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Suppression of Phagocytic and Bactericidal Functions of Rat Alveolar Macrophages by the Organic Component of Diesel Exhaust Particles

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Exposure to diesel exhaust particles (DEP) was shown to increase the susceptibility of the lung to bacterial infection in rats. In this study, the effects of DEP on alveolar macrophage (AM) phagocytic and bactericidal functions and cytokine secretion by AM and lymphocytes in response to *Listeria monocytogenes* infection were investigated in vitro and the roles of different DEP components in these processes were compared. Exposure to DEP or the organic extracts of DEP (eDEP) significantly decreased the phagocytosis and killing of *L. monocytogenes* by AM obtained from normal rats. Washed DEP (wDEP) also decreased AM phagocytosis and bacterial killing to a lesser extent, whereas carbon black (CB) reduced AM phagocytosis but had no significant effect on AM bactericidal activity. DEP or eDEP concentration-dependently suppressed *L. monocytogenes*-induced secretion of tumor necrosis factor-α, interleukin (IL)-1β, and IL-12 by AM and of IL-2 and interferon-γ by lymphocytes obtained from *L. monocytogenes*-infected rats, but augmented the AM secretion of IL-10. wDEP or CB, however, exerted little or no effect on these *L. monocytogenes*-induced cytokines. These results provide direct evidence that DEP, through the actions of organic components, suppresses AM phagocytic and bactericidal functions in vitro. Inhibition of AM phagocytic function and alterations of AM and lymphocyte cytokine secretion by DEP and DEP organic compounds may be implicated in the diminished AM bactericidal activity and the lymphatic arm of the host immune system, thus resulting in an suppressed pulmonary clearance of *L. monocytogenes* and an increased susceptibility of the lung to bacterial infection.

Epidemiologic studies reported a correlation of ambient particulate matter (PM), especially that with diameters less than 2.5 μm ($PM_{2.5}$), with increased incidence of respiratory mortality and morbidity, including pulmonary infections, in high-risk groups or among the general population (Krewski et al., 2005; Dominici et al., 2005; Dockery et al., 1993; Pope et al., 1991; Delfino et al., 1997; Peters et al., 1997). Diesel exhaust particles (DEP), which are generated through extensive industrial use of heavy-duty diesel engines, are a major constituent of the atmospheric $PM_{2.5}$ found in urban and industrialized areas. Therefore, DEP effect on pulmonary infections is of environmental and occupational concern (Becker & Soukup, 1999).

DEP are carbon-based particles that adsorb a variety of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), quinones, and nitro-PAHs. Both the organic and particulate components play a role in DEP-induced pulmonary toxicity (Ma & Ma, 2002). Studies previously showed that inhalation exposure of rats to DEP suppresses alveolar macrophage (AM) phagocytic function and their secretion of proinflammatory cytokines including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-12, but increased AM production of IL-10 in response to *Listeria monocytogenes* infection. Alterations of these AM functions in rats resulted in a markedly

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aggravated bacterial infection in the lung (Yang et al., 2001; Yin et al., 2002, 2004a). It was suggested that the organic compounds of DEP may play a major role in the depression of bacterial killing, as impaired macrophage function and decreased bacterial clearance were not observed in rats exposed to carbon black (CB), a carbonaceous particle with size and surface area characteristics similar to those of DEP but with few adsorbed organic compounds (Yang et al., 1999, 2001). The fact that in vitro exposure to DEP, but not methanol-washed DEP, inhibits the lipopolysaccharide (LPS)-stimulated AM production of IL-1 and TNF-α also support the notion that the inhibitory action of DEP on AM function is due to the presence of the organic compounds (Yang et al., 1997). Further, the carbonyl content of DEP significantly affects IL-8 and PGE2 release (Madden et al., 2003). In a recent study, data showed that DEP, through the organic compound-mediated reactive oxygen species, induce heme-oxygenase-1 expression and IL-10 production and at the same time inhibit AM production of TNF-α and IL-12 to dampen the host immune responses (Yin et al., 2004b).

Currently, the constituents and mechanisms through which DEP suppress the pulmonary immunity to bacterial infection are not yet fully understood. Although a few studies pointed out that exposure to DEP impaired AM phagocytic function, determined using biologically inert fluorescent latex or polystyrene particles (Chen et al., 1980; Yin et al., 2002) or heat-killed, fluorescein isothiocyanate-labeled yeast particles (Rudell et al., 1999), none of them actually showed that DEP might produce a defective AM phagocytosis of viable pathogens. Furthermore, direct evidence that DEP suppress the bactericidal activity of AM is currently lacking, while studies found that DEP exposure increases the susceptibility of the lung to bacterial infection (Yang et al., 2001; Castranova et al., 2001; Yin et al., 2002, 2004a; Saito et al., 2002). This study was carried out to test the hypothesis that DEP, through their organic component, impair the phagocytic, bactericidal, and secretory functions of AM, leading to an increased susceptibility of the lung to bacterial infection. The concentration- and time-dependent effects of DEP on AM phagocytosis and killing of *L. monocytogenes* and cytokine secretion by AM and lymphocytes in response to *L. monocytogenes* infection were investigated and the roles of different DEP components in these processes were compared.

MATERIALS AND METHODS

Preparation of DEP, CB, and DEP Components

The diesel particulate matter SRM 2975, collected from an industrial forklift, was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). This material, which has a median diameter of 19.4 μm and a surface area of 91 m^2/g, is a well-characterized DEP sample and has been certified by the NIST for use in the development, evaluation, and certification of analytical methods for complex environmental diesel mixtures (Claxton et al., 1992). CB (particle size: 0.1–0.6 μm) was obtained from Cabot Co. (Boston). DEP or CB was suspended in sterile phosphate-buffered solution (PBS, pH 7.4) by sonication using an ultrasonic processor (Heat System-Ultrasonics, Plainview, NY). The organic extracts of DEP (eDEP) and the washed DEP (wDEP) were prepared as previously described (Yin et al., 2004b). Briefly, DEP were suspended in dichloromethane, sonicated for 10 min on ice, and then centrifuged for 10 min at 500 × g. After removal of the solvent, the particles were further washed with a 1:1 (v/v) mixture of acetone and methanol following the same procedure. The two extracts were combined, evaporated to dryness, and weighed. This procedure yielded 41 mg eDEP from 100 mg DEP (Yin et al., 2004b). The eDEP and wDEP were dissolved in dimethyl sulfoxide (DMSO) or sterilephosphate-buffered saline (PBS) and stored at –20°C. When used in experiments, samples were diluted with sterile saline. All concentrations expressed for eDEP or wDEP refer to the extracts and particles, respectively, from the same concentration of DEP.

Animals and *L. monocytogenes* Infection

Male Brown-Norway rats [BN/CrlBR], weighing 225–250 g, were purchased from Charles River Laboratories (Wilmington, MA). The animals were housed in a clean-air and viral-free room with restricted access, given a conventional laboratory diet and tap water ad libitum, and allowed to acclimate for 1 wk in an animal facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care.

Listeria monocytogenes (strain 10403s and serotype 1) was cultured as previously described (Yin et al., 2002, 2004a). Briefly, *L. monocytogenes* was incubated with brain heart infusion broth (Difco Laboratories, Detroit, MI) at 37°C until the culture was in log growth. The bacterial concentration was then determined spectrophotometrically at 600 nm and diluted with sterile saline for animal infection and AM phagocytosis and bactericidal assays described later.

Rats were anesthetized with methohexital sodium (35 mg/kg, Eli Lilly Co., Indianapolis, IN) and intratracheally inoculated with ~100, 000 *L. monocytogens* in 500 μl sterile saline according to the method previously described (Antonini et al., 2000).

Isolation of AM and Lymphocytes

For AM phagocytosis and bactericidal studies, untreated normal rats were anesthetized with sodium pentobarbital (200 mg/kg; Butler, Columbus, OH) and then exsanguinated by cutting the abdominal aorta. Bronchoalveolar lavage (BAL) was performed and AM in the BAL cell suspension were counted using an electronic cell counter equipped with a cell-sizing unit (Coulter Electronics, Hialeah, FL), as described previously (Yin et al., 2002, 2004a, 2004b).

For cytokine studies, the *L. monocytogenes*-infected rats were euthanized at 7 d after intratracheal instillation. All lung-draining lymph nodes (LDLN) were removed, teased apart with forceps, and homogenized in RPMI-1640 medium (Gibco

BRL, Gaithersburg, MD) with a glass pestle in a screen cup (Sigma Chemical Co., St. Louis, MO). Single-cell suspensions were obtained by repeatedly passing the cell clumps through a 22-gauge needle attached to a 10-ml syringe, as previously described (Yin et al., 2002, 2004a). The cells were washed twice with the medium and lymphocytes were isolated by Histopaque (density, 1.083; Sigma) gradient centrifugation. Following centrifugation at 2500 rpm for 30 min, cells were washed twice and resuspended in the same medium. The lymphocytes were counted with a standard hemocytometer and the cell viability was assessed by the trypan blue dye exclusion technique. Both the purity and viability were shown to be >98% for the lymphocytes prepared with these procedures. Following the removal of LDLN, BAL procedure was performed and the number of AM in BAL cell suspension was determined as already described.

Measurement of AM Phagocytosis and Bactericidal Activity

AM phagocytosis and bactericidal activity were determined by a method modified from Ohya et al. (1998) and described previously (Antonini et al., 2002). AM were obtained by BAL from untreated normal rats. The AM, at a density of 2.5×10^5 cells/well, were allowed to attach in 24-well plates (Costar, Cambridge, MA) for 2 h in a humidified incubator (37°C and 5% CO_2) in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The nonadherent BAL cells were removed by rinsing the monolayer three times with the medium. The remaining AM-enriched cells were then treated with vehicle control (PBS or 0.25% DMSO) or varying concentrations of DEP (10, 50, or 100 μg/ml) for 1, 4, 16, or 24 h, or 50 μg/ml of eDEP, wDEP, or CB for 24 h. Following treatment, the cells were washed thoroughly with medium to remove the treatment materials. The viability of the cells was determined by the trypan blue dye exclusion technique. To ensure that the number of adherent cells was the same in various culture samples, cellular protein levels were determined as described previously (Yin et al., 2002). The cells were then incubated with 10^7 viable *L. monocytogenes* at 37°C for 1.5 h. After this incubation, one set of AM from each treatment was washed six times with medium to remove any extracellular bacteria and lysed in sterile water by sonicating with a Sonifer 450 cell disruptor. This first lysate was diluted and cultured quantitatively on brain heart infusion agar plates. The number of bacteria colony-forming units (CFU) was counted after an overnight incubation at 37°C to determine AM phagocytosis.

For the remaining AM from each treatment, the cells were incubated for an additional 4 or 18 h at 37°C. The AM were continually washed every 2 h during the incubation period with medium containing 10 μg/ml of chloramphenicol (Sigma Chemical Co., St. Louis, MO) to inhibit growth of any extracellular bacteria that had not been taken up by the AM after the first incubation and, importantly, to help prevent the cultured cells from reinfection by the bacteria that had parasitized and subsequently released from any compromised AM (Van Loveren et al., 1988). After the incubations, the AM were lysed and the lysates were cultured overnight at 37°C, and the number of CFU determined. The number of bacteria killed by the AM was determined by subtracting the number of CFU of the lysate at 4 or 18 h from that of the first lysate at 1.5 h.

Measurement of Cytokines

The BAL cells and lymphocytes obtained from *L. monocytogenes*-infected rats were suspended in RPMI-1640 medium supplemented with 10% FBS. Aliquots of 1 ml cell suspensions, containing 2×10^6 AM or lymphocytes, were added to each well of 24-well plates. Before the treatment, the BAL cells were incubated for 2 h at 37° C to allow cell attachment to plastic plate. The nonadherent BAL cells were removed by rinsing the monolayer three times with the medium. The remaining AM-enriched cells or lymphocytes were then treated ex vivo with vehicle control (0.25% DMSO) or increasing concentrations of DEP, eDEP, wDEP, or CB (5, 10, 25, or 50 μg/ml) in the presence, respectively, of LPS derived from *Escherichia coli* (1 μg/ml, Sigma Chemical Co., St. Louis, MO) or heat-killed *L. monocytogenes* (HKLM, 10^7/ml) for 24 or 48 h, respectively. Following the incubations, the culture media were collected, centrifuged at 1200 × g for 4 min, and the supernatants were stored at –70°C until assay.

The production of TNF-α, IL-1β, and IL-10 by AM and lymphocyte secretion of interferon (IFN)-γ and IL-6 were quantified by enzyme-linked immunosorbent assay (ELISA) using the OptEIA ELISA Sets according to the manufacturer's instructions (BD PharMingen, San Diego, CA). The levels of IL-12 in AM-conditioned media were quantified by ELISA using an ELISA kit from BioSource International, Inc. (Camarillo, CA). Absorbance of samples was read at 450 nm with a SpectraMax 250 plate spectrophotometer and analyzed using Softmax Pro 2.6 software (Molecular Devices Co., Sunnyvale, CA). All cytokine levels were determined from the linear portion of the standard curves generated using recombinant cytokines.

Statistical Analysis

The experimental results are expressed as mean ± SE of multiple measurements. Statistical analyses were carried out with the JMP IN statistical program (SAS, Inc., Cary, NC). The significance of the interaction among the different treatment groups for the different parameters at each time point was assessed using analysis of variance (ANOVA). The significance of difference between individual groups was analyzed using the Tukey–Kramer honestly significant difference (HSD) test. For all analyses, the criterion of significance was set at $p < .05$.

RESULTS

Effect of DEP on AM Phagocytosis

Treatment of AM with DEP, eDEP, wDEP, or CB at concentrations up to 100 μg/ml for 24 h was found to be noncytotoxic. The viability of the cells from each treatment group remained 95% or greater as determined by trypan blue dye exclusion technique. In addition, the treatment was also found not to affect the number of adherent cells, as determined by measurements of cellular protein levels in various cultures (data not shown). The effect of DEP exposure on phagocytosis of viable *L. monocytogenes* by AM obtained from normal rats is shown in Figure 1. Compared to the control, in vitro treatment with DEP at 10–100 μg/ml significantly decreased the uptake of *L. monocytogenes* by AM in a concentration-dependent manner (Figure 1A). The suppression of AM phagocytosis by DEP was also shown to have a time-dependent feature, with the number of the bacteria phagocytized by AM decreasing with time (Figure 1B). AM exposed to a high concentration (50 μg/ml) of DEP showed a rapid decrease in phagocytotic activity within a short time period (starting at 1 h), while cells exposed to the low concentration (10 μg/ml) of DEP exhibited a significant decrease in phagocytosis only at later time points (after 4 h). The effects of DEP and different DEP components (eDEP and wDEP) on AM phagocytosis were also compared (Figure 1C). At a concentration of 50 μg/ml, eDEP markedly decreased the uptake of *L. monocytogenes* by AM and these organic extracts appeared to be more effective than DEP in suppressing AM phagocytosis of the bacteria. In comparison, treatment with wDEP, which appeared to be less effective than DEP, only moderately decreased the number of AM bacterial uptake. CB, a carbonaceous particle with size and surface area characteristics similar to those of DEP but few adsorbed organic compounds, acted similarly to wDEP.

Effect of DEP on AM Bactericidal Activity

The effect of DEP exposure on the bacterial killing activity of AM obtained from normal rats, determined at 4 h post-bacteria uptake, is shown in Figure 2. Compared to the control, in vitro exposure to DEP concentration-dependently decreased the number of *L. monocytogenes* killed by AM (Figure 2A). Kinetic studies showed that this DEP effect was also time dependent, with the inhibition of AM bactericidal activity becoming increasingly apparent with the extension of time periods for DEP exposure (Figure 2B). This concentration- and time-dependent feature of DEP effect is also reflected in the fact that AM exposed to the high concentration (50 μg/ml) of DEP exhibited an impaired bactericidal activity shortly (1 h) after exposure, while those exposed to the low concentration (10 μg/ml) of DEP showed a delayed (after 16 h) and a less pronounced effect. In comparison to the effects of DEP and DEP components, eDEP and, to a lesser extent, wDEP signifi-

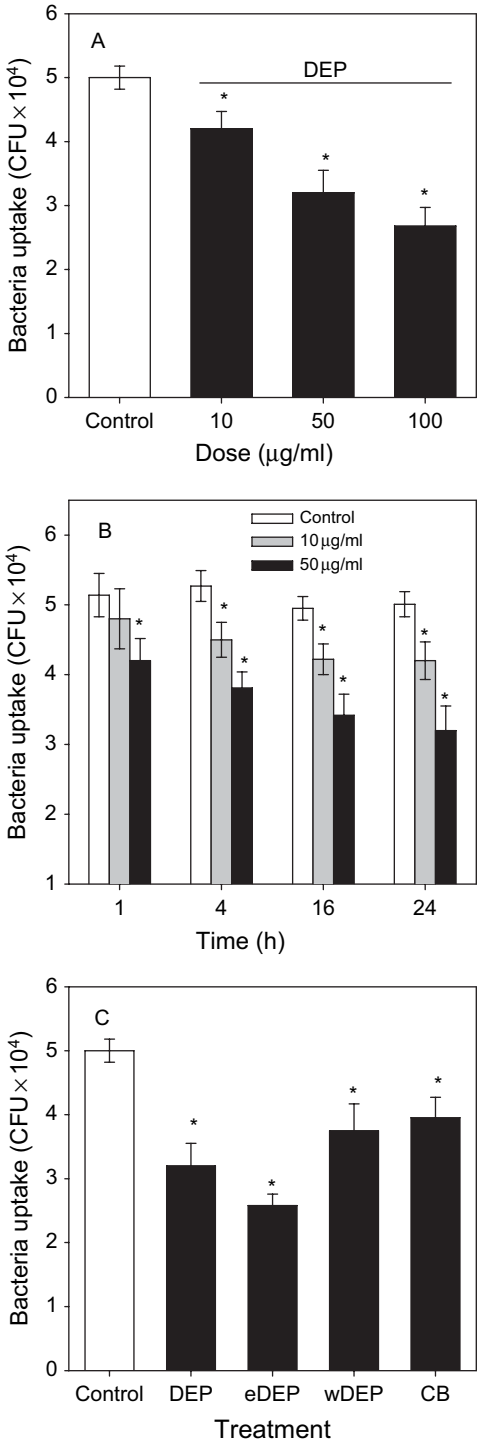

FIG. 1. Effects of DEP and DEP components on phagocytic function of AM obtained from normal rats. (A) Concentration-dependent effect: AM were treated with vehicle control (PBS) or varying concentrations of DEP for 24 h. (B) Time-dependent effect: AM were treated with vehicle control (PBS) or 10 or 50 μg/ml DEP for different time periods. (C) Comparison of effects of different DEP components: AM were treated with vehicle control (0.25% DMSO) or 50 μg/ml of DEP, eDEP, wDEP, or CB for 24 h. The number of *L. monocytogenes* taken up by AM was determined at 1.5 h after incubation with the bacteria. Values are mean ± SE of three separate experiments. Asterisk indicates significantly different from control, $p < .05$.

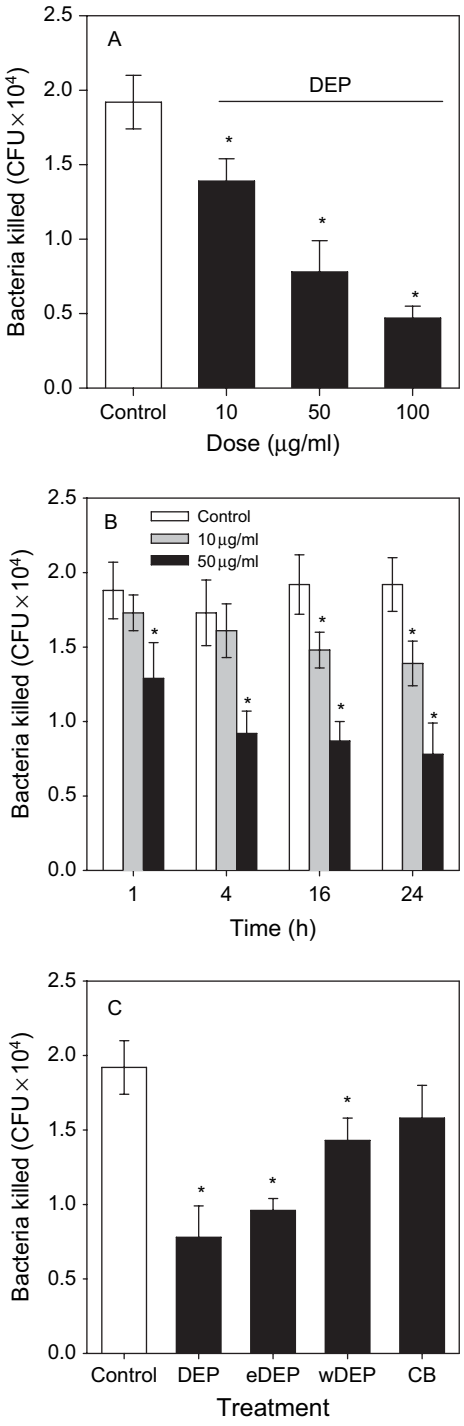

FIG. 2. Effects of DEP and DEP components on bactericidal function of AM obtained from normal rats. (A) Concentration-dependent effect: AM were treated with vehicle control (PBS) or varying doses of DEP for 24 h. (B) Time-dependent effect: AM were treated with vehicle control (PBS) or 10 or 50 μg/ml DEP for different time periods. (C) Comparison of effects of different DEP components: AM were treated with vehicle control (0.25% DMSO) or 50 μg/ml of DEP, eDEP, wDEP, or CB for 24 h. The number of *L. monocytogenes* killed by AM was determined at 4 h after the first incubation with the bacteria. Values are means ± SE of three separate experiments. Asterisk indictes significantly different from control, $p < .05$.

TABLE 1
Effects of DEP and DEP Components on Bactericidal Activity of AM

Treatment	Concentration (μg/ml)	Percent killed bacteria[a]
Control	—	38.4 ± 3.6
DEP	10	33.1 ± 3.5
	50	24.1 ± 7.3[b]
	100	17.5 ± 3.1[b]
eDEP	50	37.2 ± 2.6
wDEP	50	38.5 ± 4.4
CB	50	40.0 ± 5.3

Note. AM obtained from normal rats were treated with vehicle control (0.25% DMSO), 10–100 μg/ml of DEP, or 50 μg/ml of eDEP, wDEP, or CB for 24 h, followed by incubation with *L. monocytogenes*. The percentages of killed bacteria were calculated from the number of phagocytized bacteria determined after 1.5 h of incubation and the number of killed bacteria determined after an additional 4 h of incubation. Values are mean ± SE of three separate experiments.

[a]Percent killed bacteria = (number of killed bacteria/number of phagocytized bacteria) 100%.

[b]Significantly different from control, $p < .05$.

cantly reduced the bacterial killing activity of AM compared to the control, even though both of them were less effective than DEP in exerting this effect. However, treatment of AM with CB was not significant when compared to control (Figure 2C). In addition, DEP, but not eDEP, wDEP, or CB, were shown to significantly decrease the percentage of killed bacteria in a concentration-dependent manner (Table 1). At 18 h post-bacteria uptake, all the AM from both control cultures and various treatments were able to kill almost all (>97%) of the bacteria phagocytized and no difference in bactericidal activity was seen among these cells (data not shown).

Effects of DEP on Cytokine Secretion by AM

Since cytokine secretion is directly related to the bactericidal activity of AM, the cellular response to DEP in the production of cytokines important to bacterial killing was examined and the effects of different DEP components were compared (Figure 3). Our previous studies indicated that secretion of various cytokines by AM and lymphocytes obtained from noninfected Brown-Norway rats or spontaneous production of cytokines by the cells obtained from *L. monocytogenes*-infected rats was relatively low (Yin et al., 2002, 2003, 2004a). Therefore, only cells obtained from the infected rats, which produced a great amount of various cytokines following LPS or HKLM stimulation (Yin et al., 2002, 2003, 2004a, 2004b), were used as a model in the present study. Compared to the control, treatment with DEP or eDEP concentration-dependently decreased the *L. monocytogenes*-induced production of

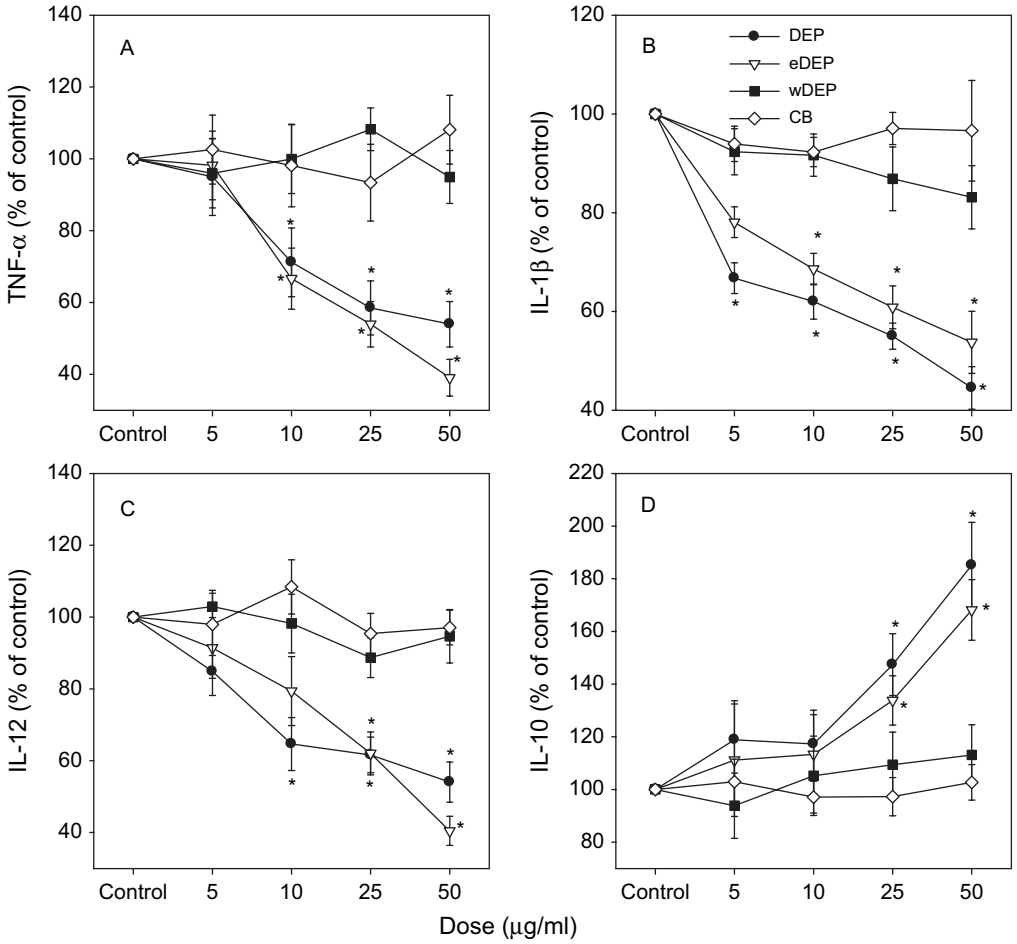

FIG. 3. Concentration-dependent effects of DEP and DEP components on AM secretion of (A) TNF-α, (B) IL-1β, (C) IL-12, and (D) IL-10. AM obtained from *L. monocytogenes*-infected rats at 7 d postinfection were treated ex vivo with vehicle control (0.25% DMSO) or 5, 10, 25, or 50 μg/ml of DEP, eDEP, wDEP, or CB in the presence of lipopolysaccharide (LPS, 1 μg/ml) for 24 h. Levels of cytokines in the culture media were determined by ELISA. Values are mean ± SE of three separate experiments and are presented as production relative to that in the control-/LPS-treated cells. Asterisk indicates significantly different from control, $p < .05$.

TNF-α, IL-1β, and IL-12 by AM, but increased IL-10 secretion. In contrast, wDEP and CB did not show significant effect on the *L. monocytogenes*-induced secretion of these cytokines in response to LPS.

Effects of DEP on Cytokine Secretion by Lymphocytes

To further study the effect of DEP on antigen-specific response against *L. monocytogenes* infection, lymphocyte-mediated immune responses were assessed by examining cellular production of IL-2 and IFN-γ in response to HKLM, which activates *Listeria*-specific lymphocytes (Figure 4). DEP exposure significantly decreased lymphocyte production of IL-2 and IFN-γ in a concentration-dependent manner. eDEP, which acted similarly to DEP, also concentration-dependently decreased the cytokine production by lymphocytes. In comparison, wDEP and CB exerted little or no effect on the cytokine production. At high concentrations, however, wDEP were found to significantly decrease lymphocyte secretion of IFN-γ when compared to the control.

DISCUSSION

The adverse effect of exposure to DEP on pulmonary infections is an environmental and occupational concern. Studies previously showed that in vivo exposure to DEP markedly aggravated pulmonary *L. monocytogenes* infection in rats (Yang et al., 2001; Yin et al., 2002, 2004a). Suppression of AM phagocytic function and alterations of AM and lymphocyte cytokine secretion by DEP may provide partial explanations for this adverse effect. This study was carried out to elucidate how DEP may affect AM phagocytic and bactericidal functions and secretion of cytokines by AM and lymphocytes, with emphasis on the roles of different DEP components in these processes. Exposure of AM to DEP in vitro produced a concentration- and time-dependent suppression in AM

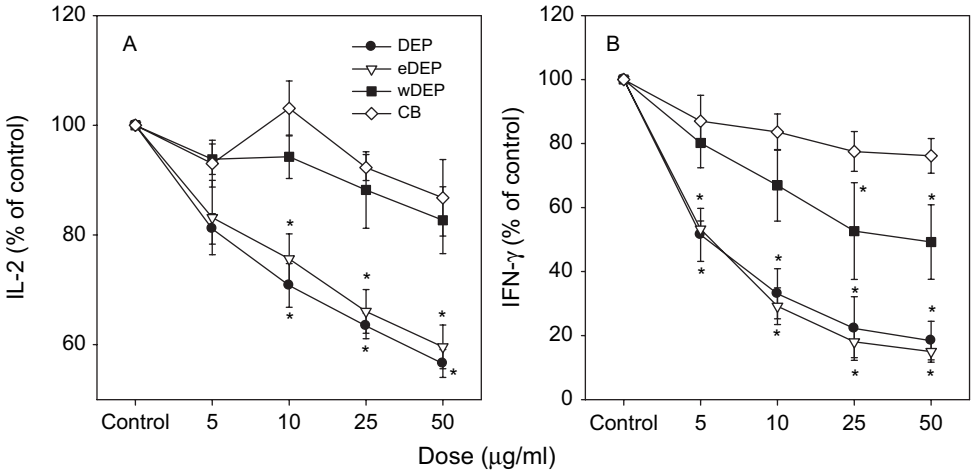

FIG. 4. Concentration-dependent effects of DEP and DEP components on lymphocyte secretion of (A) IL-2 and (B) IFN-γ. Lymphocytes obtained form LDLN of *L. monocytogenes*-infected rats at 7 d postinfection were treated ex vivo with vehicle control (0.25% DMSO) or 5, 10, 25, or 50 μg/ml of DEP, eDEP, wDEP, or CB in the presence of HKLM (10^7/ml) for 48 h. Levels of cytokines in the culture media were determined by ELISA. Values are means ± SE of three separate experiments and are presented as production relative to that in the control-/HKLM-treated cells. Asterisk indicates significantly different from control, $p < .05$.

phagocytosis and killing of *L. monocytogenes*, accompanied with altered profiles of cytokine secretion by AM and lymphocytes in *L. monocytogenes*-infected and DEP-exposed cells. One of the important findings of our current study is that the effects of DEP on the phagocytic and bactericidal functions of AM and cytokine secretion by AM and lymphocytes stem from the actions of the organic component. The washed DEP or CB, which are largely devoid of organic compounds, exerted little or no effect on these processes. This was also shown by Madden et al. (2003). This study is in agreement that DEP exposure directly impairs AM phagocytosis of a viable pathogen and their bactericidal activity in which the organic component plays a major role.

It is also interesting and worthwhile to examine the effects of DEP and DEP components on the bactericidal activity of AM in terms of percentages of killed bacteria, since the numbers of *L. monocytogenes* phagocytized by AM at 1.5 h were not the same among the different treatments. In this regard, DEP were shown to significantly decrease the percentages of killed bacteria by AM in a concentration-dependent manner. Although eDEP markedly decreased the absolute number of bacteria killed by AM, it did not significantly affect the percentages of killed bacteria when compared to control. This appeared to be associated with a pronouncedly reduced number of phagocytized bacteria in eDEP-treated cells, making the resultant percentages of killed bacteria greater than expected. Since phagocytosis is the primary and key function of AM in the host defense against various microorganisms, an impaired phagocytic activity may have a negative influence on their immune defense functions (Becker & Soukup, 1999). Although the adverse effect of DEP on AM phagocytosis has been demonstrated using certain model foreign materials (Chen et al., 1980; Rudell et al., 1999; Yin et al., 2002), a reduced capacity to phagocytize pathogens is of a particular concern for the defense against harmful microorganisms, as it may affect communication with T cells which need to respond to peptide derivatives presented by the macrophages during direct cell to cell interaction. The depression of AM phagocytosis of *L. monocytogenes* by DEP and DEP compounds, therefore, may not only directly affect bacterial killing of AM, but also interrupt the subsequent lymphocyte responses to the infection.

Bacterial killing by AM is known to be associated with the production of various mediators, including reactive oxygen and nitrogen species, chemokines, and cytokines (Sibille & Reynolds, 1990; Laskin & Pendino, 1995; Zhang et al., 2000). A number of AM-derived cytokines are known to be necessary for the generation of a protective immune response against *L. monocytogenes* (Bancroft et al., 1989; Czuprynski et al., 1992). Studies showed that mice deficient in the 55-kD TNF receptor are extremely sensitive to *L. monocytogenes* and succumb easily to infection (Campbell, 1994; Shen et al., 1998), suggesting that TNF-α is necessary for the elimination of *L. monocytogenes*. Both IL-1 and TNF-α activate NK cells to release interferon (INF)-γ, which activates macrophages to kill the bacteria. These cytokines are also T-cell activators (Akira et al., 1990; Hsieh et al., 1993). IL-10, on the other hand, is a potent immunosuppressive factor that was shown to downregulate macrophage bactericidal activity (Fleming et al., 1999). Our current study shows that DEP suppressed AM production of proinflammatory cytokines IL-1β and TNF-α and, at the same time, exacerbated *L. monocytogenes*-induced AM production of IL-10. Considering these opposite effects, the reduction of IL-1β and TNF-α by DEP under the current conditions was not likely caused by binding of the cytokines to the particles, as suggested by a recent study where DEP at high concentrations were shown to bind IL-8 and reduced levels of the cytokine

in vitro (Seagrave et al., 2004). The effect of DEP exposure on the production of IL-10 by AM is also of interest because some intracellular pathogens including *L. monocytogenes* specifically target macrophages for infection and use IL-10 to dampen the host immune response and thus prolong their survival (Redpath et al., 2001). These data correlate with the impaired bacterial killing by the DEP-exposed AM as demonstrated in the present study and those demonstrated previously that the early pulmonary clearance of *L. monocytogenes* was significantly suppressed in DEP-exposed rats (Yin et al., 2002, 2004a).

Although the innate immunity is efficient in limiting the initial spread of infection, sterilization of *L. monocytogenes* infection depends on the later development of acquired T-cell responses involving $CD4^+$ Th1 and $CD8^+$ cells (Kaufmann, 1993; Unanue, 1997; Shen et al., 1998). Considering the important role of IL-12 in the communication between innate and cell-mediated immune systems (Hsieh et al., 1993; Park & Scott, 2001; Trinchieri, 1995, 1998), the suppressive effect of DEP on AM production of IL-12 indicates that DEP exposure might elicit an adverse influence on the development of T cell-mediated immunity. The suppression of AM phagocytosis of *L. monocytogenes* by DEP and DEP compounds, as discussed earlier, may also have a negative influence on the development of cell-mediated immunity.

Indeed, the current study shows that DEP exhibited a direct and concentration-dependent effect on the production of cytokines by lymphocytes that are crucial for the host to mount an effective immune response toward *L. monocytogenes* infection. DEP exhibited a direct inhibitory effect on the production of IL-2, which promotes T cell proliferation, and the production of IFN-γ by *L. monocytogenes*-infected lymphocytes in response to HKLM stimulation. These results show that DEP interrupt the development of bacterial-specific T-cell responses, and suppress T cell functions. Since IFN-γ activates macrophages to kill the bacteria, inhibition of lymphocyte secretion of IFN-γ by DEP suggests that it may be also responsible for the diminished AM bactericidal activity in vivo.

In summary, the current study demonstrates that DEP, through the actions of organic compounds, directly inhibit the ability of AM to kill *L. monocytogenes*. DEP suppressed AM phagocytic activity and their secretion of proinflammatory cytokines, but increased production of IL-10, resulting in a reduced macrophage bactericidal activity and thus a prolonged bacterial survival. Inhibition of lymphocyte production of IFN-γ by DEP might be another mechanism underlying the effects of DEP on AM bactericidal activity and cell-mediated immune responses to *L. monocytogenes* in vivo.

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