the haze.

Blood Counts

The serial changes in the band cells expressed as % PMN (95% CI) and the 24-h PM_{10} levels measured on the same dates on which blood samples were collected are shown in Figure 2. The bone marrow and blood cells responses (mean and SE) where there are highly significant and consistent differences in band cell counts on haze days compared to that after haze exposure are shown in Table 2. There was also variation in monocyte and platelet counts that were significant on some days, but less consistent than those observed for band cells.

The results of the serial analysis (Table 3) shows the correlation of band cell changes with PM_{10} and SO_2 with different lag days. The relationship between band cells and PM_{10} showed the best correlation with no lag days, whereas SO_2 showed the best relationship to band cells with 3 lag days. Similar analyses for the other indices of air pollution (NO_2, O_3, CO) show no difference. Although there were some variations

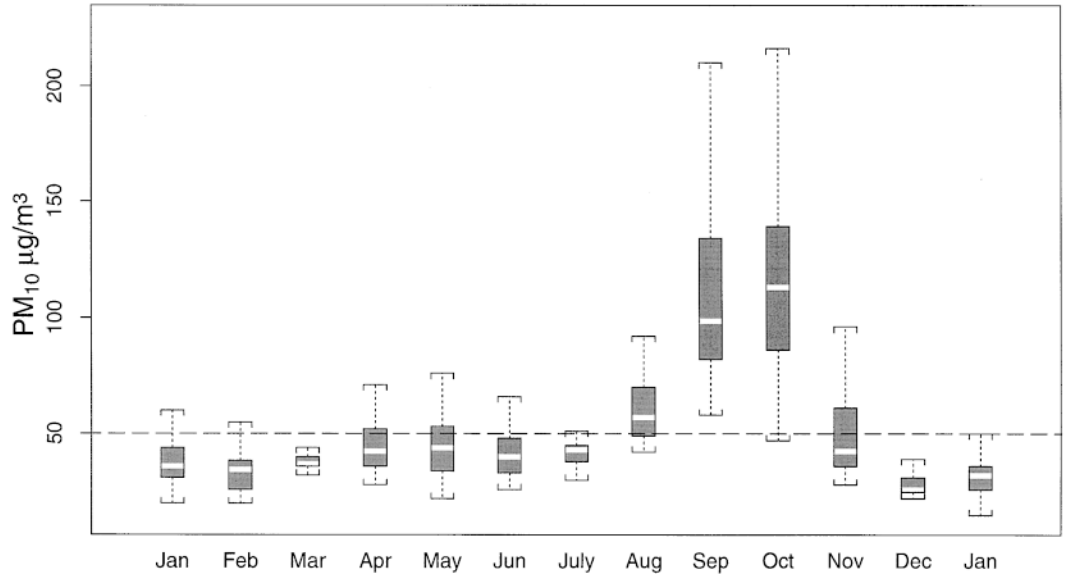

Figure 1. Box and whisker plots showing data for each month from January 1997 to January 1998: the *shaded box* represents inner quartile range (25–75 percentiles); the open bar within the box is the median; and the whiskers (antennae) indicate the minimum and maximum or 95 percentile). Outliers are indicated by a *horizontal line*. The mean and median PM_{10} values for the haze period in Singapore were August: mean, 59; median, 57; September: mean, 113; median, 99; October: mean, 116; median, 113.

TABLE 1

SINGAPORE AIR POLLUTION DATA DURING AND AFTER THE HAZE*

	PM_{10} ($\mu g/m^3$)	SO_2 ($\mu g/m^3$)	NO_2 ($\mu g/m^3$)	O_3 ($\mu g/m^3$)	CO (mg/m^3)
Haze, Sep-Oct	125.4 ± 44.9	78.7 ± 31.2	128.9 ± 23.5	108.3 ± 38.9	21.7 ± 6.0
Post-Haze, Nov-Dec	40.0 ± 14.3	40.1 ± 23.1	100.9 ± 24.3	66.3 ± 26.7	16.2 ± 6.5
p Value	< 0.001	< 0.001	< 0.001	< 0.001	0.002

* All values are mean ± SD.

in monocytes by days, there were no statistically significant relationship between the monocytes and the change in PM_{10} and SO_2 levels.

Lung Functions

FEV_1 and FVC values were all within predicted normal range for subjects studied. There was no statistically significant difference in FEV_1 and FVC during and after haze exposure (Table 2).

DISCUSSION

The cumulative weight of evidence supports the concept that contamination of the atmosphere with PM_{10} and other gaseous pollutants produce adverse health effects at relatively low concentrations (1–4, 20–23). Some studies had shown that elevated PM_{10} levels correlate with a decline in several indicators of pulmonary function in a more consistent fashion than gaseous pollutants such as ozone and sulfates (1–3). Residents of communities exposed to high levels of PM_{10} show faster rates of lung function decline, chronic respiratory and cardiovascular disease (24–27), and higher rates of hospital admissions for pneumonia, COPD, myocardial infarctions, and heart failure (2, 3) after adjusting for individual risk factors, including smoking (1). The data reported here show that the systemic response to breathing polluted air with high levels of PM_{10} and SO_2 elevate the circulating PMN band cells without having a biologically significant effect on lung function. These observations are consistent with animal experiments showing an increased release of WBC and their precursors from the bone marrow in response to the deposition of particles in the lung (5, 6).

The band cell is an immature PMN recognized by an incomplete separation of the lobes of the nucleus that is characteristic of the mature PMN granulocyte (17, 28). These cells are commonly found in the marrow and form 2 to 6% of the normal circulating WBC, and an increase in their number indicates that the marrow has been stimulated to increase the release of PMN cells (28). The serial comparison of peripheral blood samples from subjects in Singapore show that the band cell counts are consistently higher during the haze period than during that after the haze had cleared.

The results of the serial analysis of the relationship between band cell response and PM_{10} and SO_2 is interesting, and we believe it is a significant new finding. The differential association between band cells changes and PM_{10} and SO_2 for different lag days suggests that there are different "most influential days" for the two indices. These data suggest that PM_{10} has an immediate effect on the release of band cells from the marrow into the circulating blood, whereas the effect of SO_2 is delayed. These findings are similar to the commonly reported delayed effects observed for SO_2 on mortality and morbidity (hospitalizations, clinic visits) in other epidemiologic studies (29–32).

Peripheral white cell counts can also be influenced by levels of exercise, stress, and health-related events such as acute infections. We controlled for variations in subject activities and life-style stresses during the period of the study by selecting well-defined, closely supervised, healthy subjects of the same sex and similar age, working outdoors and living in the same

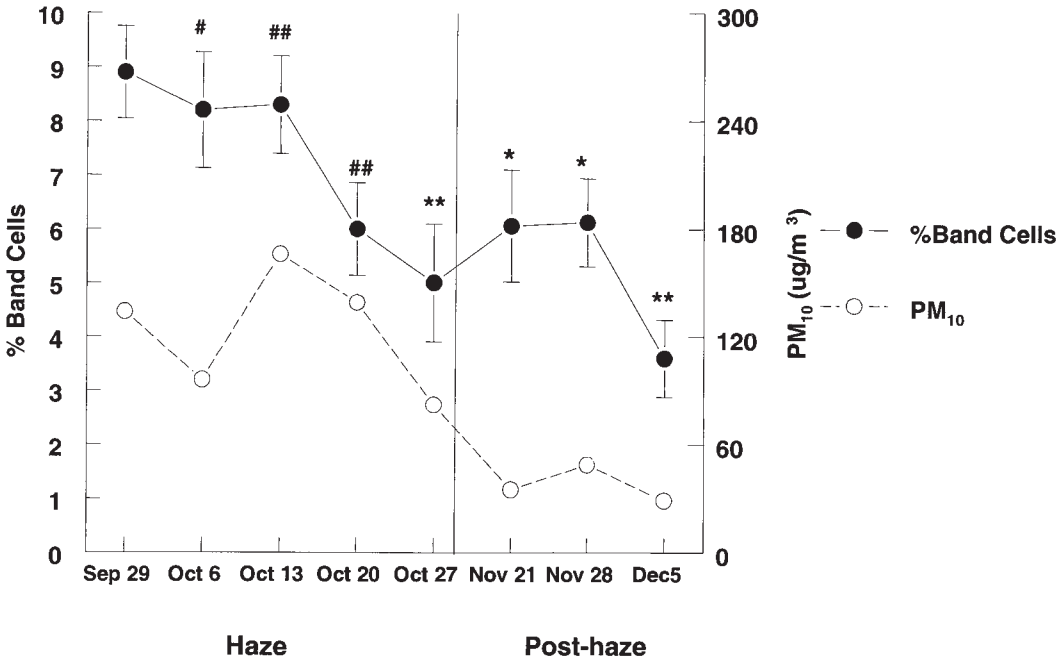

Figure 2. Band cell response and Pm_{10} level (without lag) during Southeast Asian Haze of 1997, Singapore. Symbols indicate that Band cells on October 6, 13, and 20 were higher than those on December 5 ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$). Band cells on September 29 were higher than those on October 27, November 21, November 28, and December 5 ($^{*}p < 0.05$, $^{**}p < 0.001$); p values of statistical significance are adjusted for multiple comparisons using the Bonferroni method.

TABLE 2

BONE MARROW AND BLOOD CELL RESPONSES TO HAZE EXPOSURE DURING THE SOUTHEAST ASIAN HAZE OF 1997*

	Band Cells (% PMN)		WBC ($\times 10^3/mm^3$)	Platelets ($\times 10^3/mm^3$)		Neutrophils ($\times 10^3/mm^3$)	Lymphocytes ($\times 10^3/mm^3$)	Monocytes ($\times 10^3/mm^3$)		Eosinophils ($\times 10^3/mm^3$)	Basophils ($\times 10^3/mm^3$)	FEV_1 (% pred)	FVC (% pred)
Haze													
Sep. 26	8.98	a,b,	7.36	259.3	i	3.97	2.64	0.43		0.52	0.069	107.9	105.2
	(0.85)	c,d	(0.30)	(7.5)		(0.34)	(0.28)	(0.03)		(0.08)	(0.006)	(2.9)	(3.2)
Oct. 6	8.19	e	7.41	260.2		4.17	2.66	0.46		0.52	0.059	109.1	108.6
	(1.07)		(0.30)	(7.5)		(0.36)	(0.23)	(0.03)		(0.07)	(0.005)	(3.0)	(3.6)
Oct. 13	8.31	f,g	6.94	250.6		4.24	2.26	0.45		0.42	0.068	109.2	108.1
	(0.91)		(0.32)	(7.9)		(0.32)	(0.19)	(0.03)		(0.07)	(0.007)	(3.0)	(3.0)
Oct. 20	6.00	h	7.21	248.2		4.25	2.23	0.40		0.45	0.077	108.5	107.6
	(0.86)		(0.32)	(8.1)		(0.27)	(0.16)	(0.02)		(0.06)	(0.006)	(3.3)	(3.5)
Oct. 27	5.00		7.84	254.2		4.69	2.27	0.53	j	0.39	0.087	107.2	107.2
	(1.09)		(0.31)	(7.8)		(0.49)	(0.21)	(0.04)		(0.06)	(0.012)	(2.9)	(3.4)
Post-Haze													
Nov. 21	6.06		7.35	244.3		4.39	2.12	0.47		0.38	0.064	104.8	104.6
	(1.04)		(0.34)	(8.6)		(0.37)	(0.19)	(0.02)		(0.06)	(0.005)	(3.6)	(3.7)
Nov. 28	6.12		6.72	233.1		3.81	2.22	0.45		0.42	0.070	104.3	104.3
	(0.82)		(0.32)	(8.1)		(0.30)	(0.19)	(0.03)		(0.07)	(0.009)	(3.7)	(3.4)
Dec. 5	3.62	a,e,	6.51	265.9		3.99	2.11	0.38		0.32	0.060	106.5	104.5
	(0.72)	g,h	(0.32)	(8.1)		(0.24)	(0.18)	(0.02)		(0.04)	(0.007)	(2.6)	(3.1)

* Figures in parentheses are standard errors.
a versus Oct. 27: p = 0.000.
b versus Nov. 21: p = 0.024.
c versus Nov. 28: p = 0.023.
d versus Dec. 5: p = 0.000.
e versus Dec. 5: p = 0.024.
f versus Oct. 27: p = 0.003.
g versus Dec. 5: p = 0.006.
h versus Dec. 5: p = 0.009.
i versus Nov. 28: p = 0.003.
j versus Dec. 5: p = 0.041.

environment, and with unchanging activities for the whole period of the study. Respiratory tract infection did not occur during the study period, and no other confounding factors relating to work or daily activities could be identified. The absence of these potential confounding influences on WBC kinetics and the clear temporal relationship between the band cell count and the indices of air pollution in the longitudinal data strongly suggest the elevations in band cell count can be attributable to the effects of the haze.

Although PM_{10} was considered the predominant pollutant on most days during the haze in the reports by the Ministry of the Environment in Singapore, other air pollutants such as SO_2 and O_3 were closely linked to the rise in PM_{10}. Particle characteristics such as acidity and composition may also influence the local inflammatory response in the lung, especially with an acute episode of air pollution (4, 23). Our results suggest that there is a differential temporal effect on the bone marrow response between particulate and gaseous components of air pollution, but it does not provide the exact mechanism by which either might stimulate the marrow.

TABLE 3

RESULTS OF GENERALIZED LINEAR MODELING OF THE RELATIONSHIP BETWEEN BAND CELL RESPONSE AND AIR POLLUTANT EXPOSURES WITH DIFFERENT LAG DAYS

		ANOVA		
	Lag Days	R-squared	F Statistics	p Value
PM_{10}	Zero	0.144	35.54	0.000
	1	0.132	32.28	0.000
	2	0.018	3.95	0.048
	3	0.005	1.15	0.284
	4	0.017	3.73	0.055
SO_2	Zero	0.016	3.53	0.062
	1	0.012	2.52	0.114
	2	0.029	6.28	0.013
	3	0.103	24.3	0.000
	4	0.133	32.4	0.000

An important limitation in our study was the lack of the pre-haze WBC counts. Because the southeast Asian haze came suddenly and largely unexpectedly because of the changes in the prevailing winds carrying pollutants from the forest fires from neighboring Indonesia, it was not possible for us to obtain WBC counts before the onset of the haze. We took advantage of a unique situation to study the association between acute air pollution and hematologic changes. Although we could not control the circumstances, we were able to standardize the type of subjects studied and their activities. Hence, we felt that the data collected from a well-defined group of subjects would control for confounding factors that could influence the WBC counts. We believe that the close and highly significant temporal relationship of the serial band cells counts with the levels of air pollution indices are unlikely to be random, and the absence of any identifiable fluctuations in life style or work stresses or illnesses lead us to conclude that there is a clear association between the two events, although a true cause and effect relationship cannot be established from these data.

Despite these limitations, the observations made during this natural disaster are unique and support hypotheses concerning mechanisms that have been generated from animal experiments (5–8). The findings suggest that the excess respiratory and vascular morbidity associated with air pollution (9, 10) is associated with bone marrow stimulation. Weiss and colleagues (9) have shown that an increase in circulating leukocyte count is a predictor of total mortality, independent of smoking, and longitudinal studies have linked elevations of the peripheral blood leukocytes count to increased mortality

(9, 10). Recent reports have shown that immature cells released from the marrow marginate more readily in the lung and have greater tissue-damaging capabilities than do mature PMN (5–8). Therefore, we suspect that these immature cells released in the marrow have an important role in the pathogenesis of the lesions responsible for the pulmonary and cardiovascular mortality associated with air pollution.

In summary, we attribute the differences in PMN band cells between the haze and post-haze period in Singapore to the elevation of atmospheric particulate and gaseous pollutants caused by biomass burning. This conclusion is consistent with animal studies showing an increased release of WBC and their precursors from the marrow by a non-lineage-specific mediator generated by alveolar macrophages as they phagocytose fine particulates. Further studies examining contributions of the various air pollution components would be needed before biologic effects could be attributed to a single component. Nevertheless the findings in this study have given useful insight into the probable relative contribution of the various components of air pollution on the bone marrow response.

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