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Metal Effects of Urban Air Particulates on Cytokine Production and DNA Damage

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ABSTRACT *: Epidemiologic studies have demonstrated an association between short-term exposure to particulate air pollutants and increased mortality. However, the biological mechanisms underlying these associations have not been fully established and also the chemical and physical characteristics of the pollutant particles are not well understood. The metal constituents of air pollutant particles and their bioavailability are considered to play an important role as possible mediators of particle-induced airway injury and inflammation. Sprague-Dawley rat alveolar macrophage cells (NR8383) were exposed to airborne and acid-leached particulate matter (PM). Titanium oxide and nickel subsulfide were used as negative and positive controls. Particle-induced reactive oxygen species formation in cells was detected using the fluorescent probe 2',7'-dichlorofluorescin diacetate. Expressions of TNF-α and IL-6 were measured by enzyme-linked immunosorbent assay, and PM-induced DNA double-strand breaks were determined with λDNA/Hind III marker. Metals associated with air pollutant particles mediated intracellular oxidant production in alveolar macrophages, and the cytotoxicity and proinflammatory cytokine production induced by PM were associated with oxidative stress. The oxidants produced by air pollutant particles also are likely to induce DNA double-strand breaks. Our findings in alveolar macrophage cells exposed to PM and acid-leached PM support the hypothesis that metal components in urban air pollutants and their bioavailabilities might play an important role in the induction of the adverse health effects.*

Key Words *: Alveolar macrophage, Particulates, Reactive oxygen species, TNF-α, IL-6, DNA double-strand breaks*

I. INTRODUCTION

Epidemiologic studies using time-series analysis have demonstrated an association between short-term exposure to particulate air pollution and increased mortality after adjusting for confounders (Pope *et al.*, 1992; Pope *et al.*, 1995). The production of free radicals induced by inhalable particles might cause an inflammatory response, contributing to enhance blood coagulation, and increasing the risk of acute circulatory deaths, especially in persons with pre-existing circulatory disease (Seaton *et al.*, 1995; Bouthillier *et al.*, 1998). Epidemiologic studies also have yielded evidence, which suggest that exposure to high levels of urban air pollution may result in an increased lung cancer risk (Georgiadis and Kyrtopoulos, 1999). However, the biological mechanisms for the associations have not been fully established and the characteristics of the pollutant particles are not understood. Because associations between particle concentrations and health effects have been found consistently in locations with widely varying emission sources, causative chemical species may promote common pathways and mechanisms.

Transient metals of air pollutant particles and their bioavailabilities are considered to play an important role in the health effects as possible mediators of particle-induced airway injury and inflammation (Carter *et al.*, 1997; Kennedy *et al.*, 1998; Dye *et al.*, 1999; Monn and Becker, 1999). The adverse health effects of particulate air pollutants may be related to inflammation mediated by metal-induced oxidative stress. Therefore, the investigation of the role of intracellular oxidative stress in the activation of the proinflamma-

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tory capacities of alveolar macrophages (AM) is vital to obtain an understanding of the pathogenesis of particulate pollutants.

Inhaled particles stimulate the recruitment and subsequent activation of inflammatory cells through the activation of lung macrophages or epithelial cells to release tumor necrosis factor-α (TNF-α) or other cytokines, such as interleukin-6 (IL-6) (Driscoll *et al.*, 1997). Cytokines in turn regulate inflammation by interacting with membrane receptors to control cellular processes, such as proliferation, differentiation, and secretion (Driscoll *et al.*, 1997).

Because lung AM cells are on the first line of defense against respirable particles, AM cells were frequently used to assess particle effects. Various studies that characterize the release of TNF-α by AMs have indicated that some particles, such as quartz, crocidolite, chrysotile, and coal dust activate AMs TNF-α production via reactive oxygen formation (Dubois *et al.*, 1989; Gosset *et al.*, 1991; Zhang *et al.*, 1993; Simeonova and Luster, 1995). TNF-α subsequently contributes to the particle-associated inflammation through the recruitment of inflammatory cells. However, TNF-α does not directly induce inflammation, instead it activates several other cytokines to promote chemotaxis (Driscoll *et al.*, 1997).

We hypothesize that oxidative stress induced by the metals of urban particulate matters may be one mechanism responsible for inflammatory reaction after exposure to air pollutant particles. We also postulate that particle-mediated oxidative stress plays a role in the mechanisms which cause DNA damage. These inflammatory particle effects and DNA damage may cause an increase of acute mortality and cancer as has been shown in several epidemiologic studies upon the effects of air pollutant particles.

In the present study, we tested three related hypotheses; a) that metals in urban air particulates will mediate oxidative stress in alveolar macrophage; b) that particle-mediated oxidative stress will induce cytokine production in alveolar macrophage; and c) that metals in air pollutant particles will mediate DNA double-strand breaks.

To test our hypothesis, we exposed AMs to particulate matter (PM) and acid-leached PM, and subsequently measured levels of intracellular oxidant and the expression of TNF-α and IL-6. We also measured PM-induced DNA double-strand breaks using λDNA/*Hin*dIII marker. We found that metals of PM are responsible for inducing cytotoxicity, cytokine production, and DNA damage through the mediation of oxidative stress.

II. MATERIALS AND METHODS

1. Metal analysis and particle preparation

Stock concentrations of Urban Particulate Matter (Standard Reference Material 1648; NIST, Gaithersburg, MD) were prepared in phosphate buffered saline and sonicated for 1 min in an ultrasonic bath immediately before use. To make metal-extracted particles by acid-leaching, one gram of PM was dissolved in 10 m*l* of (1 : 1 v/v) 5.7 N HCl and 13.4 N HNO_3, filtered through a 47 mm membrane filter paper, then diluted to 50 m*l* with 0.1 N HNO_3 and stored at -80°C until analysis. The residue was dried on a hotplate for 4 hr at 110°C and then collected. These acid-leached PM samples were used and treated in the same way as PM. The eluate was analyzed for Al, Fe, Pb, Zn, As, Cd, Cr, Cu, Mn, Ni, Se and V by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Seiko SPS 1200A, Chiba, Japan). Titanium oxide (TiO_2) (Aldrich Chemical Co., Milwaukee, WI) and nickel subsulfide (Ni_3S_2) (INCO Canada, Ltd., Toronto, Ontario) were used as negative and positive controls, respectively.

2. NR8383 cell cultures

Sprague-Dawley rat alveolar macrophage cell line NR8383 (CRL-2192; American Type Culture Collection, Manassas, VA) were maintained in Ham's F12K supplemented with 15% fetal bovine serum, 100 units/m*l* penicillin, 100 μg/m*l* streptomycin and 0.25 μg/m*l* amphotericin B (Gibco BRL, Grand Island, NY). The cells were grown at 37°C in tissue culture flasks in a humidified incubator (Forma Scientific, Marietta, OH) supplied with 5% CO_2.

3. Cytotoxicity

The effects of PM or acid-leached PM on cell proliferation were examined in the NR8383 cells. Cells

were seeded in 96-well microtiter plates (10^3 cells/well) in 90 μl of culture medium and grown for 4 hr. Ten microliters of four different concentrations of PM or acid-leached PM (25, 50, 100 and 200 μg/ml) were added in triplicate to the NR8383 cells, and titanium oxide 25 μg/ml or 25 μg/ml Ni_3S_2 was used as negative or positive control, respectively. Cytotoxicity was quantified by measuring the release of formazan dye. After 24 hr of incubation, cells were incubated with an XTT labeling mixture (Roche Molecular Biochemicals, Mannheim, Germany) for 24 hr. Tetrazolium salt XTT was then cleaved to formazan by the metabolic action of the mitochondria. Formazan dye formation was quantified using an ELx 808 microplate reader (Bio-Tek Instruments, Cortland, NY) at 450 nm.

4. Detection of ROS formation

Particle-induced reactive oxygen species (ROS) formation in the NR8383 cells was detected using a fluorescent probe 2',7'-dichlorofluorescin diacetate (DCFH-DA) (Acros Organics, Fair Lawn, NJ). Cells were collected from the culture flasks using a cell scraper, and 5×10^4 cells/3 ml were seeded per well in 6-well multi-dishes and incubated for 4 hr. Four concentrations of PM or acid-leached PM (25, 50, 100 and 200 μg/ml) were added simultaneously to the cells with DCFH-DA and incubated at 37°C for 24 hr. At the same time, the negative or positive control samples were tested in duplicate (25 μg/ml TiO_2 and 25 μg/ml Ni_3S_2). The fluorescence intensity was monitored using a Bio-Tek FL 500 fluorometer at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

5. Determination of TNF-α and IL-6

Particle-induced TNF-α and IL-6 concentrations in the NR8383 cells were carried out using enzyme-linked immunosorbent assay (ELISA) kits, purchased from R&D Systems Inc. (Minneapolis, MN). Briefly, the NR8383 cells were collected using a cell scraper, and 5×10^5 cells were inoculated per well in 24-well multi-dishes and grown for 4 hr. The cells were then exposed to four different concentrations of PM or acid-leached PM (25, 50, 100 and 200 μg/ml), 25 μg/ml TiO_2 or 25 μg/ml Ni_3S_2. After 24 or 48 hr of incubation, the supernatant was harvested from each well separately. Standards and supernatant samples were added to the microtiter plates, which were pre-coated with monoclonal TNF-α or IL-6 antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat TNF-α or IL-6 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. The reaction was terminated by adding stop solution, and the concentrations of TNF-α and IL-6 determined using a microplate reader at 450 nm. The inhibitory effects of dimethyl sulphoxide (DMSO) on TNF-α or IL-6 secretion were also evaluated.

6. Determination of double-strand breaks

DNA double-strand breaks were performed in reaction mixtures containing 50 μg/ml of λDNA/*Hin*dIII marker (Promega, Madison, WI) and 5 mM of H_2O_2 with 50 mM Tris-HCl (pH 7.4) in the presence of 1 mg/ml TiO_2, Ni_3S_2, PM or acid-leached PM. The reaction mixtures were incubated for 24 hr at 37°C and reactions terminated by adding loading dye. They were then analyzed using 0.7% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) at 40 V for 4 hr. Gels were stained with aqueous solutions of ethidium bromide (0.5 μg/ml) and photographed with Polaroid film under UV light. The inhibitory effects of DMSO on PM-induced DNA double-strand breaks were also evaluated.

7. Statistical analysis

Results were analyzed statistically using ANOVA for grouped data to determine the differences in cytotoxicity, intracellular oxidation, and cytokine production among the experimental groups.

III. RESULTS

1. Particle analysis

Using scanning electron microscopic finding, PM was found to be composed of variously sized spherical materials. Examination of the acid-leached PM also showed similar spheres (Fig. 1). As expected,

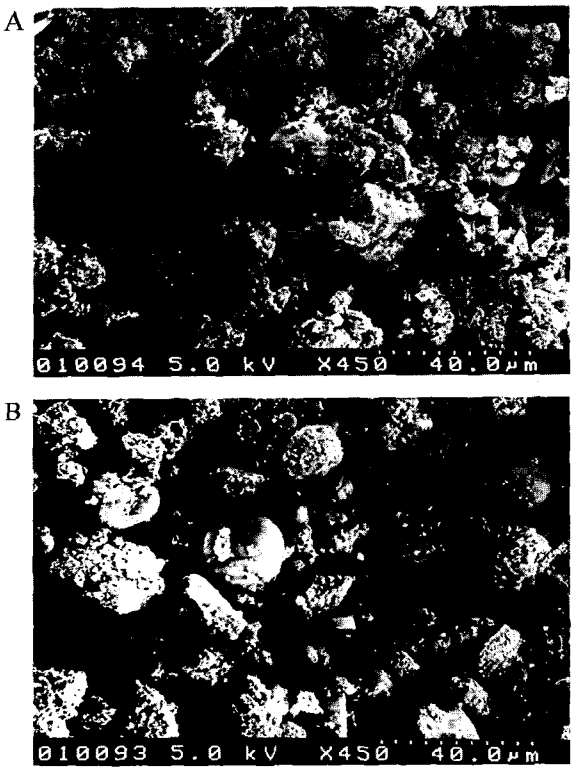

Fig. 1. Scanning electron microscopic findings of (A) particulate matter and (B) acid-leached particulate matter (×450).

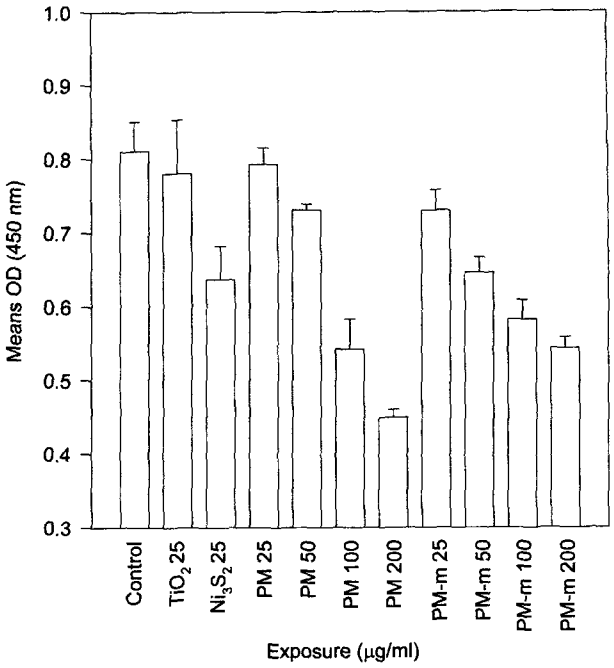

Fig. 2. Formation of formazan dye in NR8383 cells exposed to particulate matter (PM) or acid-leached particulate matter (PM-m) after incubation for 24 hr with tetrazolium salt XTT. The results shown are mean±SD for each treatment (n = 3).

acid-leached PM contained much lower metal levels than the original PM. Table 1 details the metal compositions of PM before and after acid-leaching.

2. Cytotoxicity

To determine whether metals in the PM are cytotoxic to the NR8383 cells, we compared the cytotoxicity induced by exposure to PM and acid-leached PM. Formazan dye formed in PM-exposed cells decreased significantly in a concentration-dependent manner. Cytotoxicity was more prominent in PM-exposed cells at the concentrations of 100 and 200 µg/ml, whereas the dye formation was higher at the concentrations of 25 and 50 µg/ml in acid-leached PM-exposed cells. Formazan dye formation in TiO_2-exposed cells did not decrease (0.78±0.07) compared the controls (0.81±

Table 1. Analysis of constituent metal elements of urban particulate matter before and after acid-leaching

Metals	Certified values of Particulate Matter (SRM 1648)	Concentrations of acid-leachate of Particulate Matter	Concentrations of leachate after second acid treatment of acid-leached Particulate Matter
Aluminium	34.200	28.200	1.000
Iron	39.100	34.200	2.600
Lead	6.550	6.000	0.016
Zinc	4.760	4.000	0.028
Arsenic	0.115	0.086	Trace
Cadmium	0.075	0.047	Trace
Chromium	0.403	0.248	Trace
Copper	0.609	0.394	Trace
Manganese	0.786	0.472	0.012
Nickel	0.082	0.030	Trace
Selenium	0.027	0.017	Trace
Vanadium	0.127	0.063	Trace

All values are expressed in mg/g particles.

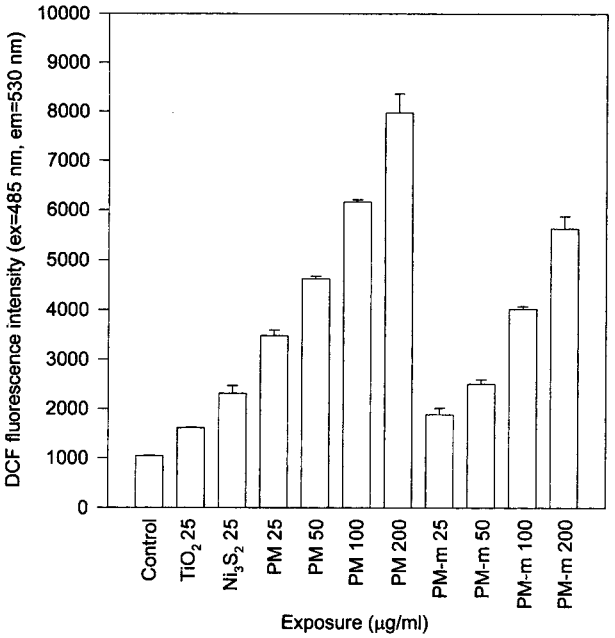

Fig. 3. Formation of reactive oxygen species in NR8383 cells exposed to particulate matter (PM) or acid-leached particulate matter (PM-m) for 24 hr. The results shown are mean±SD for each treatment (n = 2).

0.04), whereas a significant cytotoxicity was observed in Ni_3S_2-exposed NR8383 cells (0.64±0.05) (Fig. 2).

3. Intracellular oxidation

To assess the PM-mediated oxidative responses in the NR8383 cells, dichlorofluorescein (DCF) fluorescence was used for measurement of oxidant. We compared changes in DCF fluorescence induced by the exposure of cells to PM and acid-leached PM.

Figure 3 shows intracellular oxidant production by the NR8383 cells after exposure to different concentrations of PM or acid-leached PM. The DCF fluorescence intensity after 24 hr in both PM and acid-leached PM treated cells increased in a concentration-dependent manner. However, PM showed greater oxidant-producing capacity in the NR8383 cells than acid-leached PM. Ni_3S_2 (25 μg/ml) also slightly increased oxidant production as determined by dichlorofluorescin (DCFH) oxidation. In contrast, TiO_2 exposure induced only minor increases in oxidant production.

4. Cytokine production

To assess the contribution of the NR8383 cells to the development of pulmonary inflammatory response after exposure to PM, we measured the production of TNF-α and IL-6 by ELISA after 24 hr or 48 hr of exposure. The results obtained TNF-α and IL-6 production significantly increased at 24 and 48 hr after exposure, respectively. TNF-α production was higher at 24 hr than at 48 hr, whereas, IL-6 production was higher at 48 hr than at 24 hr (Table 2). As shown in Table 2 and Fig. 4, PM increased the TNF-α and IL-6 production in a concentration-dependent manner. In contrast, acid-leached PM did not show the production of TNF-α and IL-6.

To assess whether oxidative stress was involved in cytokine production, NR8383 cells were exposed to DMSO and various particles at 5% volume in medium. Figure 4A shows an inhibition of TNF-α release by the addition of the antioxidant DMSO to cells in which

Table 2. TNF-α and IL-6 release by NR8383 cells exposed to particulate matter or acid-leached particulate matter for 24 hr and 48 hr

Exposure (μg/ml)	TNF-α (pg/ml)		IL-6 (pg/ml)	
	24 hr	48 hr	24 hr	48 hr
Control	1.56±0.00	2.10±0.93	14.26±0.00	18.15±0.84
TiO_2 25	4.25±0.94	7.53±0.95	15.23±0.84	17.18±0.00
Ni_3S_2 25	4.25±0.94	7.53±0.95	20.10±0.00	27.82±0.83
PM 25	85.96±1.00	62.93±1.00	138.09±0.80	155.63±2.11
PM 50	148.73±1.01	103.32±1.00	289.66±2.73	323.24±0.79
PM 100	293.57±1.02	207.36±1.77	541.10±6.16	666.54±3.08
PM 200	871.22±3.78	644.01±1.81	855.22±2.76	1064.91±2.02
PM-m 25	4.25±0.94	7.53±0.95	25.41±0.83	22.03±0.84
PM-m 50	8.63±0.95	11.94±0.96	24.45±0.00	26.37±0.84
PM-m 100	16.39±1.68	14.16±0.96	29.26±0.83	37.40±0.00
PM-m 200	27.62±0.98	33.27±1.70	39.78±0.83	52.13±1.64

Abbreviations: PM, particulate matter; PM-m, acid-leached particulate matter. Data shown are mean±SD (n = 3).

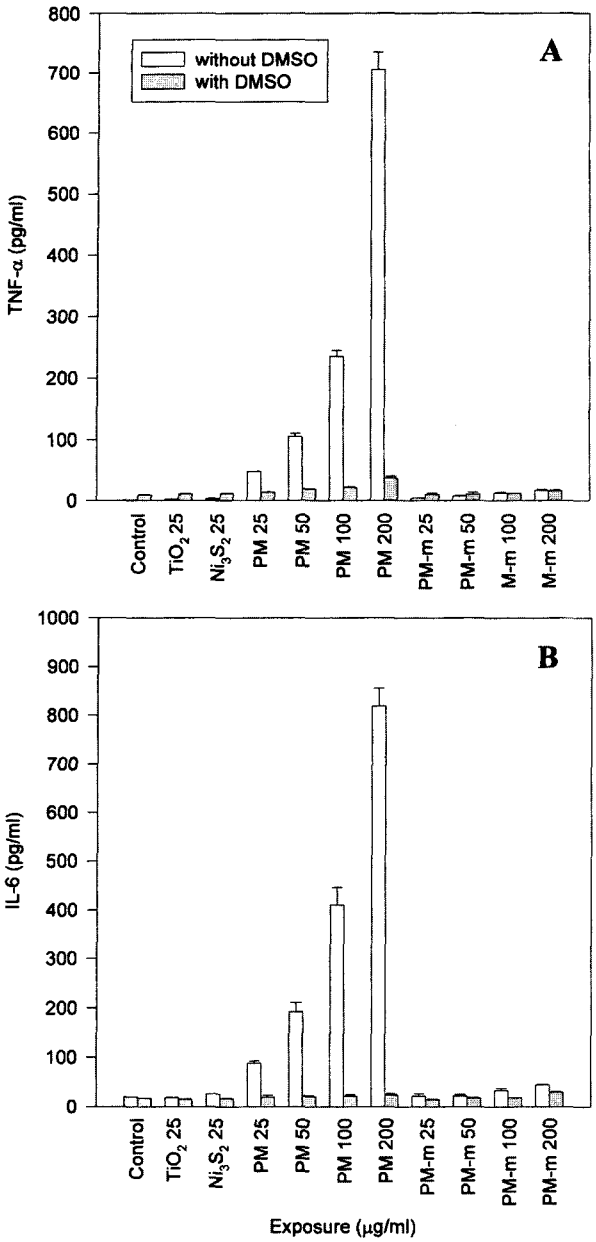

Fig. 4. Effect of antioxidant on particulate matter on the TNF-α (A) and IL-6 (B) production in NR8383 cells. Cells were treated with particulate matter (PM) or acid-leached particulate matter (PM-m) for 24 hr with or without DMSO (5% v/v). The results shown are mean±SD for each treatment (n = 3).

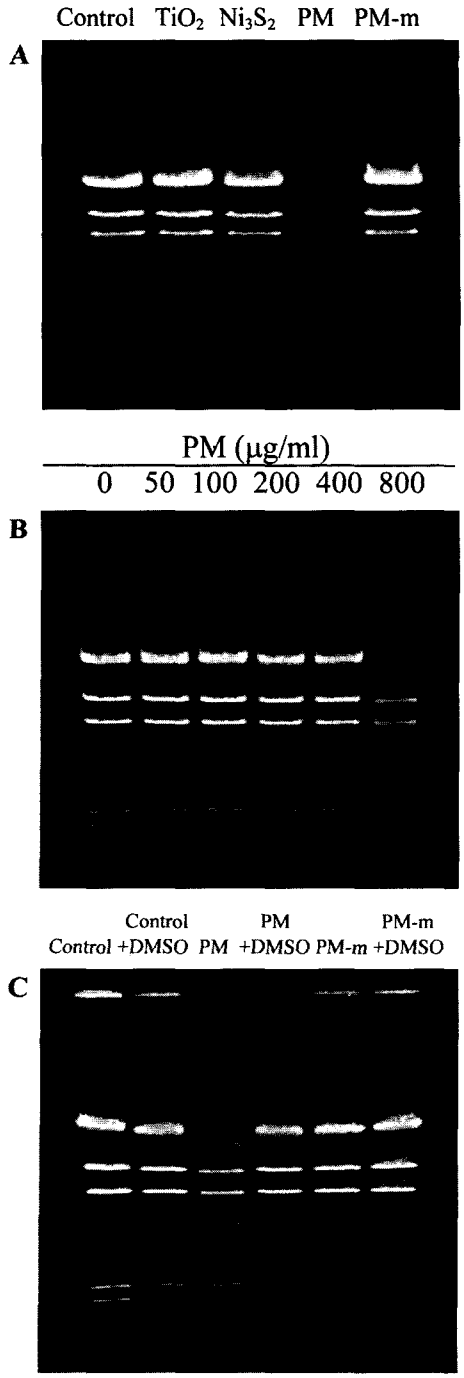

Fig. 5. Agarose gel of DNA double-strand breaks in λDNA/*Hin*dIII markers at 24 hr treatment with 1 mg/ml particulate matter (PM) or acid-leached particulate matter (PM-m) (A). The dose-responsive pattern of DNA double-strand breaks in PM-treated λDNA/*Hin*dIII markers (B). The inhibitory effects of DMSO (5%, v/v) on PM (1 mg/ml)-induced DNA double-strand breaks (C).

PM and acid-leached PM had also been treated. Figure 4B also indicates that DMSO significantly inhibits PM-mediated IL-6 production.

5. DNA double-strand breaks

PM at 1 mg/ml caused DNA double-strand breaks to the λDNA/*Hin*dIII marker, whereas acid-leached PM, TiO_2 and Ni_3S_2 did not cause DNA breaks at the same dosage (Fig. 5A). To investigate the concentra-

tion-dependence of DNA double-strand breaks, we treated λDNA/*Hin*dIII marker with 50, 100, 200, 400, and 800 μg/ml of PM. PM started to cause DNA double-strand breaks at 100 μg/ml, and this increased with increasing dose (Fig. 5B). The addition of 5% (v/v) DMSO to PM effectively ameliorated DNA double-strand breaks (Fig. 5C).

IV. DISCUSSION

Epidemiologic studies have repeatedly demonstrated statistically meaningful associations between PM and morbidity or mortality, especially in the susceptible population (Pope *et al.*, 1995). One hypothesis is that a high particle burden on AMs imposed during episodes of high concentrations of particulates in the air may cause inflammatory changes, precipitating increases in morbidity and mortality in the elderly (Becker *et al.*, 1996).

We studied air particles collected in urban locations for three important reasons. First, increased particulate air pollution has been known to be associated with excess morbidity and mortality. Second, some studies have suggested that metals contribute to the biologic effects of particulate air pollution. Third, we believed that oxidative stress caused by the phagocytosis of particulate pollutants may be a prerequisite of subsequent health effects, such as inflammatory cytokine production and DNA damage. Our results show that metals of PM are largely responsible for inducing cytotoxicity, cytokine production, and DNA damage via the mediation of oxidative stress.

Urban air particulates are complex mixture of metals, sulfates, nitrates, and organic materials, including polycyclic aromatic hydrocarbons, however, the properties responsible for its effects upon health remain unresolved (May *et al.*, 1992; Becker *et al.*, 1996; Kennedy *et al.*, 1998). Metal components adsorbed to urban air pollution particulates are known to significantly contribute to the ability of particles to cause oxidant stress and cytokine production in AM cells (Goldsmith *et al.*, 1998). A number of studies have also suggested that the toxicity of particulates is associated with metals (Ghio *et al.*, 1996), such as iron (Dusseldorp *et al.*, 1995; Simeonova and Luster, 1995), vanadium (Samet *et al.*, 1997) or copper (Kennedy *et al.*, 1998).

The objective of the present study was to compare toxicity, intracellular oxidation, and cytokine release in AM cells and the DNA damage conferred by PM with those of acid-leached PM. Urban air particulates collected in St Louis were used for this study. These are Standard Reference Materials (SRM 1648) available from the National Institute of Standards and Technology. The particles examined in this study also included TiO_2 and Ni_3S_2 as negative and positive controls, respectively.

AM cells are believed to be involved in the particle-associated response (Becker *et al.*, 1996). AMs respond to particulate stimuli by increasing its oxidative metabolism, as characterized by elevated oxygen consumption and the production of reactive oxygen species (Imrich *et al.*, 1999). Intracellular oxidant production in hamster AM cells was measured upon exposure to concentrated ambient particulates, residual oil fly ash, and their water-soluble and particulate fractions (Goldsmith *et al.*, 1998).

The fluorescent probes of intracellular oxidant production are commonly used for ROS investigations. In the present study, we used the well-established oxidant probe DCFH-DA, which can easily enter the cell and is hydrolyzed to nonfluorescent DCFH. This in turn is oxidized to the fluorescent reporter DCF in the presence of oxidants, and DCF fluorescence intensity is believed to reflect the amount of intracellular oxidant production (Kobzik *et al.*, 1990; LeBel *et al.*, 1992; Goldsmith *et al.*, 1998; Stringer and Kobzik, 1998).

Exposure to particulate air pollution has been proposed to result in the development of lung inflammation through the generation of oxygen radicals (Dye *et al.*, 1999). Intracellular oxidant stress is known to regulate genes involved in the production of inflammatory mediators (Janssen *et al.*, 1995; Timblin *et al.*, 1995), which contribute to the signaling, and the activation and subsequent release of inflammatory cytokines, mediating the deleterious health effects associated with PM (DeForge *et al.*, 1993; Kobzik *et al.*, 1993; Barrett *et al.*, 1999).

Because AMs have the capacity to phagocytose particles in the lungs and secrete mediators responsible for inflammation into the lung (Carter *et al.*, 1997), it has long been believed to be responsible for the release of mediators pertinent to the inflammatory

influx that occurs after particle exposure. Lung epithelial cells primed by inflammatory mediators, possibly from AMs, can also show enhanced cytokine production after exposure to air pollution particulates (Stringer and Kobzik, 1998). Various kinds of particles, such as residual oil fly ash or asbestos fibers were reported to induce inflammatory cytokines in AMs or lung epithelial cells (Simeonova and Luster, 1995; Carter *et al.*, 1997; Goldsmith *et al.*, 1998). An *in vivo* experiment also demonstrated that aqueous extracts of total suspended particulates induced cytokine-mediated inflammation when instilled into the airways of rats (Kennedy *et al.*, 1998).

Our results show that PM induces the release of inflammatory cytokine, such as, TNF-α and IL-6 from AM, whereas the inert TiO_2 and acid-leached PM induces neither. We also confirmed that the proinflammatory cytokines, TNF-α and IL-6 increased in a concentration-dependent manner after exposure to PM, and that the peak TNF-α release preceded that of IL-6, which result is compatible with the TNF-α-mediated cytokine networking in particle-induced inflammatory cell recruitment (Driscoll *et al.*, 1997). Blanc *et al.* (1993) also demonstrated that the increase of TNF-α was followed by increased IL-6 in BAL fluid.

Cytokine production can be inhibited by deferoxamine, a metal chelator, and dimethylthiourea (DMTU), a free radical scavenger (Carter *et al.*, 1997), which also indicate that cytokine release is mediated by metal-induced free radicals. The ability of asbestos to stimulate AM to release TNF-α can be abolished by iron chelation on the fibres (Simeonova and Luster, 1995), showing the importance of oxidative stress in cytokine production induced by metal. We also found that TNF-α and IL-6 releases were inhibited by DMSO, a well-established free radical scavenger in mammalian cells, which supports the hypothesis that intracellular oxidant production contributes to inflammatory cytokine release in macrophages.

As we focused on the metal components of PM, to evaluate whether metals adsorbed on PM cause health effects associated with PM exposure, it is important to understand the pharmacokinetics of particle-bound metals (Dye *et al.*, 1999). The low pH of macrophage lysosomes may favor the mobilization of metals in lung (Gilmour *et al.*, 1996). In the present work, acid-treatment was used to prepare metal-leached particles for comparison with metal-adsorbed particles. However, the metal contents of the acid leachate indicated that some of the metals remained in the PM after the leaching process. In addition, the treatment could have affected not only the particle chemistry by removing metals from the particles, but have also changed factors, such as surface charge, surface potential, and crystallinity, thus influencing AM responses (Prahalad *et al.*, 1999). Therefore, we cannot attribute all the differences of cytotoxicity, intracellular oxidation, and cytokine release to the metals, even though a large proportion of these effects are believed to be due to the metal components of PM. The similar particle images obtained by scanning electron microscopy for acid-leached and original PM support the notion that any difference is unlikely to be due to a change in physical characteristics.

Some studies have used deferoxamine, as a metal chelator, to differentiate metal-induced free radical formation from other particle effects (Gilmour *et al.*, 1996; Carter *et al.*, 1997). However, the use of deferoxamine presents a problem in terms of evaluating the metal-induced free radical effect, because deferoxamine is known to have free radical scavenging properties in addition to its ability to act as a chelator (Halliwell and Gutteridge, 1986), and therefore, particulate-induced AM oxidant production can be inhibited by deferoxamine acting in two ways. In addition, deferoxamine has much greater affinity for iron than for other metals (Goldsmith *et al.*, 1998). Even though iron is the principal metal associated with oxidant production, it is also possible that other metal components of the PM contribute to oxidant production. In the present study, we used acid-leached PM instead of deferoxamine, therefore, any inhibition of oxidant production can be attributed to decreases not only in the iron content, but also in the general metal content of the particulates.

Particulates from vehicle exhausts and asbestos fibers are known to induce oxidative DNA damage, with the mediation of ROS (Nagashima *et al.*, 1995; Kuo *et al.*, 1998; Xu *et al.*, 1999). Gilmour *et al.* (1996) showed that PM_{10} collected in Edinburgh induced DNA damage due to the generation of oxidant radicals. Our results also indicated that PM caused double-strand DNA breaks via oxidant formation. The use of DMSO to scavenge oxidants pro-

duced by PM resulted in the amelioration of DNA damage, indicating that free radicals from metal components of PM were responsible for DNA double-strand breaks. Because DNA double-strand breaks are associated with chromosome aberration, cell transformation, and cancer (Bryant, 1984; Bryant and Riches, 1989; Toyokuni and Sagripanti, 1993), it is biologically plausible to relate particulate pollutants to the increased incidence of cancer.

Endotoxins from gram-negative bacteria are potent natural stimulators of macrophage cytokine production. Gram-negative bacteria, molds, and fungi are likely to be a part of the PM_{10} pollution in both the outdoor and indoor environments (Teeuw *et al.*, 1994; Monn and Becker, 1999). However, particulate-associated AM cytokine production cannot be attributed to endotoxin contamination in our study because DMSO effectively obliterated TNF-α and IL-6 release.

In summary, our findings in AM cells exposed to PM or acid-treated PM support the hypothesis that metal components and their bioavailabilities might play a central role in the adverse effects induced by PM exposure. Metals associated with air pollutant particles mediate intracellular oxidant production in AMs, and the cytotoxic effects and proinflammatory cytokine production induced by PM were found to be associated with oxidative stress in AMs, even though the *in vitro* effect of PM is not fully representative of its likely *in vivo* influence. Oxidants produced by air pollutant particles were also found to be likely to induce DNA double-strand breaks.

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