EFFECTS OF DIESEL EXHAUST PARTICLES (DEP), CARBON BLACK, AND SILICA ON MACROPHAGE RESPONSES TO LIPOPOLYSACCHARIDE: EVIDENCE OF DEP SUPPRESSION OF MACROPHAGE ACTIVITY

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The effects of diesel exhaust particle (DEP) exposure on alveolar macrophage (AM) response to ex vivo and in vivo lipopolysaccharide (LPS) challenge were determined by monitoring LPS-stimulated production of interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α). The roles of the insoluble particulate and the organic compounds of DEP in altering pulmonary responses were evaluated by comparing the DEP-induced pulmonary responses to those of carbon black (CB), a carbonaceous particle with few adsorbed organic compounds, or to silica, a known pneumotoxic dust. Male Sprague-Dawley rats were exposed to a single intratracheal dose (5 or 35 mg/kg body weight) of DEP, CB, or silica, or to saline vehicle. Rats were sacrificed 1, 3, or 7 d postexposure. To study the responsiveness to the bacterial product LPS, AM isolated from particle-exposed rats were challenged ex vivo with LPS (0.1 μg/10^6 AM) and LPS-stimulated cytokine release was monitored. In addition, rats were exposed intratracheally to a single dose of DEP (5 mg/kg) and 3 d later exposed in vivo to 1 mg/kg LPS for 3 h prior to measurement of cytokine production by AM. DEP exposure resulted in neutrophil infiltration and elevated levels of albumin and lactate dehydrogenase (LDH) activity in the bronchoalveolar lavage fluid; these responses were not substantially different from those elicited by CB or silica exposure. AM from DEP-exposed rats showed increased spontaneous production of IL-1, but not TNF-α, while the opposite was true for CB or silica. Upon ex vivo challenge with LPS, AM from DEP-exposed rats showed a significant decrease in the secretion of TNF-α and, to a lesser extent, IL-1, compared to the sum of the DEP and LPS effects. In contrast, AM from CB- or silica-exposed rats did not

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show this decreased responsiveness to subsequent LPS challenge. This inhibitory action of DEP on LPS-stimulated AM production of IL-1 and TNF-α was further confirmed by the results obtained from rats exposed to both DEP and LPS in vivo. In summary, these results indicate that while DEP, CB, and silica all induce pulmonary inflammatory responses due to particle stimulation, only DEP suppress AM cytokine release in response to LPS stimulation. The contrasting cellular response with respect to DEP and CB exposures may be due to the presence of adsorbed organic compounds on DEP, which may contribute to the increased susceptibility of hosts to pulmonary infections after DEP exposure.

Diesel exhaust particles (DEP) are of great occupational health concern due to the extensive use of heavy-duty diesel engines, which emit 30 to 100 times more particles than do gasoline engines, in underground mining, trucking, railroad, construction, and auto repair industries. The worst-case mean exposures to DEP in underground coal mines and other metal and nonmetal mines have been reported to be about 2000 $\mu g/m^3$, with maximum measurements as high as 3650 $\mu g/m^3$ (Department of Labor, 1998). Such high exposures can put these miners at excess health risk. The potential health effects of DEP exposure may include pulmonary inflammation (Henderson et al., 1988; Nagai et al., 1996), increased susceptibility to respiratory infections (Hahon et al., 1985), fibrosis and lung cancer (Mauderly et al., 1987, 1994), and allergic asthmatic sensitization (Takano et al., 1997).

Many types of particulate matter are known to induce a macrophage respiratory burst. Studies carried out in our laboratory have shown that DEP, the DEP carbonaceous core (methanol- or CH_2Cl_2-washed DEP), and carbon black (CB) were all capable of activating alveolar macrophages (AM) to produce reactive oxygen intermediates (Dong, 1998). In contrast, the organic extracts from DEP did not induce a respiratory burst from AM (Dong, 1998), suggesting that particles alone can exert toxic effects in the lungs. Particles can also serve as carriers to deliver toxic chemicals to phagocytes through particle-induced phagocytosis. DEP has been reported to decrease AM phagocytic activity (Prasad et al., 1988; Jakab et al., 1990), and to downregulate AM generation of chemiluminescence in chronically exposed rats (Castranova et al., 1985). It has also been shown that DEP can alter the host defense mechanisms and increase the susceptibility of the lung to viral infection (Hahon et al., 1985). Such results suggest that exposure to DEP may increase the susceptibility of hosts to pulmonary infections.

The importance of macrophages in host defense against bacterial infection has been well documented (Sibille & Reynolds, 1990; Laskin & Pendino, 1995). These phagocytes are capable of releasing various proteolytic enzymes and reactive intermediates in response to inhaled microorganisms or microbial products (Beckerman et al., 1993; Boockvar et al., 1994). In addition, AM are activated to release various bioactive lipids, growth factors, and cytokines, which are important elements in initiating and regulating host defense against bacterial infections. Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), produced by AM, are necessary for the generation of a protective immune response against bacterial infec-

tions (Bancroft et al., 1989; Blanchard et al., 1988; Williams et al., 1990; Van der Meer et al., 1988). Therefore, alterations of AM release of IL-1 and TNF-α can have direct consequences on regulating host immune responses. Previously, it has been shown that DEP induce AM production of IL-1 but not TNF-α in vitro (Yang et al., 1997). However, in lipopolysaccharide (LPS)-challenged AM, DEP inhibit the LPS-stimulated production of both IL-1 and TNF-α. This effect has not been observed in methanol-washed DEP, suggesting that the inhibitory action of DEP on AM function is due to the presence of the organic compounds. Thus, while various respirable dusts, such as DEP, CB, and silica, can induce pulmonary inflammation, their effects on the ability of macrophages to secrete cytokines in response to bacterial products may be different. DEP may elicit a distinct effect on the pulmonary immune/inflammatory system due to the presence of adsorbed organic compounds and the capacity of the particles to facilitate intracellular delivery of these organic compounds to pulmonary phagocytes. Thus a suppression of AM response to bacterial stimulation by DEP may impair host responses to bacterial infection.

The effects of DEP exposure on macrophage secretion of proinflammatory cytokines and on AM responses to bacteria or bacterial products have not been fully characterized. It is hypothesized that DEP, due to the combined effects of the particulate and organic components, suppress AM response to LPS stimulation, resulting in decreased secretion of IL-1 and TNF-α, and that this effect is different from those of other respirable dusts such as CB or silica. To test this hypothesis, a comparative study of the pulmonary responses to DEP, CB, or silica exposures was conducted. The objectives were to establish, through in vivo studies, the action of DEP on macrophage production of IL-1 and TNF-α and the effect of DEP on macrophage responses to subsequent LPS stimulation. CB particles used in this study had particle size and surface area characteristics similar to those of DEP, but contained 10-fold to several hundredfold less adsorbed polycyclic aromatic hydrocarbons than DEP (Nikula et al., 1995). Comparison of the effects of CB and DEP on LPS-stimulated cytokine production should elucidate the importance of particle core versus the organic components on DEP-induced suppression of macrophage activity. Crystalline silica was used as a well-known pneumotoxic dust with chemical properties strikingly different from those of DEP. The results of this study should elucidate further the effects of DEP on the secretory functions of AM and substantiate earlier studies that suggest that DEP may weaken the pulmonary host defense system.

MATERIALS AND METHODS

Preparation of Particulate Samples

A standardized DEP sample (standard reference material 1650), representative of heavy-duty engine emissions, was purchased from the National

Institute of Standards and Technology (Gaithersburg, MD). DEP had a mass median aerodynamic diameter of approximately 0.5 μm. Carbon black was obtained from a commercial source (Elftex-12 furnace black, Cabot, Boston), with particles ranging from 0.1 to 0.6 μm in diameter. Crystalline silica (Min-U-Sil; <5 μm in diameter) was obtained from U.S. Silica Corporation (Berkeley Springs, WV). All particles were autoclaved before preparing particle suspensions. The sterilized particles were suspended in pyrogen-free sterile saline (Baxter Healthcare Corporation, Deerfield, IL). The suspensions of DEP and CB samples were sonicated for 5 min using an ultrasonic processor with micro tip (Heat System-Ultrasonics, Plainview, NY) prior to intratracheal instillation.

Animals and Treatments

Specific pathogen-free male Sprague-Dawley rats (~250 g) were purchased from Hilltop Labs (Scottsdale, PA). Rats were kept in cages upon arrival and housed in an AAALAC-approved facility maintained at 23 ± 1°C with 50% relative humidity and a 12-h light/dark cycle. Food and water were given ad libitum. Before intratracheal instillation, rats were lightly anesthetized with methohexital sodium (35 mg/kg body weight, ip; Eli Lilly Co., Indianapolis, IN), and then placed in a vertical position. Particulate suspensions were briskly injected into the trachea through a curved ball-tipped cannula (18 gauge). Rats received a single dose of either 5 or 35 mg/kg body weight of DEP, CB, or silica. Control animals received the sterile saline vehicle only. The instillation volume was 2 ml/kg body weight of rats. After instillation, the rats revived spontaneously and were returned to their cages. The intratracheal dose of 5 mg/kg of dust particles corresponds to a lung burden of about 1.25 mg, which is below the cumulative lung burden of 6.3 mg reported for a 2-yr exposure to DEP at 2–3 mg/m^3 (Heinrich et al., 1995). The intratracheal dose of 35 mg/kg body weight corresponds to a lung burden of about 8.75 mg. These doses were used to provide an evaluation of the acute pulmonary responses to moderate and high levels of dust exposure. Particle-exposed rats were sacrificed at 1, 3, or 7 d postexposure. In experiments involving in vivo LPS exposure, rats were intratracheally instilled with a single dose of either DEP (5 mg/kg) or saline vehicle. Three days later, half of the saline-treated and DEP-treated rats received a single intratracheal dose of 1 mg/kg body weight of LPS (*Escherichia coli* 026:B6, DIFCO Laboratories, Detroit, MI), and the other half of the rats received an equivalent amount of saline. These animals were sacrificed 3 h after LPS challenge.

Bronchoalveolar Lavage and Biochemical Assay of Bronchoalveolar Lavage Fluid

Rats were anesthetized with sodium pentobarbital (50 mg/kg, ip; Butler, Columbus, OH) and euthanized by exsanguination of the abdominal aorta. The trachea was cannulated and the lungs were lavaged with Ca^{2+}/Mg^{2+}-

free phosphate-buffered solution (PBS, 145 m*M* NaCl, 5 m*M* KCl, 1.9 m*M* NaH_2PO_4, 9.35 m*M* Na_2HPO_4, and 5.5 m*M* glucose; pH 7.4) at a volume of 6 ml for the first lavage and 8 ml for the subsequent lavages. A total of 80 ml of bronchoalveolar lavage fluid (BALF) was collected from each rat and centrifuged at 500 × g for 10 min at 4°C. The supernatant of the first lavage (~4 ml/rat) was separated from the others and saved for various assays. All cell pellets from an individual rat were combined and suspended in 1 ml of PBS. Aliquots of cell suspensions from each rat were taken for the determination of the total cell and differential cell counts using an electronic cell counter (Coulter Electronics, Hialeah, FL) equipped with a cell sizing unit (Lane & Mehta, 1990). Alveolar macrophages and neutrophils were determined by their unique cell diameters (Castranova et al., 1990). The remaining BAL cells were used for primary cell culture to determine AM secretion of IL-1 and TNF-α.

The albumin content and the activity of lactate dehydrogenase (LDH) in the acellular BALF from the first wash were measured using an automated Cobas Fara II analyzer (Roche Diagnostic Systems, Montclair, NJ). The albumin content was determined using Sigma Diagnostic reagents and procedures (Sigma Chemical Company, St. Louis, MO) and expressed as milligrams per milliliter BALF, whereas the LDH activity was measured by the formation of NADH and expressed as units per liter BALF using the Roche Diagnostic reagents and procedures (Roche Diagnostic Systems, Indianapolis, IN).

Primary BAL Cell Culture

The bronchoalveolar lavage (BAL) cells from each rat were suspended in RPMI-1640 culture medium (RPMI, Sigma Chemical Co.) containing 2 m*M* glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin, and 10% heat-inactivated fetal bovine serum. Aliquots of 1 ml cell suspensions from each rat, adjusted to 1×10^6 AM, were added to each well of a 12-well tissue culture plate. Macrophages were allowed to adhere to plastic plate for 60 min in a humidified incubator (37°C and 5% CO_2). The nonadherent BAL cells were then removed by rinsing the monolayers three times with RPMI media. These AM-enriched cells (Driscoll et al., 1990) were incubated (37°C and 5% CO_2) in fresh RPMI medium with or without 0.1 μg/ml of LPS (DIFCO Laboratories) for 24 h. The AM-conditioned media were collected, centrifuged (1200 × g for 4 min), and the supernates stored in aliquots at –70°C until the time for analysis of IL-1 and TNF-α activities. To ensure that the number of adherent cells was the same in various exposure samples, studies were carried out to determine the cellular protein levels after incubation. The adherent cells were treated with 0.5% Triton X100 at 37°C for 30 min and the media were collected and centrifuged. The supernates were used for protein determination according to Sigma Diagnostic reagents and procedures (Sigma Chemical Co.) using a Cobas Fara II analyzer. The results did not show a significant dif-

ference among the samples from various treatment groups (data not shown).

Determination of Cytokines

The activity of IL-1 was determined based on its ability to act as a comitogen for murine thymocytes (Kang et al., 1992). Briefly, mouse thymocytes were suspended in RPMI-1640 medium with 2 m*M* glutamine, 10% heat-inactivated fetal calf serum, 100 unit/ml penicillin, 100 μg/ml streptomycin, and 2×10^{-5} *M* mercaptoethanol. Aliquots containing 1×10^6 thymocytes were added to each well of a 96-well microplate and cultured with AM-conditioned media at 37°C in 5% CO_2 for 50 h and pulsed with 1 μCi/well [^{3}H]thymidine (specific activity = 2.0 Ci/mmol, Dupont NEN Products, Boston) during the last 20 h of incubation. The cells were collected and the uptake of [^{3}H]thymidine was determined by liquid scintillation counting. The relative IL-1-like activity in samples was calculated as disintegration per minute (dpm) per 10^5 AM.

A murine fibroblast cell line, L929 (American Cell Type Culture Collection, Rockville, MD), was used for the TNF-α assay (Shahan et al., 1994). L929 cells were grown in Iscove's modified Dulbecco's medium (IMDM, Gibco, Grand Island, NY) supplemented with 30% heat-inactivated horse serum, 7.5% $NaHCO_3$, and 4.4×10^{-5} *M* mercaptoethanol. They were seeded onto a 96-well microtiter plate at 2×10^4 cells/well and incubated at 37°C in 5% CO_2 for 24 h. Following the development of confluent monolayers, the medium was removed and replaced with serial dilutions of AM-conditioned media in IMDM containing 1 μg/ml actinomycin D (Sigma Chemical Co., St. Louis, MO). After incubation for another 24 h, media were removed and cells were incubated with IMDM containing 10% Alamar blue (Alamar Bioscience, Sacramento, CA) for 16 h. The samples were assayed using a fluorescence microplate reader (IDEX Laboratories, Westbrook, ME). The amount of cell lysis was inversely proportional to the dye uptake. A complete standard curve, using recombinant murine TNF-α (R&D Systems, Minneapolis, MN), was obtained for each plate of assay. The concentrations of TNF-α for samples in the same plate were determined from the standard curve and expressed as nanograms per 10^6 AM.

Statistical Analysis

The data were analyzed using JMPs, a statistical package obtained from SAS Institute, Inc. (Cary, NC). All data were expressed as the mean ± standard error (SE) except for IL-1 activities, where the dpm data were normalized as a fold of the respective controls. One-way analysis of variance (ANOVA) was used to analyze differences among the treatments at the same dosage and the same postexposure day, followed by multiple comparisons among means using Tukey–Kramer's Honestly Significant Difference (HSD) test. Two-way ANOVA was used to evaluate nonadditive interaction of dif-

ferent particle treatments on ex vivo and in vivo LPS-stimulated AM secretion of IL-1 and TNF-α. The significance was set at $p < .05$.

RESULTS

Comparison of Overall Inflammatory Responses to DEP, CB, and Silica Exposures

To provide an in-study baseline comparison of DEP with CB and silica on pulmonary responses to particle stimulation, differential cell analyses of the BALF of all exposure groups were performed. Table 1 shows that exposure to DEP, CB, or silica resulted in significant alterations in pulmonary infiltrates. For the saline-treated rats, the BAL cells consisted mostly of macrophages with very few neutrophils and lymphocytes (data not shown) and showed no significant changes in cell populations through the 7-d postexposure period. The number of lavagable macrophages decreased for all particle-exposed groups compared to the saline group at 1 d postexposure.

TABLE 1. Yield of Alveolar Macrophages and Neutrophils Obtained by Bronchoalveolar Lavage of Rats Intratracheally Instilled with Saline Vehicle or Particles

Days postexposure	Treatment			
	Saline	DEP	CB	Silica
		Macrophages		
5 mg/kg exposure				
1 d	103.2 ± 6.9	77.1 ± 6.3[a]	57.7 ± 2.9[a]	75.3 ± 9.3[a]
3 d	113.3 ± 4.8	128.0 ± 10.4	100.2 ± 10.6	165.0 ± 15.9[a]
7 d	92.8 ± 9.8	149.1 ± 18.0[a]	137.1 ± 16.2[a]	102.0 ± 8.0
35 mg/kg exposure				
1 d	103.2 ± 6.9	60.6 ± 3.6[a]	57.4 ± 7.5[a]	60.8 ± 8.8[a]
3 d	113.3 ± 4.8	101.3 ± 9.1	83.1 ± 10.2	115.8 ± 14.4
7 d	92.8 ± 9.8	194.1 ± 23.9[a]	112.2 ± 18.7	123.4 ± 10.3
		Neutrophils		
5 mg/kg exposure				
1 d	2.10 ± 0.32	22.78 ± 2.40[a]	66.33 ± 6.74[a]	87.26 ± 14.21[a]
3 d	2.15 ± 0.42	48.08 ± 6.51[a]	25.34 ± 3.31[a]	27.80 ± 3.05[a]
7 d	1.37 ± 0.27	15.35 ± 3.32[a]	2.60 ± 0.60	26.20 ± 2.30[a]
35 mg/kg exposure				
1 d	2.10 ± 0.32	40.31 ± 3.90[a]	29.38 ± 2.69[a]	89.38 ± 11.60[a]
3 d	2.15 ± 0.42	76.57 ± 9.40[a]	53.45 ± 5.91[a]	52.84 ± 4.95[a]
7 d	1.37 ± 0.27	49.12 ± 6.72[a]	54.11 ± 6.20[a]	78.37 ± 5.93[a]

Note. Data represent means ± SE of cell numbers ($\times 10^5$). Each group had at least 5 animals. The results were analyzed by one-way ANOVA followed by Tukey-Kramer's HSD test for multiple mean comparisons for each treatment group with same dose and at same exposure times.

[a]Significant difference from the saline group, $p < .05$.

These decreases in AM were transient, and the cell counts returned to normal at 3 and 7 d postexposure. For rats exposed to DEP for 7 d, there appeared to be an increase in macrophage counts compared to the saline control. Table 1 also shows a significant increase in neutrophil counts in all particle-exposed rats. These increases persisted through 7 d, and the magnitude of increase correlated with the exposure dose. The neutrophil counts for the silica group at 1 and 7 d postexposure were in general higher than those of the DEP and CB groups.

Table 2 shows the dose- and duration-dependent increase in albumin contents and LDH activities in the acellular BALF. The albumin content in BALF, which indicates injury at the alveolar/capillary barrier, was increased with increasing exposure dose in all particle-exposed groups. There was, however, a significant decrease in albumin content for low dose DEP- and CB-exposed rats at 7 d postexposure, indicating partial recovery from the injury. Silica exposure at both low and high dose was associated with a persistent progressive elevation of albumin level, with the highest levels being observed at 7 d postexposure. The activity of LDH in the acellular

TABLE 2. Levels of Albumin and LDH in Bronchoalveolar Lavage Fluid Obtained From Rats Intratracheally Instilled With Saline Vehicle or Particles

Days postexposure	Treatment			
	Saline	DEP	CB	Silica
	Albumin (mg/ml BALF)			
5 mg/kg exposure				
1 d	0.238 ± 0.014	0.407 ± 0.060[a]	0.453 ± 0.020[a]	0.492 ± 0.021[a]
3 d	0.202 ± 0.011	0.352 ± 0.021[a]	0.388 ± 0.033[a]	0.342 ± 0.043[a]
7 d	0.213 ± 0.024	0.171 ± 0.033	0.280 ± 0.038	0.528 ± 0.059[a]
35 mg/kg exposure				
1 d	0.238 ± 0.014	1.837 ± 0.197[a]	1.360 ± 0.078[a]	0.776 ± 0.048[a]
3 d	0.202 ± 0.011	0.705 ± 0.059[a]	0.810 ± 0.042[a]	0.718 ± 0.056[a]
7 d	0.213 ± 0.024	0.395 ± 0.045	0.698 ± 0.058[a]	1.276 ± 0.190[a]
	LDH (U/L BALF)			
5 mg/kg exposure				
1 d	81.6 ± 7.0	204.8 ± 17.0[a]	311.7 ± 18.7[a]	406.0 ± 50.2[a]
3 d	69.3 ± 4.1	233.7 ± 14.0[a]	247.6 ± 15.0[a]	244.0 ± 15.2[a]
7 d	80.9 ± 10.2	119.5 ± 8.2	108.8 ± 19.4	137.4 ± 11.9[a]
35 mg/kg exposure				
1 d	81.6 ± 7.0	353.0 ± 31.2[a]	411.4 ± 28.4[a]	864.2 ± 53.6[a]
3 d	69.3 ± 4.1	610.3 ± 45.6[a]	471.2 ± 27.5[a]	558.1 ± 38.8[a]
7 d	80.9 ± 10.2	274.2 ± 16.4[a]	469.5 ± 61.4[a]	536.0 ± 62.2[a]

Note. Data represent means ± SE. Each group had at least five animals. The results, in each treatment group with same dose and at same exposure times, were analyzed by one-way ANOVA followed by Tukey-Kramer's HSD test for multiple mean comparisons.

[a]Significant difference from the saline group, $p < .05$.

BALF, an indicator of cytotoxicity, was elevated with increasing exposure dose for all particle groups. For the low-dose DEP and CB exposure groups, the LDH levels returned to normal after 7 d, suggesting a recovery from cytotoxic injury. For the high-dose exposure, silica resulted in the highest levels of LDH and the level remained elevated even 7 d after particle exposure. Tables 1 and 2 indicate that in general, the alveolar/capillary injury and cell infiltration caused by DEP were similar in magnitude to those resulting from CB. Silica, on the other hand, exhibited a more severe and sustained effect on the pulmonary system. These data allow us to further compare the effects of DEP on cellular responsiveness with those of CB and silica and to determine the role of the organic components in DEP suppression macrophage secretory activity.

Effects of Particle Exposure on AM Secretion of IL-1 and TNF-α

Figure 1 shows the effects of DEP, CB, or silica on the spontaneous release of IL-1 and TNF-α by AM from particle-exposed rats. DEP exposures at both low and high doses induced a marked increase in AM production of IL-1 (Figure 1, A and B). This increase was time dependent and showed levels that were 15- and 10-fold of the control at 1 and 3 d postexposure, respectively. At 7 d postexposure, the spontaneous IL-1 release decreased from earlier levels but remained significantly above the control level. In contrast, neither CB nor silica consistently stimulated spontaneous IL-1 production by AM.

The effects of DEP, CB, or silica on AM release of TNF-α are shown in Figure 1 (C and D). DEP exposure had a small effect on the spontaneously release of TNF-α by AM (an elevated level of secretion was observed only at d 1 and only for the low dose exposure). However, both silica and CB showed significant stimulatory effects on AM production of TNF-α at 7 d postexposure. These results show that the cellular action of DEP was different from that of CB or silica, in that DEP did not affect the secretion of TNF-α by AM.

Secretion of IL-1 and TNF-α by AM from Particle-Exposed Rats in Response to Ex Vivo LPS Challenge

Figure 2 shows the IL-1 released by AM from the low particle-exposed rats (5 mg/kg) in response to ex vivo LPS challenge. Both DEP and LPS induced AM release of IL-1, but the IL-1 level for the combined DEP and LPS stimulation was less than the added total of the DEP alone and LPS stimulation alone at 3 and 7 days post-exposure (Figure 2, B and C), suggesting that AM from DEP-exposed rats were less responsive to LPS challenge. In contrast, AM from CB- and silica-exposed rats did not exhibit a suppressed response to LPS challenge in the secretion of IL-1. Moreover, at 7 d postexposure, silica augmented IL-1 secretion in response to LPS (Figure 2C).

Similar results were also observed for the effect of in vivo particle exposure (5 mg/kg) on ex vivo LPS stimulation of TNF-α production by

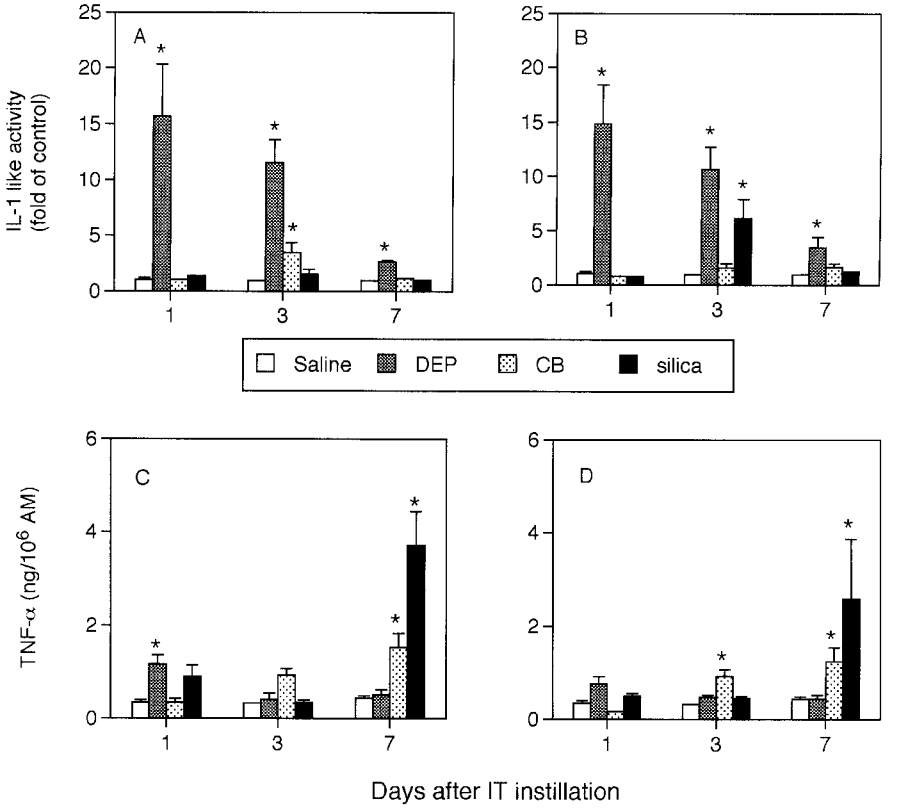

FIGURE 1. Effects of a single intratracheal low dose (5 mg/kg, A and C) or high dose (35 mg/kg, B and D) of DEP, CB or silica on the spontaneous release of IL-1 and TNF-α by rat AM obtained 1, 3, and 7 d after particle exposure. The IL-1-like activity is expressed as an increase relative to the saline control (fold of control), whereas the activity of TNF-α is expressed as ng/10^6 AM for each treatment group. All values represent the mean ± SE of data obtained from at least five rats. Asterisk indicates a significant increase from the respective saline controls, $p < .05$.

AM (Figure 3). DEP exposure had no marked effect on AM production of TNF-α, while both CB and silica had a moderate but significant stimulatory effect on AM secretion of this cytokine at 7 d postexposure. However, silica- or CB-induced TNF-α release was small compared to the LPS effect. The release of TNF-α from CB- and silica-exposed AM in response to ex vivo LPS stimulation was markedly higher than the additive effects of the particulate or LPS challenge alone. Exposure to DEP, on the other hand, inhibited AM secretion of TNF-α in response to LPS challenge. This effect is clearly indicated by the data obtained at 3 and 7 d postexposure (Figure 3, B and C), where the secretion of TNF-α by DEP-exposed and LPS-challenged AM approached to the levels of the saline controls.

Similar effects were also observed for the high-dose (35 mg/kg) particulate exposures (data not shown).

Effect of In Vivo DEP and LPS Exposure on Macrophage Release of IL-1 and TNF-α

To confirm the inhibitory effect of DEP on macrophage response to LPS stimulation, rats were exposed to DEP (5 mg/kg) or saline and to an intratracheal dose of LPS (1 mg/kg body weight) or saline 3 d after DEP exposure and sacrificed 3 h after LPS treatment. The effects of the particles,

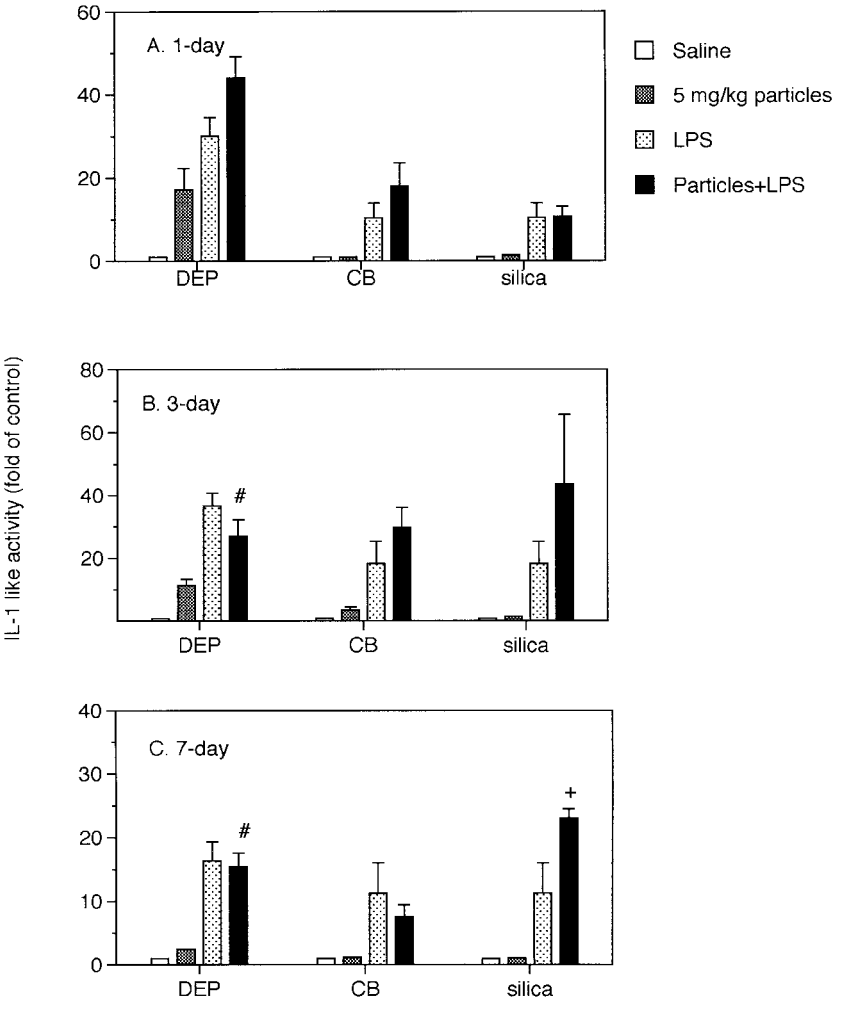

FIGURE 2. Effects of saline (control) and low-dose (5 mg/kg body weight) particle exposure on ex vivo LPS-stimulated IL-1 release from AM. Rats received a single intratracheal instillation of saline, DEP, CB, or silica. AM were harvested from rats (A) 1 d, (B) 3 d, and (C) 7 d after particle exposure and then cultured with or without 0.1 μg LPS/10^6 AM/ml for 24 h. Then AM-conditioned supernates were collected and assayed for IL-1. The IL-1 levels are expressed as fold of the respective saline controls. All values represent the mean ± SE for the respective group ($n \geq 5$). Number sign (#) indicates significantly smaller than additive effect among the treatment groups, while plus sign indicates significantly greater than additive effect (analyzed by two-way ANOVA, $p < .05$).

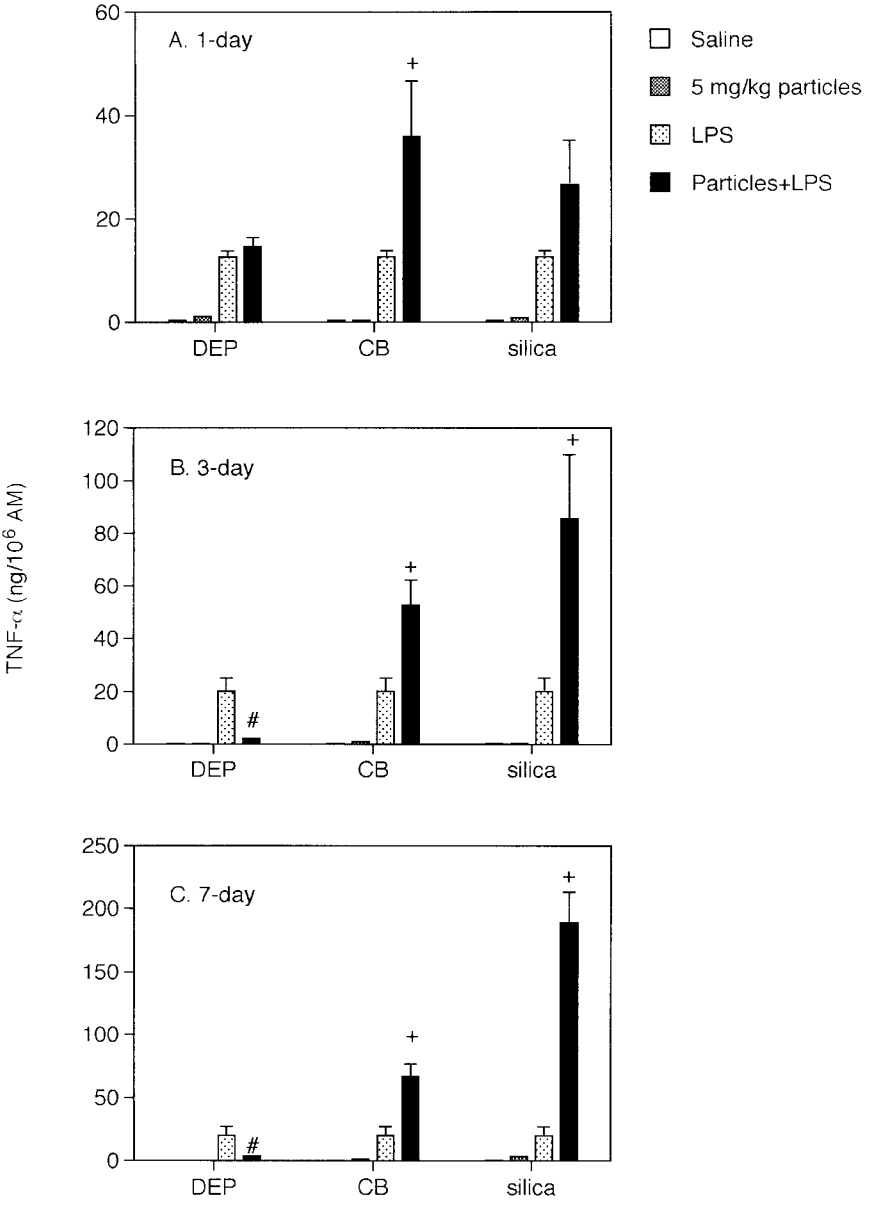

FIGURE 3. Effects of saline (control) and low-dose (5 mg/kg body weight) particle exposure on ex vivo LPS-stimulated TNF-α release from AM. Rats received a single intratracheal instillation of saline, DEP, CB, or silica. AM were harvested from rats (A) 1 d, (B) 3 d, and (C) 7 d after particle exposure and then cultured with or without 0.1 μg of LPS/10^6 AM/ml for 24 h. Then AM-conditioned supernates were collected and assayed for TNF-α. The TNF-α levels are expressed as ng/10^6 AM. All values represent the mean ± SE for the respective group ($n \geq 5$). Number sign (#) indicates significantly smaller than additive effect among the treatment groups, while plus sign indicates significantly greater than additive effect (analyzed by two-way ANOVA, $p < .05$).

LPS, and combined particle–LPS in vivo exposures on basal macrophage secretion of IL-1 and TNF-α are illustrated in Figure 4. Here, the open bars indicate the calculated sum of cytokine release by AM from the DEP–saline and saline–LPS exposed groups. This calculated sum of IL-1 release was significantly greater than that from the DEP + LPS exposed group (Figure 4A). In Figure 4B, the DEP exposure was shown to moder-

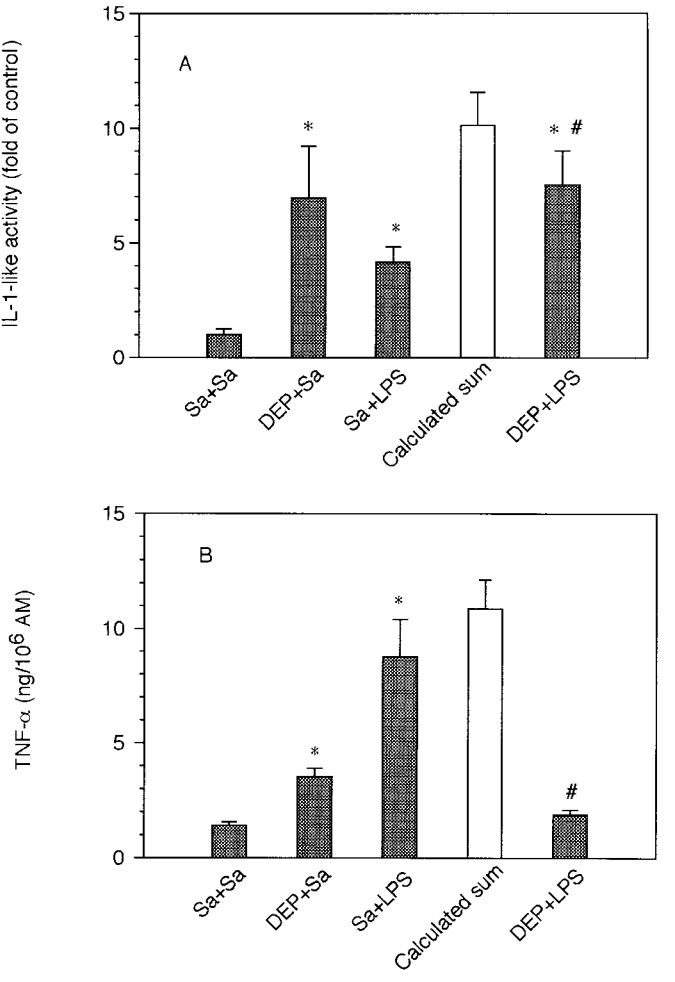

FIGURE 4. Effects of in vivo DEP and/or in vivo LPS exposures on AM release of (A) IL-1 and (B) TNF-α. Rats were exposed to a single dose of DEP (5 mg/kg) and challenged with 1 mg/kg of LPS 3 d post DEP exposure. AM were harvested from rats sacrificed at 3 h post LPS exposure and cultured without further stimulants for 18 h. AM-conditioned supernates were collected and the activities of IL-1 and TNF-α were measured. All values represent the mean ± SE of data from at least five rats for each group. Asterisk indicates a significant increase from the saline control ($p < .05$); (#) number sign indicates a significant nonadditive effect, that is, significantly lower than the calculated sum (open bar) among the treatment groups (analyzed by two-way ANOVA, $p < .05$).

ately enhance AM production of TNF-α, but the release of this cytokine by AM from the DEP + LPS exposed group was markedly lower than the values for either DEP–saline, saline–LPS exposure, or the calculated sum.

DISCUSSION

This study was carried out to investigate the effect of DEP exposure on AM response to ex vivo and in vivo LPS challenge by monitoring LPS-stimulated secretion of proinflammatory cytokines, and to determine whether adsorbed organic components of DEP play a role in the suppressive effect of DEP on macrophage responses. Diesel particulate consists of a carbonaceous core and a variety of organic compounds, including mutagenic and carcinogenic agents, that are adsorbed onto the particle surfaces. CB particles, on the other hand, are similar to the diesel soot particles, but contain little or no adsorbed organic compounds. Thus, a comparison of the DEP and CB pulmonary responses should allow one to determine the effect of the adsorbed organic components of DEP on the pulmonary system. Silica, a known pneumotoxic dust, was used to further compare the effects induced by particles of a different chemical nature. The exposure studies were carried out by intratracheal instillation, which has been shown to produce inflammatory profiles that are comparable to those derived from inhalation experiments (Henderson et al., 1995).

The results of this study show that pulmonary injury and inflammation manifested by neutrophil infiltration, increased albumin leakage, and elevated LDH activity in the BALF can be induced by particles with very different chemical properties. The pulmonary injury induced by DEP exposure was not substantially different from that induced by CB or silica, although the response to silica tended to be slightly greater. For low-dose DEP and CB exposure groups, the results showed a gradual decrease from the acute elevation of albumin content and LDH activity, suggesting a possible recovery from the pulmonary injury. These results also indicate that the alveolar/capillary injury and cell infiltration are induced by the carbonaceous core of DEP with little influence from organic components of DEP, since DEP and CB induced a similar magnitude of damage. For the silica exposure, these levels remained elevated through 7 d postexposure. In addition, silica resulted in significantly greater neutrophil infiltration in the BALF than DEP or CB, suggesting that silica caused greater and more persistent lung damage than did DEP or CB exposure. Ample studies have shown that silica produces oxygen free radicals at the particle surface (Vallyathan et al., 1988). It is possible that this and/or other surface features result in the enhanced responsiveness to silica.

While DEP, CB, and silica all resulted in similar lung damage as indicated by the inflammatory and cytotoxic indicators in the BALF, the effects of DEP on AM secretion of IL-1 and TNF-α and on AM responsiveness to LPS challenge were, in general, different from those of CB or silica.

This is despite the fact that DEP and CB particles share a similar carbonaceous core, and that the CB particles are markedly different from silica in chemical nature. As can be seen from Figure 1, both CB and silica stimulated AM secretion of TNF-α at 7 d postexposure. DEP, on the other hand, stimulated AM secretion of IL-1 at 1, 3, and 7 d postexposure, but did not consistently induce AM secretion of TNF-α. In experiments involving ex vivo and in vivo LPS challenges, the production both of IL-1 and TNF-α by AM in response to LPS challenge was suppressed by DEP, but not by CB or silica. These results suggest that the organic components of DEP may suppress AM secretion of IL-1 and TNF-α. The organic components of DEP had a lesser effect on IL-1 release than on TNF-α secretion, probably due to the fact that the secretion of IL-1 is an earlier cellular response than the TNF-α release. Time may be required for the dissolution of the adsorbed organic compounds from the diesel particles. This difference in response of macrophages to LPS challenge after DEP or CB exposure is consistent with the previous study of Castranova et al. (1985). That study showed that inhalation of DEP depressed alveolar macrophage activity, measured as zymosan-stimulated chemiluminescence or the degree of surface ruffling in cultured macrophages. In contrast, inhalation of coal dust enhanced chemiluminescence and increase cellular spreading and surface ruffling, indicating activation of alveolar macrophages. Since coal dust does not have organic compounds adsorbed onto the particle, the depressive effects of DEP exposure on macrophage functions were attributed mainly to the organic components of DEP.

The underlying mechanisms by which DEP, CB, and silica affect AM responses to LPS challenge are not yet clear. Kobzik (1995) suggested that DEP and silica exhibit different binding characteristics toward the macrophage scavenge receptors, which have been shown to play an important role in the clearance and detoxification of LPS (Hampton et al., 1991). The organic components of DEP may in fact have a broad inhibitory effect on macrophage-mediated immune/inflammatory responses. Results of a previous study have shown that DEP can inhibit LPS-induced macrophage production of nitric oxide and macrophage inflammatory protein-2 (Yang et al., 1998). In the literature, DEP has been shown to induce a variety of biological responses, including stimulation of immunoglobulin E (IgE) production (Takenaka et al., 1995), release of superoxide (Sagai et al., 1993), and suppression of AM phagocytic activity (Prasad et al., 1988; Jakab et al., 1990). All these effects have been attributed to the organic components of DEP.

The ability of DEP to suppress macrophage production of IL-1 and TNF-α in response to LPS stimulation suggests that DEP may predispose the lung to bacterial infections. Both IL-1 and TNF-α released by macrophages are found necessary for the generation of a protective immune response against infections (Blanchard et al., 1988; Bancroft et al., 1989; Van der Meer et al., 1988). Indeed, DEP exposure has been shown to

decrease viral-induced interferon production by the lung and to increase the susceptibility of the lung to viral infection (Hahon et al., 1985). An impairment of macrophage function by DEP can thus have an influence on the development of respiratory infectious diseases.

In summary, data demonstrated that exposure of rats to DEP, CB, or silica induced pulmonary inflammation and injury. The insoluble particles, rather than the organic compounds adsorbed to DEP, seem to be responsible for these deleterious changes. However, DEP suppressed LPS-stimulated AM production of IL-1 and TNF-α, whereas CB or silica either augmented or had no effect on the ability of LPS challenge to stimulate AM secretion of these proinflammatory cytokines. The suppressive effect of DEP on AM activity was attributed to the organic compounds adsorbed onto the diesel particles. Both IL-1 and TNF-α are important cytokines in macrophage-mediated immune responses against microbial insults. Thus, the suppression of AM secretory activity may lead to increased susceptibility of hosts to pulmonary infection after long-term exposure to DEP.

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