Phagocytosis of particulate air pollutants by human alveolar macrophages stimulates the bone marrow

HIROSHI MUKAE,1 JAMES C. HOGG,1 DEAN ENGLISH,1 RENAUD VINCENT,2 AND STEPHAN F. VAN EEDEN1
1Pulmonary Research Laboratory, University of British Columbia, St. Paul’s Hospital, Vancouver, British Columbia V6Z 1Y6; and 2Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada K1A OL2

Received 30 November 1999; accepted in final form 9 June 2000

Mukae, Hiroshi, James C. Hogg, Dean English, Renaud Vincent, and Stephan F. van Eeden. Phagocytosis of particulate air pollutants by human alveolar macrophages stimulates the bone marrow. Am J Physiol Lung Cell Mol Physiol 279: L924–L931, 2000.—Epidemiologic studies have shown an association between the level of ambient particulate matter < 10 μm (PM10) and cardiopulmonary mortality. We have shown that exposure of rabbits to PM10 stimulates the bone marrow. In this study, we determined whether human alveolar macrophages (AMs) that phagocytose atmospheric PM10 produce mediators capable of stimulating the bone marrow. AMs incubated with PM10 for 24 h produced tumor necrosis factor-α in a dose-dependent manner (86.8 ± 53.29 pg/ml with medium alone; 1,087.2 ± 257.3 pg/ml with 0.1 mg/ml of PM10, P < 0.02). Instillation of the supernatants from AMs incubated with 0.1 mg/ml of PM10 into the lungs of rabbits (n = 6) increased circulating polymorphonuclear leukocyte (PMN) and band cell counts as well as shortened the PMN transit time through the bone marrow (87.9 ± 3.3 h) compared with unstimulated human AMs (104.9 ± 2.4 h; P < 0.01; n = 5 rabbits). The supernatants from rabbit AMs incubated with 0.1 mg/ml of PM10 (n = 4 rabbits) caused a similar shortening in the PMN transit time through the bone marrow (91.5 ± 1.6 h) compared with human AMs. We conclude that mediators released from AMs after phagocytosis of PM10 induce a systemic inflammatory response that includes stimulation of the bone marrow.

particulate matter less than 10 micrometers; air pollution; bone marrow; leukocytes; cytokines

Epidemiologic studies have associated particulate air pollution, especially particulate matter < 10 μm (PM10), with increased cardiopulmonary morbidity and mortality (6, 24). Residents of communities exposed to high levels of PM10 showed faster rates of lung function decline (19), more hospital admissions for pneumonia, chronic obstructive lung disease, myocardial infarctions, and heart failure after adjustment for several individual risk factors including smoking (1, 7, 20). The biological mechanisms responsible for this PM10-induced pulmonary and cardiovascular morbidity and mortality are not clear (33). Weiss et al. (36) have shown that an increase in peripheral blood leukocyte count is a predictor of total mortality, independent of smoking in large population-based studies, and several independent longitudinal studies (8, 9, 12) have linked elevations of the peripheral blood leukocyte count to increased mortality. A leukocytosis, specifically a granulocytosis, serves as a marker of the systemic inflammatory response (13) and is thought to contribute to this increase in morbidity and mortality. During an episode of acute air pollution in Southeast Asia, Tan et al. (26) demonstrated a leukocytosis in young military recruits that was associated with a release of granulocytes from the bone marrow (26). This suggests that this systemic inflammatory response that includes bone marrow stimulation is a feature of breathing polluted air and that this leukocyte response may play a pathogenetic role in the cardiopulmonary mortality associated with air pollution.

Our laboratory has developed a method to measure the response of the bone marrow in rabbits with the thymidine analog 5-bromo-2′-deoxyuridine (BrdU) to label dividing polymorphonuclear leukocytes (PMNs) in the bone marrow. This method provides the measurement of the transit time of PMNs through the mitotic and postmitotic pools of the bone marrow (27) and has been used to measure the effect of inflammatory stimuli such as pneumococcal pneumonia (27), cigarette smoking (28), glucocorticoids (17), and interleukin (IL)-8 (30) on the transit time of PMNs through the marrow pools. This model has also been used to show that the deposition of inert carbon particles in the lung enhanced the transit of PMNs through the bone marrow (18). Alveolar macrophages (AMs) were implicated in this response by showing that a similar stimulation of the marrow by supernatants from AMs fed carbon particles (29). Because AMs are important effector cells that phagocytose and process inhaled particles, we suspect that they play a key role in this bone marrow response. The present study was designed to determine whether mediators released from human AMs after phagocytosis of particles causes a systemic

Address for reprint requests and other correspondence: S. F. van Eeden, Pulmonary Research Laboratory, Univ. of British Columbia, St. Paul’s Hospital, 1081 Burrard St., Vancouver, BC, Canada V6Z 1Y6 (E-mail: svaneeden@mrl.ubc.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
response that includes stimulation of the bone marrow. Mediators that could be responsible for this systemic response include granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, IL-1, and tumor necrosis factor (TNF)-α (14, 18). In the present study, we used TNF-α as a marker of human AM stimulation and the rabbit model to determine the collective effect of the AM-derived mediators on the bone marrow. A major question that we addressed in this study is whether the rabbit model we used to study bone marrow stimulation is suitable to study the effect of mediators produced by human AMs. We provide data showing that when human AMs phagocytose atmosphere-derived PM₁₀, they release mediators that stimulate the bone marrow of rabbits. We propose that a rabbit model could be a useful tool in identifying the AM-derived mediators responsible for the systemic response to breathing polluted air.

METHODS

Urban air particles. The urban air dust preparation EHC-93 was obtained from Environmental Health Directorate (Health Canada, Ottawa, ON) (34, 35). The particles were recovered from bag-house filters of the single-pass air filtration system of the Environmental Health Center in Ottawa (100% outdoor air), and their elemental and organic contents have been reported elsewhere (35). The dispersed particles have a mass median diameter of 4–5 μm, and 20% of the mass is associated with the fraction of particulate matter <2.5 μm based on chemical profile and size distribution (34). The particles have a low direct cytotoxicity to lung macrophages (2) and contain endotoxin levels well below those detected in similar preparations such as SRM-1649 (3).

Stimulation of AMs in vitro. Human AMs were harvested from bronchoalveolar lavage (BAL) fluid from lungs resected for small peripheral tumors (n = 6 patients). All patients were smokers aged 62 ± 5 yr (3 women and 3 men). The BAL fluid was obtained from a noninvolved segment or lobe of the resected lung. Human AMs harvested from BAL fluid were >90% viable (trypan blue exclusion method) and consisted of 98% AMs and 2% lymphocytes and neutrophils (as determined by morphological identification of the cells by a qualified pathologist). Rabbit AMs were harvested by BAL from the lungs of normal 6-wk-old rabbits. After the rabbit was killed with pentobarbital sodium, a polyethylene tube was inserted into the trachea, and the lungs were washed with 50 ml of saline. More than 98% of cells recovered were AMs, and the cells were >90% viable. All specimens were tested for possible endotoxin contamination (positive specimens were excluded) with the Limulus amebocyte lysate test (detection level of 0.1 endotoxin unit/ml; E-TOXATE, Sigma, St. Louis, MO). Human and rabbit AMs were incubated with different concentrations of EHC-93. EHC-93 (0.01–0.1 mg/ml) particles were suspended in RPMI 1640 medium and 10% fetal calf serum. Cells (0.5 × 10⁶/ml) were incubated with the PM₁₀ suspension at 37°C in 5% CO₂ for 24 h in a 24-well plate. This time point was selected after a time-series experiment in which supernatants were harvested 2, 4, 8, 12, and 24 h after EHC-93 exposure. AMs only at the 24-h time point were significantly different from control AMs incubated only with RPMI 1640 medium. The supernatants were harvested, and to remove all particulate matter from the supernatants, they were filtered through 0.2-μm filters and centrifuged at 180 g for 10 min before storage at −70°C. TNF-α levels in the supernatants were determined by ELISA with a commercially available kit (Genzyme Diagnostics, Cambridge, MA). The supernatants used for the in vivo studies (see Effect of mediators from AMs on the bone marrow: experimental protocol) were generated by incubating AMs with either RPMI 1640 medium (control group) or 0.1 mg/ml of PM₁₀.

Effect of mediators from AMs on the bone marrow: experimental protocol. Female New Zealand White rabbits (weight 2.3–2.8 kg; n = 19) were used, and all studies were approved by the Animal Experiments Committee of the University of British Columbia (Vancouver, BC).

BrdU (100 mg/kg, Sigma) was administered to rabbits 24 h before the intrabronchial instillation of the supernatants by infusion through the marginal ear vein at a concentration of 15 mg/ml in normal sterile saline over a period of 5 min (29). For bronchial instillation of the supernatants, the rabbits were anesthetized with ketamine hydrochloride (80–100 mg/kg intramuscularly) and xylazine (10–15 mg/kg intramuscularly) and challenged with intrabronchial instillation of supernatants (0.6 ml/kg) from either human AMs stimulated with PM₁₀ or rabbit AMs stimulated with PM₁₀. The supernatant was instilled in the lower lobe of the lung under fluoroscopic control by inserting a pediatric nasogastric tube through the trachea (29). The peripheral blood samples were obtained from the central ear artery just before the BrdU injection and 3, 6, 9, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h after the intrabronchial instillation. One milliliter of blood was collected in standard Vacutainer tubes containing EDTA (Becton Dickinson, Rutherford, NJ) for leukocyte counts, and an additional 1 ml was collected in tubes containing acid citrate-dextrose for the detection of BrdU-labeled PMNs (PMN_BrdU). Fentanyl (0.02 mg/kg) and droperidol (1.0 mg/kg) were used subcutaneously as sedation at each time point of blood collection.

The animals were killed with an overdose of pentobarbital sodium, the chests were opened rapidly, the base of the heart was ligated, and the lungs were removed and inflated at 25 cmH₂O by intratracheal instillation of 10% phosphate-buffered Formalin for histological evaluation. The tissue blocks were sectioned (5 μm thick) and stained with hematoxylin and eosin. The tissue sections were evaluated at ×400 magnification in computer-generated random fields of view with a light microscope (Nikon, Japan, Tokyo).

Total white blood cell counts were determined on a model SS80 Coulter Counter (Coulter Electronics, Hialeah, FL). Differential counts were obtained by counting 100 leukocytes in randomly selected fields of view on Wright-stained blood smears, and 100 PMNs were evaluated in randomly selected fields of view to determine the changes in the number of band cells. Blood collected in acid citrate-dextrose was used to obtain leukocyte-rich plasma as previously described in detail (27). Leukocyte-rich plasma was cytopsinn onto 3-aminopropyltriethoxysilane-coated slides by cytocentrifugation at 180 g with a Cytospin 2 (Shandon Lab Products, Cheshire, UK) for 5 min. The cytosin specimens were air-dried and stained with the alkaline phosphatase-anti-alkaline phosphatase method to determine the fraction of PMN_BrdU in each specimen as previously described (27).

Evaluation of PMN_BrdU: PMN_BrdU were evaluated as previously described (27). Briefly, PMN_BrdU were divided into three groups according to the intensity of nuclear staining with an arbitrarily designated grading system. This grading system was designed to evaluate the transit time of the myeloid cells that were in their last division in the mitotic pool when exposed to BrdU (G3), those that were in the middle (G2), and those that were in their first division (G1). These slides were coded and examined without knowledge of the group or sampling time. Fields were selected in a system-
atic randomized fashion, and 200 cells were evaluated per specimen.

Transit time of PMN BrdU through the bone marrow was calculated as previously described (27). Briefly, the number of PMN BrdU was corrected for the disappearance (half-life) of cells in the circulation. In a previous study, Bicknell et al. (5), using a whole blood transfusion method, reported that the half-life of PMN BrdU in rabbits was 270 min or 4.5 h. We have applied this rate of exponential loss of PMN BrdU from the circulation to calculate the number of PMN BrdU released from the bone marrow and the transit time through the different pools (27).

Statistical analysis. All values are expressed as means ± SE. Differences in TNF-α values and PMN transit times were evaluated with a one-way ANOVA. In vivo data were analyzed with a two-way ANOVA for repeated measures, and Bonferroni’s correction was done for multiple comparisons. A value of \( P < 0.05 \) was accepted as significant.

RESULTS

Composition of particles. Table 1 summarizes the elemental composition of the EHC-93 particles as total content and soluble content as adapted from Vincent et al. (35). The calculated dose of soluble elements instilled into the lungs of rabbits is comparable to that in rats exposed for 4 h to 5 mg/m³ of EHC-93 and is considered environmentally relevant (34). This dose does not cause histological changes indicative of injury in the lung. Similarly, qualitative evaluation of lung tissue sections in our study showed no signs of inflammation in regions of supernatant instillation compared with contralateral control regions of the lung (Fig. 1B).

TNF-α produced by human AMs stimulated with PM_{10}. When human AMs were incubated with 0.1 mg/ml of PM_{10} for 24 h, they phagocytosed the particles (Fig. 1A). Incubation with different concentrations of PM_{10} resulted in a dose-dependent increase in TNF-α secretion by human AMs (Fig. 2), and the 0.1 mg/ml concentration of PM_{10} resulted in a response similar to that produced by 1 μg/ml of lipopolysaccharide (Sigma).

Table 1. Chemistry of EHC-93 soluble fraction and soluble fraction instilled in rabbits

<table>
<thead>
<tr>
<th>Element</th>
<th>Total EHC-93, μg/g</th>
<th>Soluble EHC-93, μg/g</th>
<th>Soluble EHC-93 Instilled in Rabbits, ng/kg</th>
<th>Inhaled Soluble EHC-93 in Rats, ng/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>9.8 × 10³</td>
<td>196</td>
<td>11.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Barium</td>
<td>295</td>
<td>20.7</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Boron</td>
<td>81.2</td>
<td>6.5</td>
<td>&lt;1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cadmium</td>
<td>7.3</td>
<td>5.5</td>
<td>&lt;1</td>
<td>0.2</td>
</tr>
<tr>
<td>Calcium</td>
<td>109 × 10³</td>
<td>26.2 × 10³</td>
<td>1.6 × 10³</td>
<td>926</td>
</tr>
<tr>
<td>Chromium</td>
<td>42.3</td>
<td>1.3</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cobalt</td>
<td>5.0</td>
<td>0.6</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Copper</td>
<td>845</td>
<td>144</td>
<td>8.3</td>
<td>5.1</td>
</tr>
<tr>
<td>Iron</td>
<td>14.9 × 10³</td>
<td>149</td>
<td>8.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Lead</td>
<td>6.7 × 10³</td>
<td>268</td>
<td>16.1</td>
<td>9.5</td>
</tr>
<tr>
<td>Magnesium</td>
<td>7.2 × 10³</td>
<td>1.0 × 10³</td>
<td>60.5</td>
<td>35.7</td>
</tr>
<tr>
<td>Manganese</td>
<td>483</td>
<td>101</td>
<td>6.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Nickel</td>
<td>69.6</td>
<td>4.9</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Sodium</td>
<td>20.6 × 10³</td>
<td>16.0 × 10³</td>
<td>964</td>
<td>569</td>
</tr>
<tr>
<td>Strontium</td>
<td>272</td>
<td>70.7</td>
<td>4.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Zinc</td>
<td>10.4 × 10³</td>
<td>4.8 × 10³</td>
<td>287</td>
<td>169</td>
</tr>
</tbody>
</table>

Total and soluble EHC-93 values are from Vincent et al. (35). Inhaled soluble EHC-93 values in rats are from Vincent et al. (34).
Supernatants from AMs and the release of PMNs from the bone marrow. Instillation of the supernatants from human AMs (6 h; \( P < 0.05 \)) and rabbit AMs (6 h; \( P < 0.05 \)) incubated with PM 10 caused a similar temporary increase in circulating PMNs (Fig. 3A). Circulating band cell counts also increased after instillation of the supernatants in the human AM plus PM 10 group at 6 and 9 h and in the rabbit AM plus PM 10 group at 6 h (Fig. 3B). There was no significant difference in the PMN and band cell counts between the human AM plus PM 10 and rabbit AM plus PM 10 groups.

Figure 4 shows the percentage (A) and number (B) of PMN_{BrdU} in the circulation over time. Supernatants from both human and rabbit AMs stimulated with PM 10 shifted these curves to the left (Fig. 4). Figure 5 shows the G3 cells that represent the transit of PMNs through the postmitotic pool, and Fig. 6 shows the G1 cells that represent the transit of PMNs through the mitotic plus postmitotic pools. Both G3 (Fig. 5) and G1 (Fig. 6) cells show that the supernatants from rabbit and human AMs stimulated with PM 10 causes a left shift of the curve compared with that from control human AMs.

Table 2 shows the calculated transit time of all the different populations of PMN_{BrdU} (all PMN, G3, G2, and G1 cells). The data show that compared with the effect of the instillation of supernatants from human AMs incubated without particles, supernatants from human (\( P < 0.01 \)) and rabbit (\( P < 0.05 \)) AMs incubated with PM 10 shortened the transit time of PMNs through the bone marrow. The transit time of PMNs through the postmitotic (G3; \( P < 0.01 \)) and mitotic plus postmitotic (G1; \( P < 0.05 \)) pools were shorter in human AMs incubated with than without PM 10. There was no difference in PMN transit times between the instillation of supernatants from human and rabbit AMs stimulated with PM 10 (Table 2) and supernatants incubated only in RPMI 1640 medium (29).

Urban air particles contain small amounts of endotoxin (4, 34, 35), and the PM 10 (0.1 mg/ml) dose used in this study contained trace amounts of endotoxin (0.096 endotoxin unit/ml). Instillation of RPMI 1640 medium containing the same amount of endotoxin present in 0.1 mg/ml of PM 10 into rabbit lungs (\( n = 4 \)) produced transit times of PMNs through the bone marrow that were similar to those of human AMs alone (all PMNs, 105.5 ± 4.1 vs. 104.9 ± 2.4 h; G3 cells, 65.5 ± 3.3 vs. 64.4 ± 2.9 h).

![Figure 2](image_url)  
**Fig. 2.** Tumor necrosis factor (TNF)-α production by human AMs exposed to urban air particles and lipopolysaccharide (LPS; 1 μg/ml). PM 10, particulate matter < 10 μm. Data are means ± SE of 5 (urban air particles) and 3 (LPS) experiments performed with AMs from different donors. The urban air particles caused a dose-dependent increase in TNF-α production, and the results with highest dose of particles were similar to those with LPS.* \( P < 0.05 \) vs. medium.

![Figure 3](image_url)  
**Fig. 3.** Circulating polymorphonuclear leukocyte (PMN; A) and band cell (B) counts after instillation of supernatants from cultured rabbit (\( n = 4 \)) and human (\( n = 6 \)) AMs with PM 10 and from human AMs cultured without PM 10 (\( n = 5 \)). 5-Bromo-2′-deoxyuridine (BrdU) was used to label the cells. Values are means ± SE. Note the increase in PMNs and band cells induced by supernatants from human and rabbit AMs 6 h after instillation. Band cells were also significantly increased 9 h after instillation of supernatants from human AMs stimulated with PM 10. * \( P < 0.05 \) compared with human AMs cultured without PM 10.
The results of this study show that when human AMs phagocytose atmospheric PM10, they secrete mediators such as TNF-α in a dose-dependent manner. They also show that when supernatants from human AMs incubated with PM10 are instilled into the lungs of rabbits, the transit time of PMNs through both the mitotic and postmitotic bone marrow pools are shortened. Together these results show that mediators released from AMs after phagocytosis of atmospheric particles are important in eliciting the systemic response associated with particulate air pollution.

The biological mechanisms responsible for the adverse health effects of breathing particulate air pollution are not clear. Seaton et al. (25) proposed the hypothesis that the inhalation of fine particles provokes a low-grade inflammatory response in the lung that causes an exacerbation of lung disease and a change in blood coagulability that results in increased pulmonary and cardiovascular deaths. Because AMs are important in processing airborne particles, we postulate that they are critical in secreting the mediators that initiate a systemic response that includes the release of leukocytes from the bone marrow. The present study shows that human AMs exposed to PM10 collected in an urban environment are stimulated to secrete mediators such as TNF-α (Fig. 1) and that the
mediators secreted by these human AMs and instilled into the lung of the rabbit stimulate the bone marrow (Table 2). This suggests that AMs are an important source of inflammatory mediators responsible for the systemic response after PM10 exposure.

We used TNF-α as a marker of AM stimulation by PM10, and the increased secretion of TNF-α is consistent with a previous report (4) with different urban air pollutants. AMs are also capable of producing numerous other mediators such as hematopoietic growth factors (granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor), IL-1, IL-6, and IL-8 (14, 18), all capable of stimulating the bone marrow. These mediators could spill over into the circulation and stimulate the bone marrow directly or indirectly via their effect on other effector cells in the lung or other organs. Other effector cells in the lung such as airway epithelial cells also produce inflammatory mediators when exposed to PM10 (21), but their role in this systemic inflammatory response is not well established. IL-1 and IL-6 play a critical role in the systemic inflammatory response by initiating the production of acute-phase proteins such as C-reactive protein, fibrinogen, complement, and antiproteases by the liver (10). Therefore, secondary mediator secretion by effector cells in the lung or distant organs such as the liver could also contribute to the observed bone marrow response.

The urban PM10 used in this study is a complex aggregate of elemental and organic carbons, metals, sulfates, nitrates, and organic contaminants (35). The metal components in these particles are thought to contribute to their ability to cause oxidant stress and cytokine production in AMs (11). Becker et al. (4) have suggested that endotoxin contributes to cytokine production by AMs because the cytokine response to PM10 was partly inhibitable by polymyxin B (4). Trace amounts of endotoxin were detected on the EHC-93 particles used in this study, but instillation of a similar amount of endotoxin into the lungs of rabbits did not alter the PMN transit time through the bone marrow. This suggests that the endotoxin contamination cannot explain the bone marrow stimulation induced by EHC-93 particles. Other soluble components of EHC-93 particles have been shown to induce an acute inflammatory response in the lungs of mice (2), although the dose used was 500 times higher than the calculated dose we used in our study (Table 1). Therefore, the small dose of particles we used in our experiments makes it unlikely that the soluble elements of the EHC-93 in the supernatants contribute to the bone marrow stimulation we observed. Furthermore, the dose of PM10 we used is not cytotoxic to AMs when structural integrity end points are measured (16), indicating that proteases and esterases released from

Fig. 6. Percentage (A) and number (B) of G1 cells (see text for description) in the circulation after instillation of supernatants from AMs cultured with and without PM10. Values are means ± SE. Note the lower values at 144 h in the human (n = 6) AM plus PM10 and rabbit (n = 5) AM plus PM10 groups compared with those in the human (n = 4) AM group. *P < 0.05 compared with the human AMs cultured without PM10.

Table 2. Transit times of PMNs through the bone marrow

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>All PMNs, h</th>
<th>G3, h</th>
<th>G2, h</th>
<th>G1, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit AMs + PM10</td>
<td>4</td>
<td>91.5 ± 1.6</td>
<td>61.6 ± 1.2</td>
<td>77.7 ± 1.6</td>
<td>111.7 ± 4.2</td>
</tr>
<tr>
<td>Human AMs + PM10</td>
<td>6</td>
<td>87.9 ± 3.3</td>
<td>56.8 ± 1.4</td>
<td>75.6 ± 3.7</td>
<td>117.1 ± 3.3</td>
</tr>
<tr>
<td>Human AMs</td>
<td>5</td>
<td>104.9 ± 2.4</td>
<td>65.7 ± 2.7</td>
<td>92.3 ± 2.3</td>
<td>130.7 ± 3.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. AMs, alveolar macrophages; PM10, particulate matter < 10 μm; all PMNs, total transit time of all 5-bromo-2′-deoxyuridine-labeled polymorphonuclear leukocytes; G3, transit time of PMNs through the postmitotic pool; G2, transit time of PMNs through the mitotic pool; G1, transit time of PMNs through the mitotic and postmitotic pools. No significant difference between transit times for rabbit AMs + PM10 and human AMs + PM10. Significant difference from human AMs: *P < 0.01; †P < 0.02; ‡P < 0.05.
damaged AMs are responsible for bone marrow stimulation. Further studies are needed to determine which component(s) of PM$_{10}$ is responsible for cytokine production by AMs and which mediator or mediators cause the bone marrow release of PMNs.

A major question that we addressed in this study is whether the rabbit model we used to study bone marrow stimulation is suitable to study the effect of mediators produced by human AMs. The results show that the supernatants from AMs incubated with PM$_{10}$ caused a shortening of these transit times and that this effect was similar when either human or rabbit AMs were incubated with the same amount of particles (Table 2). This suggests a large degree of interspecies homology between the cytokines of humans and rabbits (22, 32), allowing the rabbit to be used to study the effects of mediator release from human AMs. This model provides an advantage because the thymidine analog BrdU used to measure the transit time of PMNs through the mitotic and postmitotic bone marrow pools (17, 27–31) cannot be used in humans. It also allows us to study the functional characteristics and intravascular behavior of the newly released cells. The AMs used in this study were obtained from smokers who are urban dwellers, both factors that could potentially influence the response of these cells to particles. Although AMs from young healthy nonsmoking volunteers would minimize variables such as the effect of chronic smoking on the AM response, the subjects in our study represented the target population vulnerable to the adverse health effects of exposure to particle air pollution (6, 7, 19, 20).

This study shows a shortening of the transit time of PMNs through the bone marrow and an increased number of band cells in the circulation induced by PM$_{10}$, indicating that the number of circulating immature PMNs has increased. Lichtman and Weed (15) showed that immature PMNs harvested from the bone marrow are larger and less deformable than their circulating counterparts. Our laboratory has shown that immature PMNs newly released from the bone marrow by acute pneumonia (23) and cigarette smoke exposure (31) preferentially sequester in the pulmonary microvessels. These immature PMNs were also slow to migrate out of the capillaries into an inflammatory site (23), and it is reasonable to postulate that activation of these sequestered PMNs in lung microvessels could cause endothelial damage. We speculate that the increased burden of immature PMNs in the lung could contribute to the observed decrease in lung function associated with chronic exposure to particulate air pollutants. Furthermore, cytokines such as IL-6 stimulate the bone marrow to produce platelets and the liver to produce coagulation factors (10, 25), and these prothrombotic factors could contribute to the cardiovascular events associated with exposure to particulate air pollution.

In summary, our results show that mediators released from AMs after phagocytosis of ambient particulate matter collected in a major North American city induces a systemic inflammatory response that includes stimulation of the bone marrow. This bone marrow stimulation results in the release of immature PMNs into the circulation, and we speculate that both these leukocytes and the circulating inflammatory mediators released from AMs play an important role in the pathogenesis of the pulmonary and cardiovascular diseases associated with particulate air pollution.

We thank Jennifer Hards and Mark Elliott for technical support and Health Canada (Ottawa, ON) for making the EHC-93 available. The work was supported by Medical Research Council of Canada Grant 4219 and the British Columbia Lung Association.

REFERENCES


