Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 39, No. 6, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(02)01715-1

EXPERIMENTAL STUDIES

Particulate Air Pollution Induces Progression of Atherosclerosis

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OBJECTIVES We sought to determine the effect of exposure to air pollution particulate matter <10 μm (PM_{10}) on the progression of atherosclerosis in rabbits.

BACKGROUND Epidemiologic studies have associated exposure to ambient PM_{10} with increased cardiovascular morbidity and mortality. We have previously shown that PM_{10} exposure induces a systemic inflammatory response that includes marrow stimulation, and we hypothesized that this response accelerates atherosclerosis.

METHODS Watanabe heritable hyperlipidemic rabbits were exposed to PM_{10} ($n = 10$) or vehicle ($n = 6$) for four weeks, and bone marrow stimulation was measured. Quantitative histologic methods were used to determine the morphologic features of the atherosclerotic lesions.

RESULTS Exposure to PM_{10} caused an increase in circulating polymorphonuclear leukocytes (PMN) band cell counts (day 15: 24.6 ± 3.0 vs. 11.5 ± 2.7 × 10^7/l [PM_{10} vs. vehicle], $p < 0.01$) and an increase in the size of the bone marrow mitotic pool of PMNs. Exposure to PM_{10} also caused progression of atherosclerotic lesions toward a more advanced phenotype. The volume fraction (vol/vol) of the coronary atherosclerotic lesions was increased by PM_{10} exposure (33.3 ± 4.6% vs. 19.5 ± 3.1% [PM_{10} vs. vehicle], $p < 0.05$). The vol/vol of atherosclerotic lesions correlated with the number of alveolar macrophages that phagocytosed PM_{10} (coronary arteries: $r = 0.53$, $p < 0.05$; aorta: $r = 0.51$, $p < 0.05$). Exposure to PM_{10} also caused an increase in plaque cell turnover and extracellular lipid pools in coronary and aortic lesions, as well as in the total amount of lipids in aortic lesions.

CONCLUSIONS Progression of atherosclerosis and increased vulnerability to plaque rupture may underlie the relationship between particulate air pollution and excess cardiovascular death. (J Am Coll Cardiol 2002;39:935–42) © 2002 by the American College of Cardiology Foundation

Epidemiologic studies have shown an important relationship between excess cardiovascular morbidity and mortality after exposure to particulate air pollution, especially particulate matter <10 μm (PM_{10}) (1). A comparison of six U.S. cities with different levels of pollution (2) found an increased risk of cardiovascular events from pollution of the atmosphere with fine particles, even after adjustment for the other major cardiovascular risk factors. Calculations based on mortality statistics suggest that the effect of increasing

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fine particulate pollution by 10 μg/m^3 above the minimally accepted value increases total mortality by 1.8% (2) and cardiovascular mortality by 1.4% (1). Schimmel and Greenburg (3) compared daily excess pollution deaths in New York City and reported that 36.2% of total excess deaths were caused by coronary artery disease, 8.1% by hypertension and 11.2% by other circulatory diseases, and Peters et al. (4) showed that elevated concentrations of fine particles in the air transiently elevated the risk of acute myocardial infarction.

Atherogenesis is initiated in the vascular intima, and involves the infiltration of inflammatory leukocytes, accumulation of macrophages and foam cells, proliferation of smooth muscle cells, accumulation of extracellular matrix and lipids, disruption of the endothelial surface, hemorrhage into the plaque and thrombus formation (5). The inflammatory nature of atherosclerotic plaques is well established (6), and endothelial activation and interaction with leukocytes are early events (7). It is now recognized that the acute coronary syndrome is precipitated by the composition and vulnerability of plaques to rupture, rather than by plaque volume or associated vessel stenosis (8). Lipid-rich plaques are more dangerous than collagen-rich plaques, because they are more unstable, rupture-prone and highly thrombogenic after disruption (8,9). Infiltration of inflammatory cells into the plaque cap separating the lipid core from the lumen has also been associated with plaque disruption (8).

Work from our laboratory (10,11) suggests that there is a systemic response to the inhalation of fine atmospheric particles, initiated by cytokines generated by lung cells that phagocytose particles deposited on the lung surface. These

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Manuscript received June 25, 2001; revised manuscript received December 3, 2001, accepted January 2, 2002.

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Abbreviations and Acronyms

BrdU = 5′-bromo-2′-deoxyuridine
HDL = high-density lipoprotein
LAD = left anterior descending coronary artery
LCx = left circumflex coronary artery
LDL = low-density lipoprotein
LMCA = left main coronary artery
MCP-1 = monocyte chemoattractant protein-1
PM_{10} = particulate matter <10 μm
PMN = polymorphonuclear leukocyte
RCA = right coronary artery
TC = total cholesterol
vol/vol = volume fraction
WHHL = Watanabe heritable hyperlipidemic

cytokines modulate the local inflammatory response and enter the circulation, where they stimulate the marrow to release leukocytes and platelets and the liver to release acute-phase proteins and coagulation factors. Our working hypothesis is that this systemic response results in the progression of existing atherosclerosis. The present study included Watanabe heritable hyperlipidemic (WHHL) rabbits (12), because these animals develop atherosclerosis naturally. Our purpose was to determine whether the progression of this atherosclerotic process could be accelerated by exposure to PM_{10}, and whether this exposure would change the structural features of lesions vulnerable to rupture.

METHODS

Urban air particulate. Urban air PM_{10} (EHC-93) (13) was collected in 1993 over Ottawa, Canada, by filters with a nominal cutoff of 0.3 μm, from a single-pass air filtration system of the Environmental Health Centre in Ottawa (100% outdoor air). The particles have a mean diameter of 0.8 ± 0.4 μm (mean ± SD), with 99% (in number, not mass) of particles <3.0 μm (13).

Animals. This study was approved by the Animal Experimentation Committee of the University of British Columbia and was based on 16 female WHHL rabbits (14) with an average weight of 3.2 ± 0.1 kg (Covance Research Products Inc., Denver, Pennsylvania). All animals were 42 weeks old at the start of the experimental protocol and were fed standard rabbit chow.

Experimental protocol. The experimental animals (n = 10) were exposed to PM_{10} by intrapharyngeal instillation (11) of the particles suspended in saline and compared with control animals (n = 6) exposed to saline. Briefly, the rabbits were anesthetized with 5% halothane, and 1 ml of normal saline or PM_{10} (5 mg EHC-93 mixed with 1 ml of saline) was instilled twice a week for four weeks, as previously described (11). Dividing cells in the bone marrow were labeled using 100 mg/kg of the thymidine analogue, 5′-bromo-2′-deoxyuridine (BrdU) (Sigma Chemical, St. Louis, Missouri), infused (15) 24 h before the sixth instillation. The rabbits were sacrificed three days after the last (8th) instillation, with an overdose of sodium pentobarbital.

Immunohistochemical detection of BrdU-labeled polymorphonuclear leukocytes (PMNs). Cytospin preparations were stained by the alkaline phosphatase and anti-alkaline phosphatase method (16,17), using the anti-BrdU monoclonal antibody Bu20a (18) (DAKO, Copenhagen, Denmark) to determine the fraction of BrdU-labeled PMNs (16). The BrdU-labeled PMNs were evaluated, and their transit through the bone marrow was calculated, as previously described (15).

Histologic analysis. CORONARY ARTERIES. The hearts were fixed for 24 h in 10% phosphate-buffered formalin. The left main coronary artery (LMCA), the proximal part of the right coronary artery (RCA), the left anterior descending coronary artery (LAD) and the left circumflex coronary artery (LCx) were dissected. Three segments of each vessel were obtained using a magnifier ×4 (Luxo Lamp Corp., Port Chester, New York) to identify the arterial sites. The 12 arterial segments obtained from each animal were embedded, and 10 histologic sections per segment (120 sections per animal) were obtained. The sections were stained with hematoxylin-eosin to determine plaque cellularity, Movat's pentachrome to identify components of atherosclerotic lesions and immunohistochemical staining to identify BrdU-positive cells (19) in atherosclerotic lesions.

AORTA. The entire thoracic and abdominal aorta was fixed in 10% phosphate-buffered formalin for 24 h, divided into four roughly equal parts that were randomly sampled to obtain three blocks of tissue from each part. Frozen sections of fixed tissue were used to identify lipids, using the oil red O stain. The tissue processed into paraffin was sectioned and stained as described for the coronary arteries. One aortic specimen from a PM_{10}-exposed animal was unsuitable for this study and excluded from the analysis, because the tissue was fixed with glutaraldehyde for another study.

QUALITATIVE ANALYSIS OF ATHEROSCLEROTIC LESIONS. The slides were coded, examined without knowledge of their origin or experimental group and classified (types I to VI), according to the guidelines of the American Heart Association (AHA) (5,20). Comparisons between groups were made by the atherosclerotic score for each vessel of each rabbit, calculated as follows: atherosclerotic score = average of severity index of all segments in the vessel (corresponding severity index to atherosclerotic score: type I = 1; type II = 2; type III = 3; type IV = 4; type V = 5; and type VI = 6).

QUANTITATIVE ANALYSIS OF ATHEROSCLEROTIC LESIONS. The extent of the atherosclerosis and the fraction of the plaque taken up by each of its components was determined by using quantitative morphologic analysis. The volume fraction (vol/vol) of the vessel taken up by atherosclerosis was determined by point counting the sections at ×40 magnification. The images of all sections were captured by

a spot digital camera (Microspot, Nikon, Tokyo, Japan), coded and examined without knowledge of the group. The coded images were analyzed using a grid of 500 points superimposed onto the captured image. The density of the grid and the number of fields counted were selected to maintain the coefficient of error of the estimate of the volume below 0.1 (21). The vol/vol of the atherosclerotic lesion in each vessel was the sum of the points falling on the atherosclerotic lesions divided by the sum of the total points on the arterial wall.

The composition of atherosclerotic lesions was determined by point counting, where vol/vol was the number of points that fell on the component of interest, divided by the total number of points falling on the lesion. This was corrected to the vol/vol of that component in the atherosclerotic lesions present in the vessel of interest, as shown in the following example: vol/vol (atherosclerotic component in LMCA) = vol/vol (component in atherosclerotic lesions) × vol/vol (atherosclerotic lesions in LMCA).

The mononuclear, foam and smooth muscle cells and extracellular matrix were determined on Movat-stained sections. The cellularity of atherosclerotic lesions was determined by point counting on hematoxylin-eosin–stained sections to estimate the vol/vol of cell nuclei. The rate of cell turnover in the lesions was determined by counting a total of 1,000 nuclei in each segment and calculating the percentage of the cells that stained positive for BrdU (19) in the lesions. The extracellular lipid pool was estimated from Movat-stained sections and the total lipid content from oil red O stains. The latter stain was only used for aortic lesions, where frozen sections could be cut from formalin-fixed but unprocessed tissue. This was not possible for coronary arteries, where the arterial segments containing the lesions were processed into paraffin (which removes the lipid) before the sections were cut.

Statistical analysis. All data are expressed as the mean value ± SEM. Data were analyzed using two-way analysis of variance for repeated measures and the Bonferroni correction for multiple comparisons. The transit times of BrdU-labeled PMNs and the percentages of alveolar macrophages were compared between the groups by using the unpaired Student *t* test. The lesion type ratios were compared between the groups by using the Fisher exact test, and atherosclerotic scores were compared by using the Wilcoxon rank-sum test. The correlation between variables was examined by the Spearman rank correlation test. All computations were done using the statistical software package SAS, version 6.12 (SAS Institute, Cary, North Carolina). Statistical significance was set at $p < 0.05$.

RESULTS

Distribution of PM_{10} in the lung. In the PM_{10}-exposed group, particles were diffusely distributed in all lung regions and present mostly in alveolar macrophages (Fig. 1). The PM_{10}-exposed group showed a lower percentage of particle-negative alveolar macrophages (21.3 ± 5.3% in the PM_{10} group vs. 62.1 ± 6.0% in the control group, $p < 0.01$). The PM_{10}-exposed group also showed a higher percentage of alveolar macrophages in which >5% of the cytoplasmic surface area was occupied by particles (49.2 ± 3.4% in the PM_{10} group vs. 12.3 ± 5.7% in the control group, $p < 0.01$).

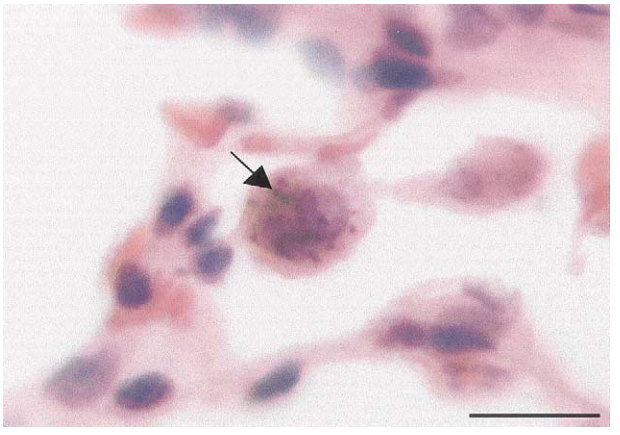

Figure 1. Photomicrograph of formalin-fixed, paraffin-embedded rabbit lung tissue stained with hematoxylin-eosin. An alveolar macrophage containing particulate matter <10 μm (>5% of the cytoplasmic surface area) **(arrow)**. Bar = 10 μm.

Systemic effects of PM_{10} exposure. BLOOD CELLS AND CHOLESTEROL LEVELS IN THE CIRCULATION. Repeated PM_{10} exposure did not change total circulating leukocyte, red blood cell, platelet or mononuclear cell counts, as compared with the control group (data not shown), but PMN counts increased two weeks after starting the exposure (day 15: 47.5 ± 5.4 in the PM_{10} group vs. 31.7 ± 4.4 × 10^8/l in the control group, $p < 0.05$), and the circulating band cell counts increased the second and third weeks of exposure (day 15: 24.6 ± 3.0 in the PM_{10} group vs. 11.5 ± 2.7 × 10^7/l in the control group, $p < 0.01$; day 18: 25.1 ± 3.0 in the PM_{10} group vs. 14.3 ± 2.4 × 10^7/l in the control group, $p < 0.05$) (Fig. 2A). Total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were not different between the groups at baseline (TC: 7.73 ± 0.93 mmol/l; HDL: 0.17 ± 0.03 mmol/l; LDL: 6.88 ± 0.94 mmol/l in the PM_{10} group vs. TC: 7.97 ± 1.75 mmol/l; HDL: 0.23 ± 0.04 mmol/l; LDL: 6.89 ± 1.67 mmol/l in the control group) and did not change with PM_{10} exposure (TC: 7.03 ± 0.69 mmol/l; HDL: 0.21 ± 0.04 mmol/l; LDL: 6.23 ± 0.71 mmol/l at the end of the study in the PM_{10}-exposed group). There were no differences between the groups (data not shown).

BONE MARROW RESPONSE. The size of the bone marrow pools reflected in the accumulation (22) of BrdU-labeled PMNs in the circulation showed a significant increase in the size of the bone marrow mitotic pool (G1 cells, $p < 0.05$) (Fig. 2B). The percentage of alveolar macrophages that phagocytosed particles in the lung also correlated with the size of the bone marrow mitotic pool of PMNs ($r = 0.53$,

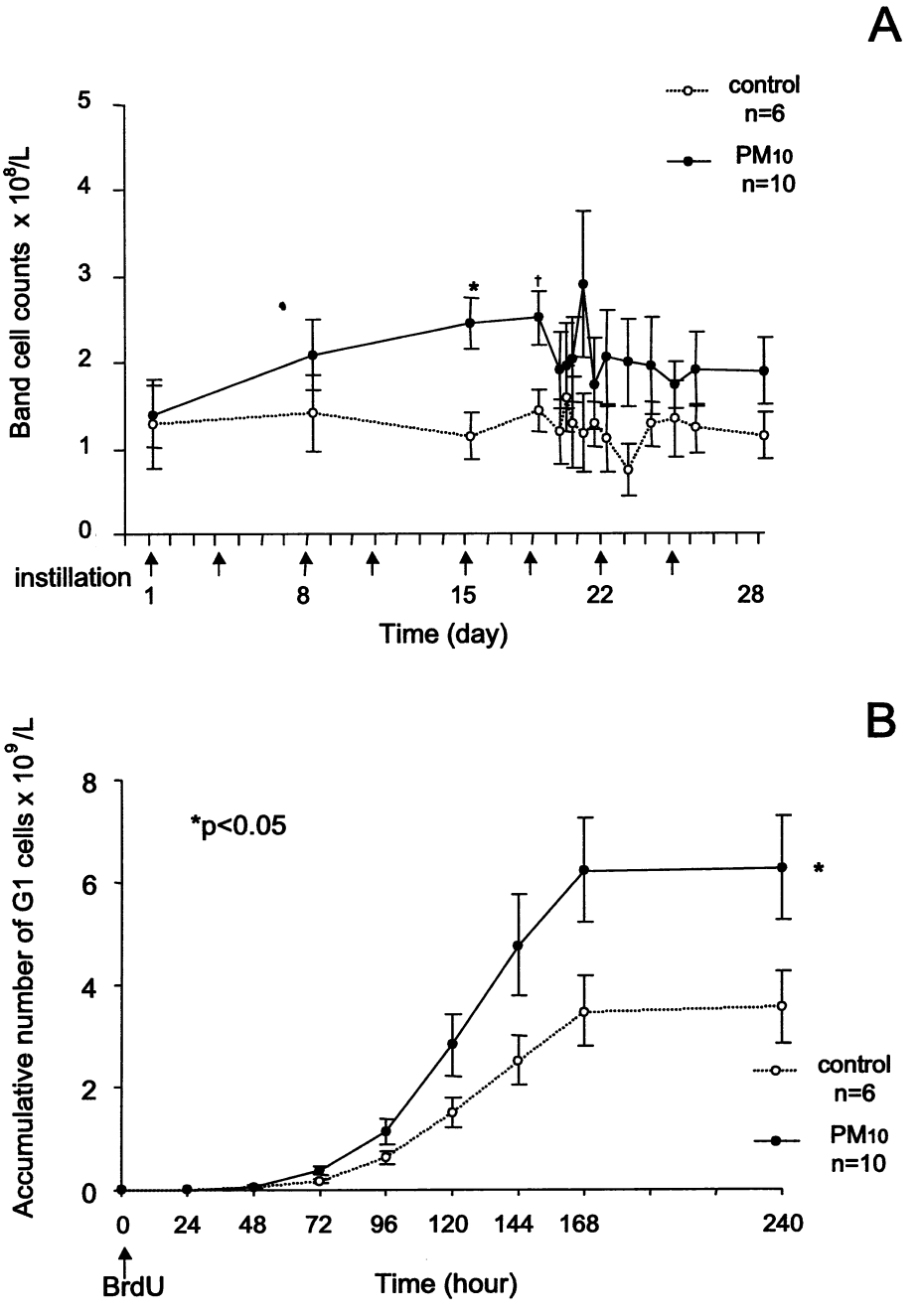

Figure 2. The circulating polymorphonuclear leukocyte (PMN) band cell counts **(A)** and accumulative number of 5′-bromo-2′-deoxyuridine (BrdU)-labeled PMNs from the bone marrow mitotic pool (weakly stained cells, G1 cells) in the circulation **(B)**. **(A)** The circulating PMN band cell counts increased during the second and third weeks of exposure (day 15: $p < 0.01$; day 18: $p < 0.05$). Rabbits were exposed to particulate matter <10 μm (PM_{10}) for four weeks (**solid circles**; n = 10) or saline (control; **open circles**; n = 6). Data are presented as the mean value ± SE. *$p < 0.01$ and †$p < 0.05$, compared with control group. **(B)** Exposure to PM_{10} increased the size of the marrow mitotic pool. Rabbits were exposed to PM_{10} for four weeks (**solid circles**; n = 10) or saline (control; **open circles**; n = 6). Data are presented as the mean value ± SE. *$p < 0.05$, compared with control group.

$p < 0.05$). The reduction in transit time (15) through the postmitotic pool (73.1 ± 3.1 h in the PM_{10} group vs. 81.9 ± 2.7 h in the saline group, $p = 0.063$) nearly reached statistical significance, but there was no reduction in the overall transit time through the marrow pools (105.7 ± 2.8 h in the PM_{10} group vs. 107.5 ± 2.9 h in the saline group).

Qualitative assessment. CORONARY ARTERIES. Examples of the lesions on which the qualitative assessment was based are shown in Figure 3. These lesions are consistent with those classified into types I–V, according to AHA guidelines (5,20). No type VI lesions showing a fissure, hematoma or thrombus formation were observed in this study.

Coronary atherosclerosis was observed most commonly (>80%) in the LMCA and RCA and less frequently (<10%) in the LAD and LCx. There was no difference in the frequency of atherosclerotic lesions in the LMCA and

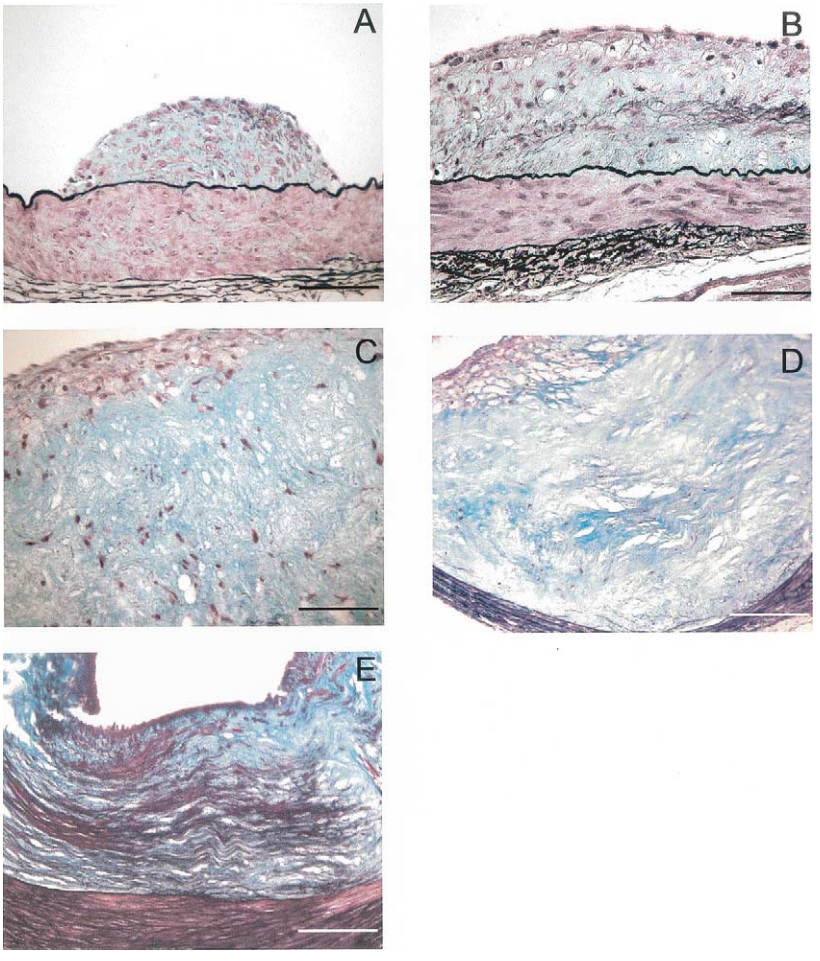

Figure 3. Photomicrographs of formalin-fixed, paraffin-embedded rabbit coronary artery tissue stained with Movat's pentachrome, showing type I to V lesions. The **bars** represent 100 μm **(A–C)** or 200 μm **(D, E)**. The type I lesion **(A)** showed intimal thickening, with an increase in the number of intimal macrophages, as well as isolated, small groups of foam cells. The type II lesion showed layers of foam cells and lipids within intimal smooth muscle cells **(B)**. The type III lesion showed extracellular lipid pools **(C)**, and the type IV lesion showed a larger, more confluent and disruptive core of extracellular lipids **(D)**. The type V lesion showed thick layers of fibrous connective tissue, in addition to the lipid core **(E)**.

RCA between the experimental group and control group (LMCA: 26/30 segments [86.7%] vs. 18/18 segments [100%]; RCA: 26/30 segments [86.7%] vs. 12/18 segments [66.7%] [PM_{10} group vs. control group]), but the lesions of the PM_{10}-exposed group showed a higher atherosclerotic score, as compared with that of the control group, in LMCA (2.67 ± 0.47 vs. 1.89 ± 0.14, $p < 0.05$). The experimental group also showed a lower percentage of type II lesions (13% vs. 50%) and higher percentages of type III (27% vs. 19%), type IV (25% vs. 3%) and type V (5% vs. 0%) lesions, as compared with the control group. The PM_{10}-exposed group showed a higher percentage of lesions that were more advanced than type II, as compared with the control group (PM_{10} group: 34/60 segments [56.7%]; control group: 8/36 segments [22.2%]; $p < 0.05$). The LAD and LCx were excluded from the analysis because of the low incidence of atherosclerotic lesions in these vessels.

AORTA. All 108 of 108 aortic samples from the PM_{10}-exposed group contained atherosclerotic lesions, as compared with 60 of 72 in the control group. This difference was not statistically significant, but showed a trend ($p = 0.09$). However, the lesions from the exposed group had a higher atherosclerotic score (3.06 ± 0.18 vs. 2.38 ± 0.27, $p < 0.05$).

Quantitative assessment. CORONARY ARTERIES. The vol/vol of atherosclerotic lesions of the LMCA and RCA was greater in the PM_{10}-exposed group as compared with the control group ($33.3 \pm 4.6\%$ vs. $19.5 \pm 3.1\%$, $p < 0.05$) (Fig. 4A). There was also a correlation between the percentage of alveolar macrophages positive for particles and the vol/vol of atherosclerotic lesions in the vessels ($r = 0.53$, $p < 0.05$) (Fig. 4B).

Analysis of the composition of atherosclerotic lesions showed that coronary arteries of the PM_{10}-exposed group contained a greater vol/vol of smooth muscle cells, extracellular matrix and extracellular lipid pools, as compared with those of the control group ($p < 0.01$) (Table 1). The atherosclerotic lesions of the PM_{10}-exposed group also had a greater vol/vol of cell nuclei, as compared with those of the control group ($3.8 \pm 0.4\%$ vs. $1.9 \pm 0.3\%$, $p < 0.05$), and

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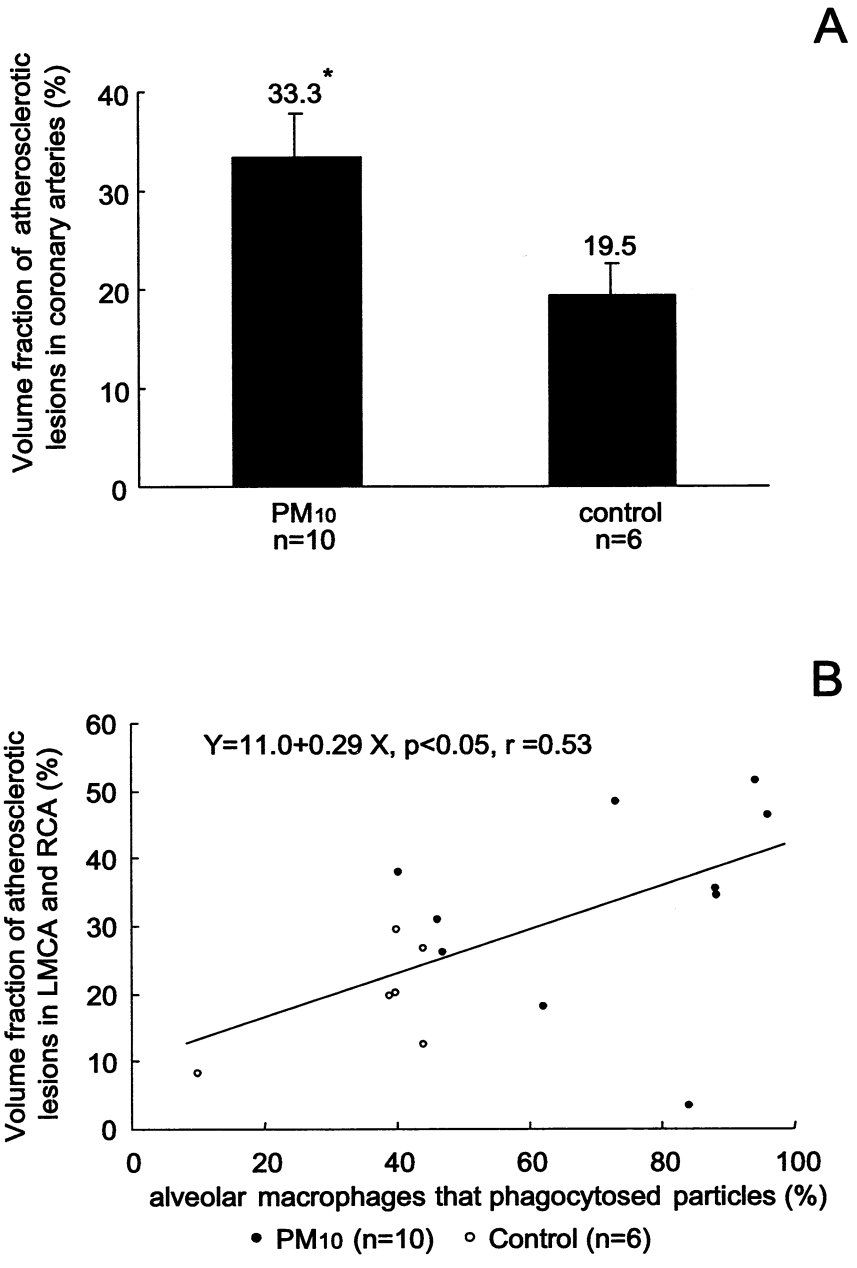

Figure 4. (A) The vol/vol of atherosclerotic lesions in the left main coronary artery (LMCA) and right coronary artery (RCA). The particulate matter <10 μm (PM_{10})-exposed group had a higher vol/vol of atherosclerotic lesions in the LMCA and RCA, as compared with the control group. Data are presented as the mean value ± SE. *$p < 0.05$, compared with control group. **(B)** The correlation between the percentage of alveolar macrophages that phagocytosed particles and the vol/vol of atherosclerotic lesions in the LMCA and RCA ($r = 0.53$, $p < 0.05$). Results were from rabbits exposed to PM_{10} for four weeks (**solid circles**; n = 10) or saline (control; **open circles**; n = 6).

the vol/vol of BrdU-positive atherosclerotic nuclei was greater in the PM_{10} group when corrected for the size of atherosclerotic lesions, as compared with that of the control group ($p < 0.01$) (Fig. 5). One rabbit in the PM_{10} group and one in the control group were excluded from the analysis of plaque components, because they did not have any atherosclerotic lesion in the LMCA nor RCA.

AORTA. The vol/vol of the aortic wall taken up by atherosclerotic lesions also correlated with the percentage of alveolar macrophages positive for particles ($r = 0.51$, $p < 0.05$). The vol/vol of the lesion taken up by extracellular lipid pools was increased in the PM_{10}-exposed group, as compared with that of the control group (3.1 ± 1.1% vs. 0.5 ± 0.1%, $p < 0.05$) (Table 1), as was the vol/vol of lipids stained with oil red O (34.2 ± 2.5% vs. 21.1 ± 2.1%, $p < 0.05$ PM_{10} vs. control), even when corrected for the vol/vol of atherosclerotic lesions in the aortic wall (11.7 ± 3.1% vs. 3.8 ± 1.2%, $p < 0.05$ [PM_{10} vs. control]). Although the vol/vol of cell nuclei was not different between the groups, the atherosclerotic lesions of the PM_{10}-exposed group showed a higher percentage of BrdU-positive nuclei, as compared with the control group (0.67 ± 0.08% vs. 0.38 ± 0.07%, $p < 0.05$), even when corrected for the

Table 1. Phenotypic Characteristics of Atherosclerotic Lesions

	Group	n	Mononuclear Cells	Foam Cells	Smooth Muscle Cells	Extracellular Matrix	Extracellular Large Lipids	Others
Coronary arteries	PM_{10}	9	2.3 ± 0.3	6.1 ± 0.6	4.2 ± 0.5*	22.7 ± 2.4*	1.1 ± 0.2*	0.2 ± 0.1
	Control	5	2.6 ± 0.6	5.3 ± 0.9	2.0 ± 0.2	11.0 ± 1.3	0.4 ± 0.1	0.2 ± 0.0
Aorta	PM_{10}	9	1.4 ± 0.4	10.0 ± 2.7	6.3 ± 1.7	13.6 ± 3.7	3.1 ± 1.1†	0.2 ± 0.1
	Control	6	0.4 ± 0.1	4.1 ± 1.3	5.0 ± 2.0	7.2 ± 2.0	0.5 ± 0.2	0.2 ± 0.1

*$p < 0.01$; †$p < 0.05$. Data are presented as the mean value ± SE (%) of each component present in the lesions. Others include fibrous connective tissue and artifacts. PM_{10} = particulate matter <10 μm.

vol/vol of atherosclerotic lesions in the aortic wall (1.63 ± 0.33 vs. 0.61 ± 0.20 × 10^{-2}, $p < 0.05$) (Fig. 5).

DISCUSSION

This study shows that repeated exposure to urban air particulates (i.e., PM_{10}) caused a systemic inflammatory response, including bone marrow stimulation, and was associated with progression of the atherosclerotic process in the coronary arteries and aorta. The extent of the atherosclerotic process at both of these sites correlated with the extent of PM_{10} phagocytosed by alveolar macrophages in the lung. These results support the hypothesis that exposure to PM_{10} causes vascular changes, and we speculate that these changes contribute to the increase in cardiovascular morbidity and mortality associated with exposure to particulate air pollution (1,2).

Systemic inflammatory response after PM_{10} exposure. Seaton et al. (23) proposed the hypothesis that inhalation of fine particles provoke a low-grade inflammatory response in the lung and changes in blood coagulability that increase cardiovascular mortality. The concept that particles deposited in the lung can lead to a systemic or acute-phase response has been supported by previous experiments (24), where supernatants from alveolar macrophages fed fine particles in vitro caused marrow stimulation, and more recently by experiments (11) showing that instillation of PM_{10} into the lungs of rabbits stimulated the release of cells from the bone marrow. Tan et al. (25) showed that this was relevant to humans by demonstrating leukocytosis and increased circulating band cells in military recruits intensely exposed to the forest fires of Southeast Asia in the summer of 1997. The evidence that inflammation is an integral component of atherogenesis (6) suggests that the systemic response induced by exposure to PM_{10} might accelerate the atherosclerotic process and contribute to the cardiovascular morbidity and mortality associated with exposure to particulate matter air pollution.

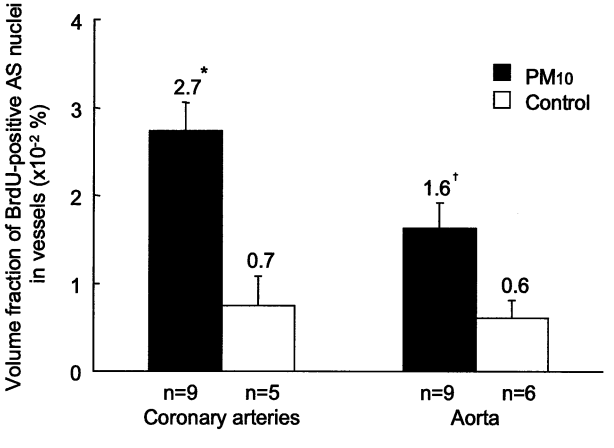

Figure 5. The volume fraction (vol/vol) of 5′-bromo-2′-deoxyuridine (BrdU)-positive atherosclerotic nuclei in the vessel, corrected for the amount of atherosclerotic (AS) lesions. The particulate matter <10 μm (PM_{10}) group showed a higher vol/vol of BrdU-positive atherosclerotic nuclei in the coronary arteries and aorta. *$p < 0.01$ and †$p < 0.05$, compared with control group.

Exposure to PM_{10}. The method of exposure we used in this study was developed in preliminary experiments that showed that ~20% of the dose deposited above the larynx was aspirated into the lung, and that ~4% of this dose reached the alveolar surface. The alveolar surface area of a 3.2-kg rabbit is ~7.0 m^2, and the alveolar exposure was 2.9 ng/cm^2 for each dose, or 23.2 ng/cm^2 over the experimental period. This is less than the estimated human exposure of 35.1 ng/cm^2 that results from 90 days of exposure at the average concentration in six U.S. cities (2).

Atherosclerosis in the coronary arteries and aorta. Our qualitative analysis of the atherosclerotic process showed that PM_{10} exposure was associated with progression to more advanced phenotypes of coronary lesions (more type III, IV and V lesions and a higher atherosclerotic score in the LMCA), with quantitative evidence of increased plaque size (Fig. 4A). This was supported by the qualitative histologic observations showing more extensive atherosclerosis in the aorta, a quantitative increase in the vol/vol of the lesion taken up by extracellular lipids, total lipids stained by oil red O and increased cell turnover. Other investigators (8) have shown that similar changes in human atherosclerotic plaques indicate lesions that are more vulnerable to rupture, with hemorrhage into the plaque, thrombotic occlusion of the artery and development of an acute coronary syndrome (26).

Cytokines and atherosclerotic lesions. Human alveolar macrophages exposed to PM_{10} in vitro produce tumor necrosis factor-alpha (TNF-alpha) in a dose-dependent manner, as well as several other cytokines, including interleukin (IL)-1-beta, IL-6 and granulocyte-macrophage colony–stimulating factor (27). Bronchial epithelial cells exposed to PM_{10} produce IL-8 and TNF-alpha (28). Tumor necrosis factor-alpha and IL-1-beta are known to upregulate the secretion of monocyte chemoattractant protein-1 (MCP-1) to endothelial cells (29) and promote the accumulation of monocytes and T lymphocytes in atherosclerotic

rotic lesions. In addition, TNF-alpha activates arterial endothelium to increase L-selectin–dependent monocyte adhesion, which is a key pathogenic event in atherosclerogenesis (30). Interleukin-6 activates the hematopoietic system to release leukocytes and platelets into the circulation (31) and stimulates the production of acute-phase proteins, such as fibrinogen and C-reactive protein (32). Schratzberger et al. (33) showed that the interaction between PMNs and the endothelium causes the release of functionally active MCP-1, which assists in the recruitment of both monocytes and T lymphocytes into atherosclerotic lesions. We postulate that the leukocytosis (34), high levels of C-reactive protein (35) and fibrinogen (34,35) that have been implicated in the pathogenesis of atherosclerosis and induction of acute coronary events can be initiated by cytokines released by alveolar macrophages when they phagocytose atmospheric particulates deposited in the lung.

Acknowledgments
The authors thank Dean English, Jennifer Hards and Kenny Lee for their technical support.

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