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EFFECT OF ASPHALT FUME INHALATION EXPOSURE AT SIMULATED ROAD PAVING CONDITIONS PRIOR TO BACTERIAL INFECTION ON LUNG DEFENSE RESPONSES IN RATS

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Asphalt fume inhalation has been suspected of affecting immune function in exposed workers. The objective of this study was to evaluate the effect of asphalt exposure on lung immune responses in rats using a bacterial infectivity model. Pathogen-free male Sprague-Dawley rats were exposed by inhalation to asphalt fumes (72.6 ± 4.95 mg/m^3) or filtered air for 6 h/day for 5 days. One day after the final asphalt exposure, rats were intratracheally inoculated with 5 × 10^5 Listeria monocytogenes. *At 0 (prior to bacterial inoculation), 3, and 7 days after* L. monocytogenes *instillation, the lungs of each animal were divided. Bronchoalveolar lavage (BAL) was performed on right lungs. The recovered BAL cells were then differentiated and counted, and alveolar macrophage (AM) function was determined. Albumin and lactate dehydrogenase (LDH), two indices of lung injury, were measured in the acellular BAL fluid. To assess bacterial clearance, the left lungs were removed, homogenized, and bacterial colony-forming units (CFUs) were counted. In addition, lung-draining lymph nodes were removed, and lymphocyte phenotype and lymphocyte-induced cytokine production were examined. Asphalt fume exposure did not cause lung injury or inflamma-*

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tion in rats in the absence of infection. Infection induced elevations in AMs, neutrophils (PMNs), albumin, and LDH. Importantly, no significant differences were seen when comparing the asphalt group with the air and nonexposed naive groups at any time before or after infection. Also, asphalt fume inhalation exposure did not affect the rate of pulmonary clearance of L. monocytogenes *or AM production of reactive oxygen and nitrogen species. However, asphalt-related increases in lymphocyte secretion of interferon (IFN)-γ, interleukin (IL)-6, and IL-10 were observed at different times after bacterial infection, whereas the total number of lymph-node cells and the percentage of CD4+ and CD8+ cells were not significantly different among the treatment groups. Despite the asphalt-induced changes observed in lymphokine secretion, adaptive immune function seemed to function properly in lung defense against bacterial infection. Because innate nonspecific lung responses and pulmonary clearance of* L. monocytogenes *were unaffected by asphalt fume exposure, lung defenses were sufficient to control the infection. It was concluded that acute inhalation of asphalt fumes at a high concentration had a minimal effect on lung immune responses to infection in rats.*

Asphalt is produced by processing petroleum crude oils, and its composition can vary depending on the source of the crude oil, refinery processes, additives, and the application practices (Gamble et al., 1999). It is principally used for road paving (~87%) and roofing (~11%) in the United States (Asphalt Institute, 1990). Asphalt fumes are a complex and variable mixture of an inorganic particulate (derived from mineral aggregates) and organic compounds that include aliphatics, polycyclic aromatic hydrocarbons (PAHs), and heteroatomic compounds containing sulfur, nitrogen, and oxygen (King et al., 1984). It is estimated that in the United States 300,000 workers are employed in the asphalt paving industry (Asphalt Institute, 1990), and approximately 144,000 roofers are exposed to asphalt, with 50,000 conducting hot asphalt work (NIOSH, 2003). Several million tons of asphalt are produced each year by the paving and roofing industries (Asphalt Institute, 1989). Thus, the potential for occupational exposure to asphalt is quite high and may pose a health risk to exposed workers.

The major route of exposure to asphalt fumes is by inhalation. Low levels of total PAHs present in asphalt fume have been detected through environmental and personal sampling during road paving operations. Enhanced levels of urinary 1-hydroxypyrene, a metabolite of PAHs, have been measured in road pavers (Jarvholm et al., 1999; Karakaya et al., 1999) and roofers (Toraason et al., 2001). Watts and colleagues (1998) measured the mean concentrations of airborne particles (2.5 μm) at three different paving sites in the United States and observed an exposure range of 111–389 $\mu g/m^3$. Burstyn and coworkers (2000) reported that European road paving workers can be exposed to 0.1–2 mg/m^3 of bitumen fume, which may contain 10–200 ng/m^3 benzo[a]pyrene. Although normal exposure levels to road paving asphalt fumes are relatively low, long-term health effects associated with chronic exposure may be a concern (Bonnet et al., 2000).

Inhalation is an important route of exposure; however, very few studies have assessed the pulmonary responses to asphalt fumes. Due to the presence of PAHs in asphalt fumes, the potential mutagenic/carcinogenic effects of asphalt exposure have been the central focus of in vitro (Qian et al., 1996,

1999), in vivo animal (Sivak et al., 1997; Qian et al., 1998), and worker studies (Fuchs et al., 1996; Burgaz et al., 1998; Toraason et al., 2001). Moreover, Ma et al. (2002) indicated that the asphalt fume-induced genotoxic effects as demonstrated by micronuclei formation in bone-marrow polychromatic erythrocytes may involve an alteration of cytochrome P-450 metabolism of PAHs.

Additional studies are needed to evaluate the noncarcinogenic effects of asphalt fume exposure. PAHs have been reported to cause suppression of humoral and cell-mediated immune responses in animals, as well as suppression of in vitro immune reactions of human blood cells (Davila et al., 1996). Karakaya and colleagues (1999) have indicated that chronic exposure to PAHs by asphalt workers may affect immune function as evidenced by altered T-cell subsets, serum immunoglobulin levels, and white blood cell differentials. In addition, it has been reported that B6C3F1 mice treated via whole-body inhalation exposure to asphalt fumes or the vapor component of asphalt fumes demonstrated an approximate 50% and 25% suppression in an immunoglobulin (Ig)M-dependent response, respectively (Diotte et al., 2001).

Therefore, the objective of the current study was to evaluate the effect of asphalt fume exposure on lung immune responses using a bacterial infectivity model. Sprague-Dawley rats were exposed by inhalation to asphalt fumes at simulated road paving conditions before pulmonary infection with the bacterial pathogen *Listeria monocytogenes*. Lung injury and inflammation, alveolar macrophage (AM) function, pulmonary clearance of *L. monocytogenes,* and lymphocyte phenotype and secretion of cytokines were examined.

MATERIALS AND METHODS

Experimental Design

Rats were exposed by inhalation to asphalt fumes (72.6 ± 4.95 mg/m^3) or filtered air for 6 h/day for 5 days. One day after the final asphalt exposure, rats were intratracheally inoculated with 5×10^5 *L. monocytogenes* cells (day 0). At 0 (prior to bacterial inoculation), 3, and 7 days after *L. monocytogenes* instillation, the lungs of each animal were divided. Bronchoalveolar lavage (BAL) was performed on right lungs. The recovered BAL cells were differentiated and counted, and chemiluminescence (CL) and nitric oxide (NO) production, indices of AM function, were determined. Also, albumin and lactate dehydrogenase (LDH), two indices of lung injury, were measured in the acellular BAL fluid. At the same time points, the left lungs were removed, homogenized, and bacterial number was determined. In addition, lung-draining lymph nodes were removed, and lymphocyte phenotype and asphalt-induced lymphocyte cytokine production were examined.

Asphalt Inhalation Exposure System

The asphalt fume generation and inhalation exposure systems used in this study have been previously described and characterized (Wang et al., 2001).

Briefly, road paving asphalt was preheated to 170°C in an oven, transferred to a reservoir (at 170°C), and passed through a heated pipe and onto a heated plate with the temperature maintained at 150°C at the inlet and 120°C at the outlet. These simulated asphalt fume generation temperatures are typical of those reported in the field during asphalt road paving. Humidity- and temperature-controlled air was blown across the plate to mix with asphalt vapor. The mixture was then transported through a heated pipe into the animal inhalation exposure chamber.

Teflon filters, having a diameter of 37 mm and a pore size of 0.45 μm, were used for gravimetric analysis of the fume in the exposure chamber. The filters were weighed immediately following the end of each sampling period during animal exposure to determine fume concentration. There were 6 sampling periods during each 6-h exposure. Fume concentration in the exposure chamber was determined by averaging the fume weight from each sampling period throughout the entire 5-day animal exposure period. Rats were exposed to a mean asphalt fume concentration of 72.6 ± 4.95 mg/m^3 for 6 h/day for 5 days. Air-exposed rats were placed in the inhalation chamber and provided an air sham exposure according to the same protocol as asphalt fume-exposed rats.

In addition, Teflon filters were backed with an XAD-2 sorbent tube and a charcoal sorbent tube to collect medium- and low-molecular-weight chemicals. The chemical analysis of these samples was described previously (Wang et al., 2001). Briefly, the gas to particle ratio of the generated asphalt fume was 1.43:1. It was determined that 70.2% of the formed particles were <1 μm as measured using an aerodynamic particle sizer (model 3320; TSI, Inc., St. Paul, MN) and a scanning mobility particle sizer (model 3071A, TSI, Inc., St. Paul, MN). The generated asphalt fume was determined to be >95% aliphatics as determined by infrared spectroscopy. Previously, it was observed that exposure to asphalt fumes generated with the asphalt inhalation system used in the current study activated lung cytochrome P-450 in a dose-dependent manner, indicating that the asphalt fumes had been delivered to the lower respiratory tract, as both Type II epithelial cells and alveolar macrophages possess cytochrome P-450 activity (Ma et al., 2003).

Animal Treatment

Male Sprague-Dawley rats [Hla:(SD)CVF], weighing 200–250 g, were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA). The animals were free of endogenous viral pathogens, parasites, mycoplasmas, *Helicobacter,* and CAR *Bacillus* and were kept in filtered ventilated cages provided with HEPA-filtered air, autoclaved Prolab 3500 diet, and tap water ad libitum under controlled light cycle (12 h light/12 h dark) and temperature (22–24°C) conditions. Facilities were AAALAC accredited, specific pathogen free, and environmentally controlled. The rats were acclimated to the animal facility for at least 1 wk after arrival. During the week before the in-

halation exposures, the animals that were to be exposed to the asphalt or filtered air were conditioned to the exposure chamber. Animals were placed in the cages of the exposure chamber for 6 h/day for 4 successive days before exposure. An additional set of animals was used as a nonexposed naive group that did not receive asphalt or filtered air to ensure that the stress of being exposed in the inhalation chamber had no effect on immune response. Evidence suggests that inhalation exposures may cause stress-induced impairment of antibacterial defenses in animals (Jakab & Hemenway, 1989). Body weight was measured each day throughout the conditioning, exposure, and infection phases of the study.

Intratracheal Inoculation with *L. monocytogenes*

Listeria monocytogenes (strain 10403S, serotype 1) was obtained as a gift from Rosana Schafer of the Department of Microbiology, Immunology, and Cell Biology at West Virginia University. One day after the final exposure to the asphalt fumes, rats were lightly anesthetized by an intraperitoneal injection of 0.6 ml of a 1% solution of sodium methohexital (Brevital, Eli Lilly, Indianapolis, IN) and intratracheally inoculated with 5×10^5 cells of *L. monocytogenes* in 500 µl of sterile saline as previously described (Antonini et al., 2000). This bacterial dose was found in an earlier study to give a uniform infection, did not kill untreated naive Sprague-Dawley rats, and was completely cleared from the lungs of infected animals by 10 days (Antonini et al., 2001a).

Bronchoalveolar Lavage

The rats were deeply anesthetized with an overdose of sodium pentobarbital (35 mg/kg body weight, ip, Butler Co., Columbus, OH) and then exsanguinated by severing the abdominal aorta. At 0 (prior to bacterial inoculation), 3, and 7 days after *L. monocytogenes* instillation, the left bronchus was clamped off, and BAL was performed on the right lungs of rats from each group. The right lungs were first lavaged with a 1-ml/100 g body weight aliquot of calcium- and magnesium-free phosphate buffered solution (PBS), pH 7.4. The first BAL fluid sample was centrifuged at 500 × g for 10 min, and the resultant cell-free supernatant was analyzed for various biochemical parameters. The lungs were further lavaged with 6-ml aliquots of PBS until 30 ml was collected. These samples also were centrifuged for 10 min at 500 × g and the cell-free BAL fluid was discarded. The cell pellets from all washes for each rat were combined, washed, and resuspended in 1 ml PBS buffer and evaluated as described next.

Cellular Evaluation

Total cell numbers were determined using a Coulter Multisizer II and AccuComp software (Coulter Electronics, Hialeah, FL). Cells were differentiated using a Cytospin 3 centrifuge (Shandon Life Sciences International, Cheshire, England). Cell suspensions (5×10^4 cells) were spun for 5 min at

800 rpm and pelleted onto a slide. Cells (200/rat) were identified after labeling with Leukostat stain (Fisher Scientific, Pittsburgh, PA).

Biochemical Parameters of Injury

Using the acellular first fraction of BAL fluid, albumin content, a measure to quantify increased permeability of the bronchoalveolar–capillary barrier, and lactate dehydrogenase (LDH) activity, an indicator of general cytotoxicity, were measured. Albumin content was determined colorimetrically at 628 nm based on albumin binding to bromcresol green using an albumin BCG diagnostic kit (Sigma Chemical Co., St. Louis, MO). LDH activity was determined by measuring the oxidation of lactate to pyruvate coupled with the formation of NADH at 340 nm. Measurements were performed with a Cobas Mira autoanalyzer (Roche Diagnostic Systems, Montclair, NJ).

Chemiluminescence

Luminol-dependent CL measures the light generated as reactive species are produced by activated cells. CL was performed with an automated Berthold Autolumat LB 953 luminometer (Wallace, Inc., Gaithersburg, MD) as previously described (Antonini et al., 1994). Luminol was used as an amplifier to enhance detection of the light, and 2 mg/ml of unopsonized zymosan (Sigma Chemical Co.) was added immediately prior to the measurement of CL to activate the AMs. Rat neutrophils (PMNs) have not been shown to respond to unopsonized zymosan in our system; therefore, the zymosan-stimulated CL produced is from AMs. Measurement of CL was recorded for 15 min at 37°C using 5×10^5 AMs, and the integral of counts versus time was calculated. Zymosan-stimulated CL was calculated as the total counts of stimulated cells minus the total counts of the corresponding resting cells.

Nitric Oxide Production

The reactive nitrogen intermediate, NO, has been shown to play an important role in antibacterial defenses against *L. monocytogenes* infection (Antonini et al., 2001b, 2002). Acellular BAL fluid was frozen immediately after collection for later determination of in vivo production of NO. The ex vivo production of NO was measured in the conditioned media of BAL cells recovered by lavage. BAL cells were incubated in 24-well plates in duplicate for 24 h at 37°C and 5% CO_2. The BAL cells were cultured in Eagle's minimum essential medium (MEM; BioWhittaker, Walkersville, MD) supplemented with 1 m*M* glutamine (GIBCO, Grand Island, NY), 10 m*M* HEPES, 100 U/ml of penicillin-streptomycin (GIBCO), 100 μg/ml of kanamycin (GIBCO), and 10% (v/v) fetal bovine serum (BioWhittaker) at a density of 1×10^6 cells/ml/well.

The NO oxidation products nitrate (NO_3) and nitrite (NO_2), collectively referred to as NO_x, were assayed in the cell-conditioned media or acellular BAL fluid samples. Samples were first incubated with *Esherichia coli* nitrate reductase to convert NO_3 to NO_2. Then NO_2 was measured colorimetrically

with the Greiss reaction (Green et al., 1982). NO_x levels were determined by comparing values to sodium nitrite standards. Conversion of NO_3 to NO_2 was confirmed in every assay by measuring the formation of NO_2 from NO_3 standards.

Lung Lavage Fluid Cytokine Analyses

Levels of cytokines—interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-2, IL-6, and IL-10—were assayed in the first fraction of BAL fluid at 0 (prior to bacterial inoculation), 3, and 7 days after *L. monocytogenes* instillation. The selection of the cytokines to be assayed was based on their potential role in lung inflammatory and immune responses to intracellular bacterial infection (Lange & Karol, 2000). TNF-α, IL-1β, and IL-6 are important proinflammatory cytokines produced by phagocytes (AMs and PMNs) and T cells, and are involved in acute-phase immune responses. IFN-γ is produced by lymphocytes (T and B cells) as well as natural killer cells and is involved in the cell-mediated immune response leading to activation of macrophages. IL-2 is secreted primarily by T helper cells and activates cell-mediated responses (in conjunction with IFN-γ) rather than humoral responses. IL-10 is an anti-inflammatory cytokine expressed by activated AMs and T and B lymphocytes and has been observed to inhibit AM activation and cytokine production involved with cell-mediated Th1 responses.

Cytokine protein concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Inc., Camarillo, CA). The results of the colorimetric assay were obtained with a Spectramax 250 plate spectrophotometer using Softmax Pro 2.6 software (Molecular Devices Corp., Sunnyvale, CA).

Pulmonary Clearance of *L. monocytogenes*

At 3 and 7 days after bacteria instillation, left lungs were removed from all rats in each treatment group. The excised tissues were suspended in 10 ml sterile water, homogenized using a Polytron 2100 homogenizer (Brinkmann Instruments, Westbury, NY), and cultured quantitatively on brain heart infusion agar plates (Becton Dickinson and Co., Cockeysville, MD). The number of viable colony-forming units (CFUs) was counted after an overnight incubation at 37°C.

Lymphocyte Isolation and T-Cell Subset Phenotypes

At 0, 3, and 7 days after bacteria instillation, all lung-draining lymph nodes were excised from each rat after the BAL procedure, teased apart with forceps, and single-cell suspensions were obtained by passing the cells through a nylon mesh bag (Tetko, Inc., Briarcliff Manor, NY) in RPMI 1640 media (GIBCO). Repeated passes of cell clumps were performed through a 22-gauge needle attached to a 10-ml syringe to disperse cells. It is possible that isolation of lymphocytes by the described procedure may affect lympho-

cyte function. The cell suspension was washed twice with the media and counted using a hemocytometer, which included trypan blue exclusion to assess viability.

To enumerate CD4+ and CD8+ T cell subsets in recovered lymphocytes at day 7, each respective cell type was stained with the appropriate monoclonal antibody which was conjugated with a fluorescent probe for visualization of 10^6 lymphocytes. After a 30-min incubation on ice in the dark, the cells were washed twice and fixed by suspending the cells in 0.4% paraformaldehyde in PBS. Flow cytometry data were collected with a Becton-Dickinson FACScan using FACScan Research Software (Becton-Dickinson Immunocytometry System, San Jose, CA) and analyzed using the PC-LYSYS software. Live lymphocytes were analyzed based on forward versus 90° scatter set to exclude dead cells as well as contaminating red blood cells, which are smaller than live lymphocytes.

Lymphocyte Production of Cytokines

Recovered lymphocytes were suspended in RPMI 1640 media containing 2 m*M* glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, and 10% heat-inactivated fetal bovine serum. Aliquots of 1 ml cell suspensions at a concentration of 2×10^6 lymphocytes were added to each well of a 24-well tissue culture plate and incubated at 37°C and 5% CO_2 for 24 h with concanavalin A (ConA; 2 μg/ml; Sigma Chemical Co.) or for 48 h with heat-killed *L. monocytogenes* (HKLM; 10^7/ml). The lymphocyte-conditioned media were collected and centrifuged (1200 × g for 4 min), and aliquots of the supernatants were stored at –70°C until assayed. The cells were treated with 0.5% Triton-X 100 at 37°C for 30 min, and the media were collected and centrifuged. The protein content in the supernatants was determined using Sigma Diagnostic reagents and procedures (Sigma Chemical Co.) on a Cobas Fara II analyzer (Roche Diagnostic System, Montclair, NJ). The results did not show a significant difference in protein content among the samples from various treatment groups. The levels of IFN-γ, IL-2, IL-6, and IL-10 in the culture supernatants were quantified by commercially available ELISA kits (Biosource International, Inc.).

Statistical Analysis

Results are expressed as means ± standard errors of measurement (SE). The significance of the interaction among the different treatment groups for the different parameters including all time points was assessed using a two-way analysis of variance (ANOVA). The significance of differences between individual groups was analyzed using the Tukey–Kramer post hoc test. For all analyses, the criterion of significance was set at $p < .05$.

RESULTS

Body Weight/Lung Clearance of Bacteria

Body weight was assessed throughout the exposure period to monitor the general health of the animals (Figure 1). During the 5-day inhalation

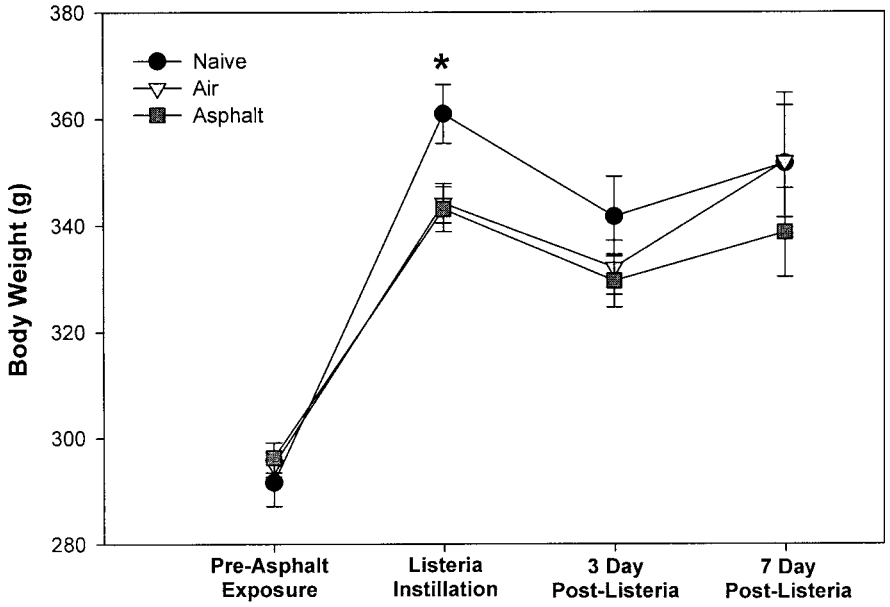

FIGURE 1. Body weight of rats preexposed to asphalt or filtered air before and after intratracheal inoculation with 5×10^5 cells *L. monocytogenes*. Naive animals served as a nonexposure group and were not preexposed to either asphalt or filtered air. Values are means ± SE (n = 6–16 rats/group at each time point); asterisk indicates significantly greater than air and asphalt values at time of bacteria instillation, $p < .05$.

exposure period, the body weight for both the asphalt and filtered air groups increased with time. The body weight for the nonexposed naive group also increased during the 5-day period, however, the weight was significantly greater at the time of *L. monocytogenes* inoculation compared with the asphalt and air groups. This difference was likely due to fact that the animals from the asphalt and air groups did not receive food during the 6-h exposure period on each of the 5 days. Body weight dropped for all groups at 3 days after bacteria inoculation, but started to rise again at 7 days. No significant difference in body weight was observed when comparing the groups at both 3 and 7 days after *L. monocytogenes* treatment.

To determine whether asphalt inhalation exposure had an effect on the ability of the lungs to clear bacteria after challenge, the number of lung CFUs was counted at 3 and 7 days after *L. monocytogenes* inoculation (Figure 2). A significantly greater number of left lung CFUs was counted for each group at 3 days after challenge compared to the number at day 7. However, a large portion of the bacteria had been cleared from the lungs of each group by day 7. No significant difference in bacterial clearance was observed when comparing the three groups at the two time points.

Evaluation of Recovered Bronchoalveolar Lavage Fluid and Cells

The number of recovered lung AMs and PMNs were counted as an index of inflammation, and albumin content and LDH activity were determined as

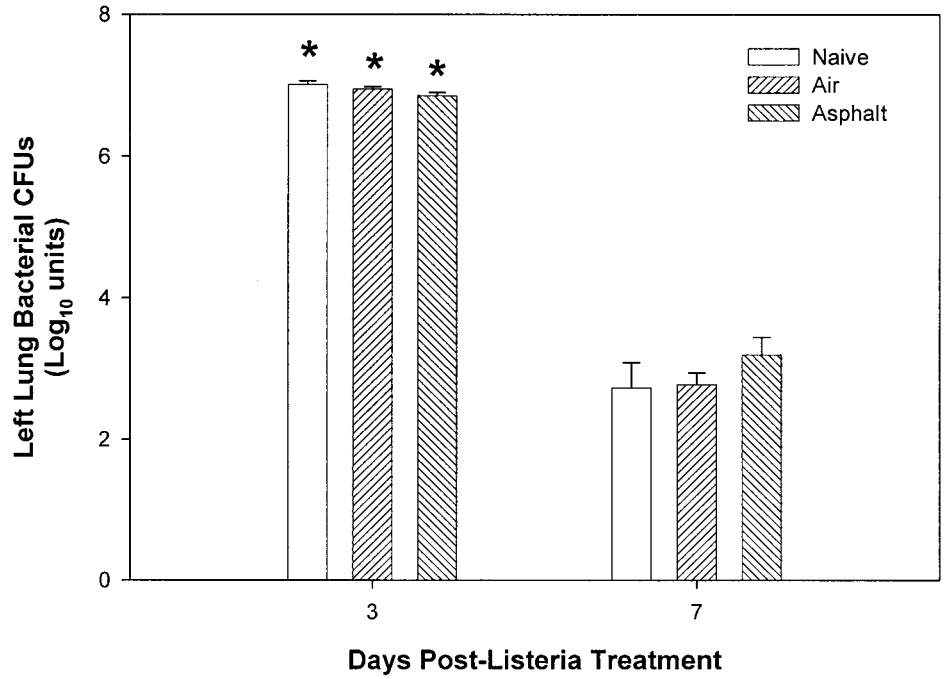

FIGURE 2. Number of bacteria CFUs in the left lung of rats preexposed to asphalt or filtered air prior to intratracheal inoculation with 5×10^5 cells *L. monocytogenes*. Naive animals served as a nonexposure group and were not preexposed to either asphalt or filtered air. Values are means in $\log_{10}$ base units ± SE ($n = 6$ rats/group at each time point); asterisk indicates significantly greater than values for all groups at day 7, $p < .05$.

measures of injury (Table 1). No significant difference was observed among the groups at day 0 for any of the parameters except for a significant elevation in AM number for asphalt. The lack of PMN, LDH, and albumin responses indicates that asphalt fume did not induce pulmonary inflammation and injury after a 5-day exposure. *Listeria monocytogenes* inoculation increased lung PMNs, albumin, and LDH activity in the 3 groups at 3 days after bacterial challenge. By 7 days after *L. monocytogenes* treatment, PMN number and albumin for all groups had returned to pre-bacteria-exposure levels, whereas LDH remained elevated. AM number for all groups was significantly elevated at day 7 compared to most values at days 0 and 3. Preexposure to asphalt fumes did not affect the inflammatory or injury response to bacterial challenge.

CL, an index of reactive species production, was measured from AMs collected from the different groups before and after bacteria treatment (Figure 3). CL production was relatively low for the groups before *L. monocytogenes* inoculation at day 0, with exposure to asphalt fumes having no effect on AM activity. Substantial elevations in CL were observed for the groups at day 3, as the AMs were highly activated in response to the pulmonary bacterial insult. However, no significant difference was observed at day 3 when comparing

TABLE 1. Lung inflammation and injury

Time (days)	Treatment	AMs (10^6)	PMNs (10^6)	Albumin (mg/ml)	LDH (U/L)
0	Naive	10.2 ± 0.71	0.17 ± 0.08	0.17 ± 0.01	130.8 ± 7.06
	Air	9.73 ± 0.79	0.11 ± 0.05	0.22 ± 0.03	144.3 ± 19.1
	Asphalt	13.5 ± 1.19[a]	0.15 ± 0.08	0.20 ± 0.02	178.0 ± 22.5
3	Naive	13.0 ± 1.33	11.6 ± 2.25[d]	0.78 ± 0.10[d]	306.7 ± 47.8[e]
	Air	8.96 ± 2.18	9.20 ± 3.37[d]	0.81 ± 0.12[d]	307.2 ± 26.6[e]
	Asphalt	8.02 ± 0.86	9.19 ± 1.72[d]	0.75 ± 0.03[d]	279.0 ± 25.1[e]
7	Naive	21.1 ± 3.32[b]	0.14 ± 0.08	0.31 ± 0.05	300.7 ± 63.8[e]
	Air	21.0 ± 4.35[b]	0.15 ± 0.02	0.27 ± 0.04	249.3 ± 56.1[f]
	Asphalt	17.9 ± 2.26[c]	0.24 ± 0.01	0.24 ± 0.03	205.5 ± 30.0[f]

Note. Values are mean ± SE (n = 4–6 rats/group at each time point).

[a]Significantly greater than the AM number for the naive and air groups at day 0; $p < .05$.

[b]Significantly greater than the AM number for all the groups at days 0 and 3; $p < .05$.

[c]Significantly greater than the AM number for the naive and air groups at day 0 and the air and asphalt groups at day 3; $p < .05$.

[d]Significantly greater than PMN number and albumin content for all groups at days 0 and 7; $p < .05$.

[e]Significantly greater than LDH activity for all groups at day 0; $p < .05$.

[f]Significantly greater than LDH activity for naive group at day 0; $p < .05$.

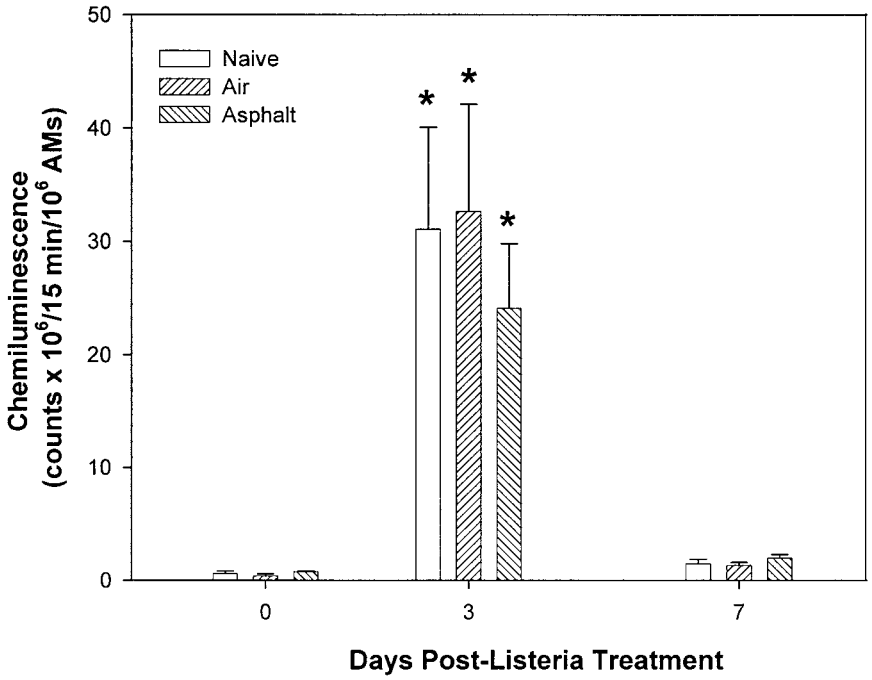

FIGURE 3. Zymosan-stimulated CL of AMs recovered from each group before and after intratracheal inoculation with 5×10^5 cells *L. monocytogenes*. Naive animals served as a nonexposure group and were not preexposed to either asphalt or filtered air. Values are means ± SE (n = 4–6 rats/group at each time point); asterisk indicates significantly greater than values for all groups at days 0 and 7, $p < .05$.

the three groups. By 7 days, CL production for all groups had returned to day 0 levels.

NO, a highly reactive nitrogen intermediate that has been shown to play an important role in AM-mediated defense against infection, was measured in the acellular BAL fluid samples and cell-conditioned media from the BAL cells of the different groups. Measurable amounts of NO were observed in both samples for each group 3 days after *L. monocytogenes* inoculation, but no significant difference was observed among the groups (Figure 4). In addition, a number of cytokines, which are important in lung immune responses, was measured in the acellular BAL fraction. No significant difference was observed in the lung cytokines measured among the groups at 3 days after bacteria challenge (Figure 5). Negligible amounts of NO and cytokine production were measured at day 0 and 7 days after *L. monocytogenes* treatment for any of the groups (data not shown).

Response of Lymphocytes Recovered from Lung-Draining Lymph Nodes

The total number of cells recovered from lung-draining lymph nodes for each group was counted after infection (Figure 6). No significant difference was observed among the groups when comparing the total number of lymph

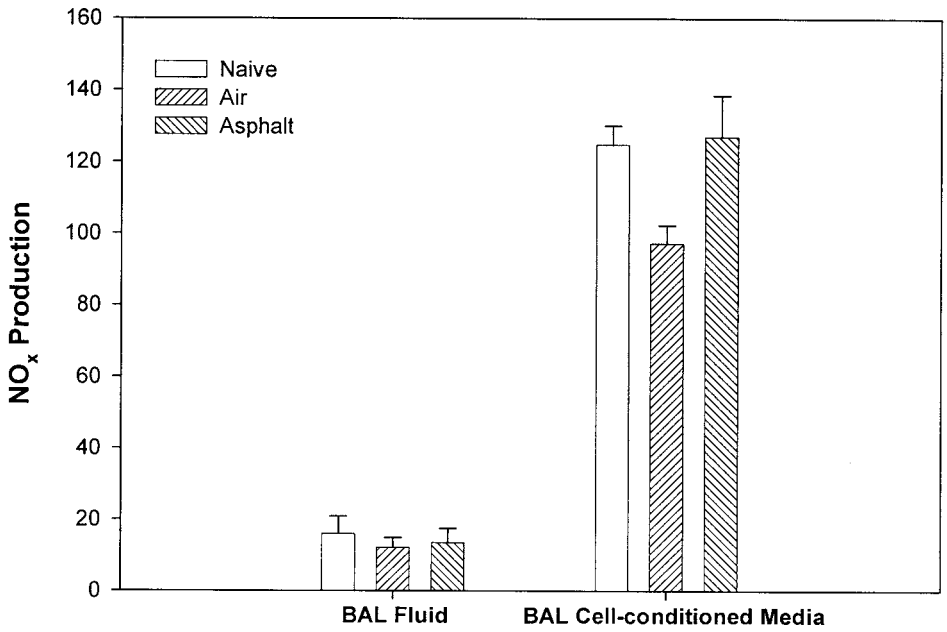

FIGURE 4. NO_x production measured in the acellular fraction of recovered BAL fluid (nmol/ml) and conditioned media from cultured BAL cells (nmol/10^6 cells) at 3 days after intratracheal inoculation with 5×10^5 cells *L. monocytogenes*. Naive animals served as a nonexposure group and were not preexposed to either asphalt or filtered air. Values are means ± SE (n = 6 rats/group at each time point).

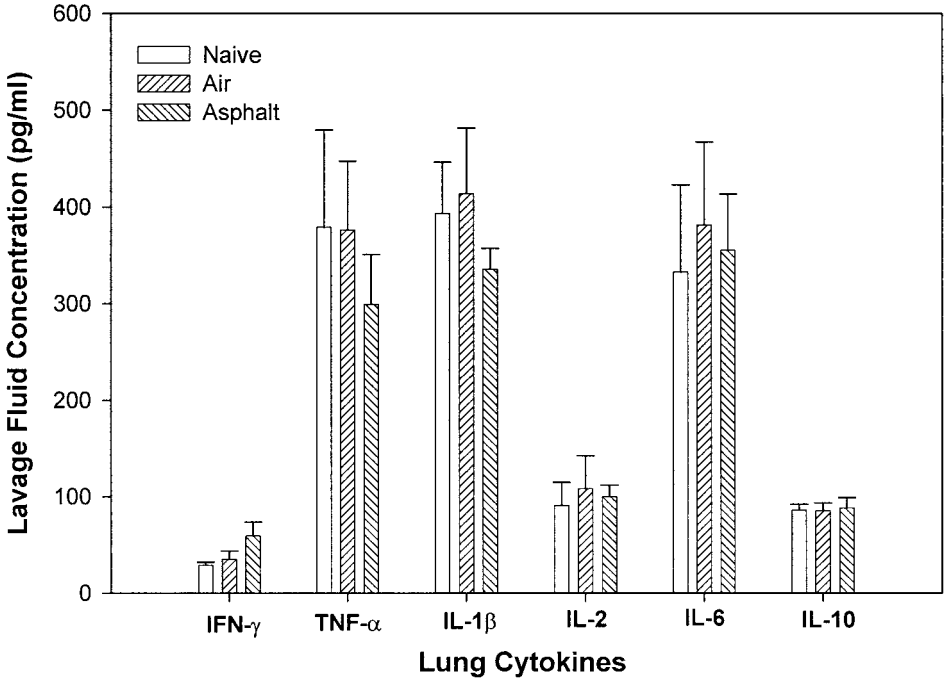

FIGURE 5. Lung cytokines measured in the acellular fraction of recovered BAL fluid at 3 days after intratracheal inoculation with 5×10^5 cells *L. monocytogenes*. Naive animals served as a nonexposure group and were not preexposed to either asphalt or filtered air. Values are means ± SE (n = 6 rats/group at each time point).

node cells at each time point (Figure 6A) and when comparing the percentage of CD4+ and CD8+ cells at 7 days after infection (Figure 6B).

The lymphocyte production of different cytokines which are possibly involved in immune responses was determined. The production of IFN-γ and IL-6 after activation of cultured lymphocytes with HKLM is depicted in Figure 7. Asphalt inhalation exposure had no significant effect on HKLM-stimulated production of IFN-γ from cultured lymphocytes recovered from rats 3 days after pulmonary bacterial inoculation (Figure 7A). However, lymphocyte IFN-γ secretion was significantly elevated in the asphalt group 7 days after infection. A significant increase in the HKLM-stimulated production of IL-6 was observed for the asphalt group only at day 3 post-*L. monocytogenes* infection (Figure 7B). No significant difference in lymphocyte IL-6 production was observed among the groups at day 7. Minimal amounts of IL-6 and IFN-γ were measured at day 0 before infection (data not shown).

The production of IL-2 and IL-10 after activation of cultured lymphocytes with ConA is shown in Figure 8. IL-2 production was minimal at 3 days after bacterial infection for each group, but by 7 days, lymphocyte IL-2 pro-

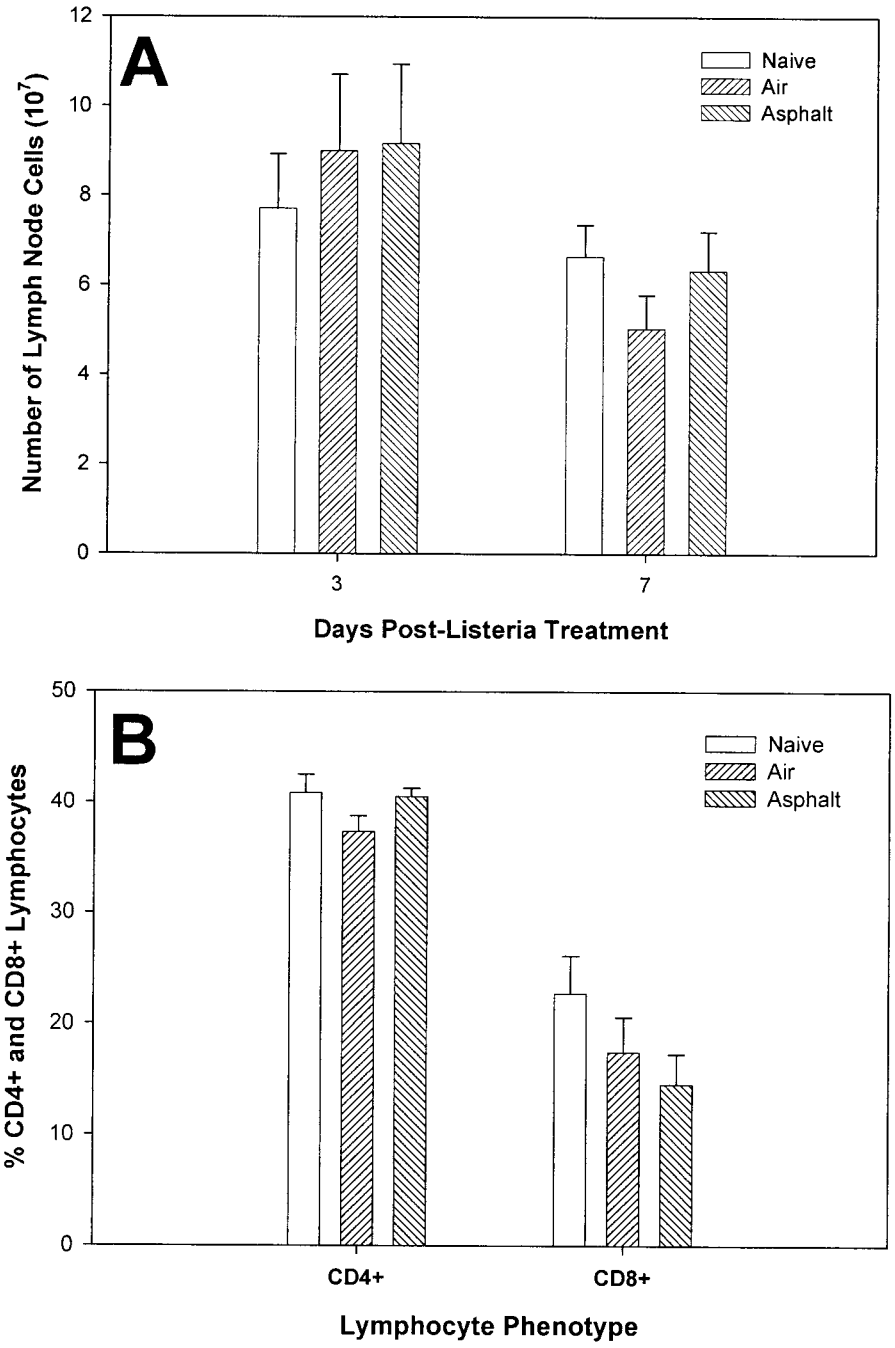

FIGURE 6. (A) Total number of lung-draining lymph node cells recovered from each group at 3 and 7 days after intratracheal inoculation with 5×10^5 cells *L. monocytogenes*. (B) Percentage of CD4+ and CD8+ cells recovered from lung-draining lymph nodes at 7 days after infection. Values are means ± SE (n = 4–6 rats/group at each time point).

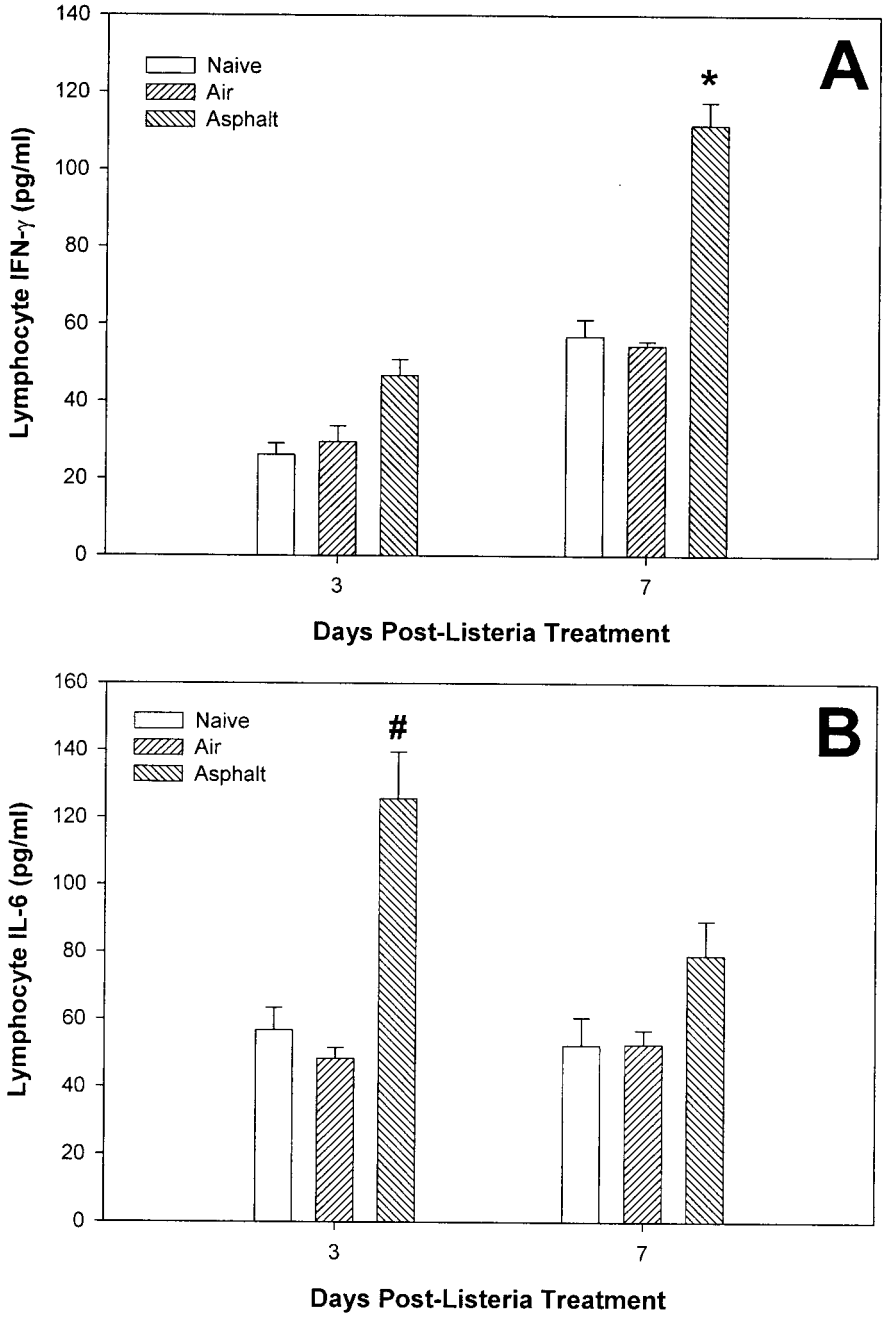

FIGURE 7. (A) Interferon-γ and (B) IL-6 production from cultured lymphocytes recovered from each group 3 and 7 days after intratracheal inoculation with 5×10^5 cells *L. monocytogenes*. Lymphokine production was determined after incubation for 48 h with heat-killed *L. monocytogenes* (10^7/ml). Values are means ± SE (n = 3–6 rats/group at each time point); asterisk indicates significantly greater than IFN-γ values for all groups, and #, significantly greater than IL-6 values for all groups, $p < .05$.

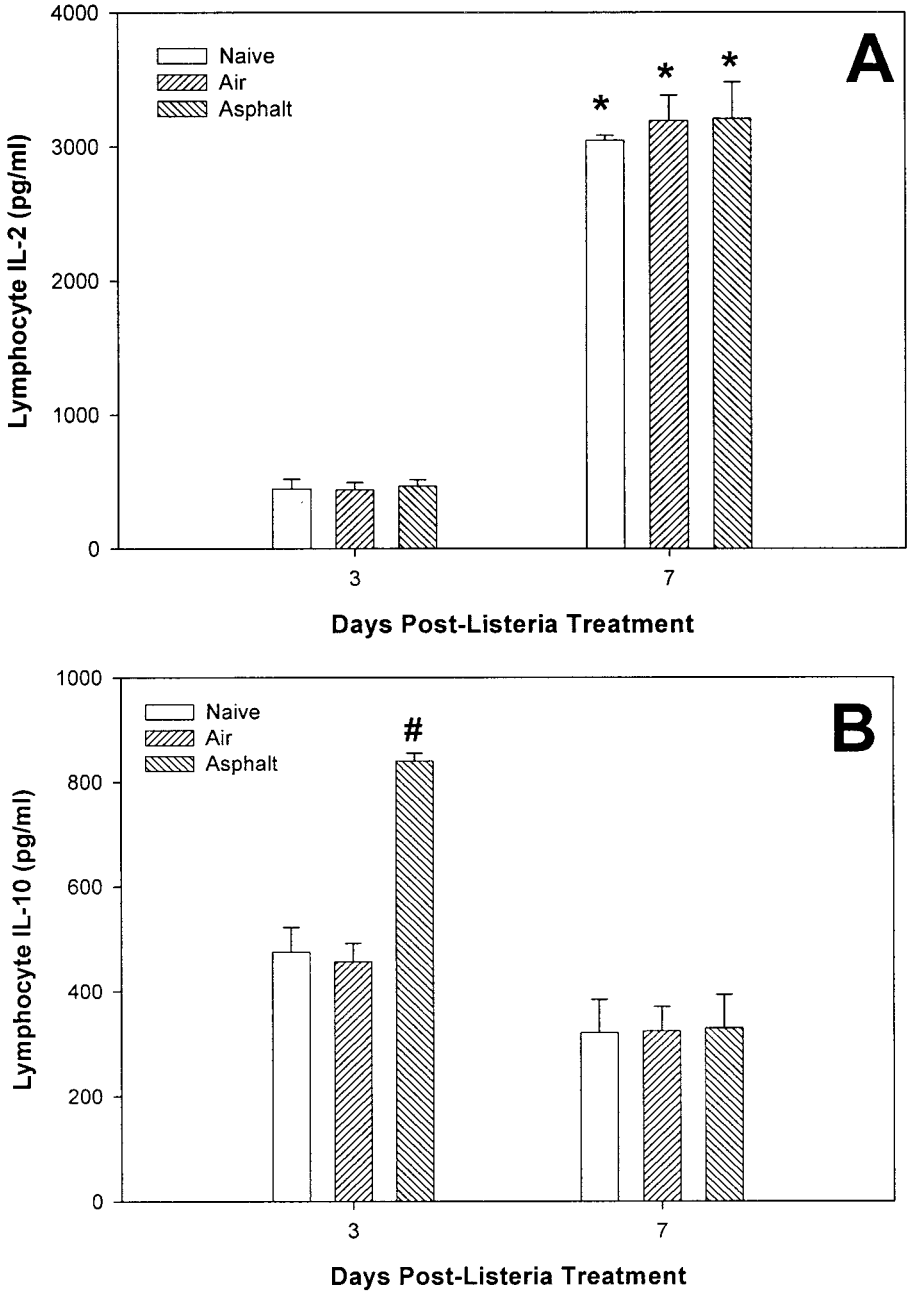

FIGURE 8. (A) IL-2 and (B) IL-10 production from cultured lymphocytes recovered from each group 3 and 7 days after intratracheal inoculation with 5×10^5 cells *L. monocytogenes*. Lymphokine production was determined after incubation for 24 h with concanavalin A (2 μg/ml). Values are means ± SE (n = 4–6 rats/group at each time point); asterisk indicates significantly greater than IL-2 values for all groups at day 3, and #, significantly greater than IL-10 values for all groups, $p < .05$.

duction was elevated in all groups (Figure 8A). No significant difference in IL-2 secretion was observed among the groups at each time point. A significant elevation in IL-10 production was observed in the asphalt group at day 3 (Figure 8B). By 7 days, the increase in IL-10 seen in the asphalt group had returned to control value. Negligible amounts of IL-2 and IL-10 were measured at day 0 before infection (data not shown).

DISCUSSION

Long-term inhalation exposure to different occupational agents may predispose workers in certain industries to an increased prevalence of respiratory infections. Animal infectivity models have been developed as a means to determine the mechanisms by which inhaled fumes and gases may increase the susceptibility to lung infection. The gram-positive, facultative intracellular bacterial pathogen *L. monocytogenes* has been extensively used to study lung defense mechanisms before and after treatment with different pneumotoxic substances (Van Loveren et al., 1988; Jakab, 1993; Reasor et al., 1996; Cohen et al., 2001, 2002). The initial response to inoculation with *L. monocytogenes* is characterized by the rapid activation of nonspecific immune responses (e.g., macrophage stimulation and PMN recruitment) to limit the spread of infection and by activation of natural killer (NK) cells (Seaman et al., 1999). In addition, the clearance of the *L. monocytogenes* also is dependent on the later development of acquired T-cell responses (Unanue, 1997; Shen et al., 1998). Thus, elimination of *L. monocytogenes* requires a strong symbiosis between innate immunity and the T-cell system (Unanue, 1997).

In previous studies, it has been demonstrated that the pulmonary clearance of *L. monocytogenes* may be affected by exposure to different environmental and occupational pollutants. Preexposure to residual oil fly ash has been shown to enhance lung injury and slow the pulmonary clearance of *L. monocytogenes* after infection (Antonini et al., 2002). In addition, ozone inhalation delayed the clearance of *L. monocytogenes* from the lungs (Van Loveren et al., 1988; Cohen et al., 2001). It was concluded that the slowing of the bacterial clearance caused by ozone and fly ash treatment was likely due to a suppression in the ability of AMs to produce bactericidal reactive oxygen (Cohen et al., 2001) and nitrogen species (Antonini et al., 2002). On the other hand, *L. monocytogenes* was observed to be cleared from the lungs at a much faster rate after subchronic exposure to a high dose of silica, despite extensive pulmonary inflammation and the presence of fibrosis (Antonini et al., 2000). Silica was found to upregulate innate immune responses, specifically AM phagocytosis and oxidant production.

It has been suggested that asphalt fumes may alter immune responses in laboratory animals and exposed workers (Davila et al., 1996; Karakaya et al., 1999; Diotte et al., 2001). It was our goal to assess the effect of asphalt fume inhalation on innate and adaptive lung defense mechanisms after infection. Asphalt fumes are a complex mixture comprised of numerous organic com-

pounds and inorganic particulates. Previously, it has been observed that pulmonary exposure to diesel exhaust particles, another chemically complex air pollutant with organic and inorganic components, suppressed lung defense mechanisms (Yang et al., 2001; Yin et al., 2002). In response to pulmonary inoculation with *L. monocytogenes,* diesel exhaust particles decreased the ability of AMs to produce antimicrobial oxidants and the proinflammatory cytokines, TNF-α and IL-1β (Yang et al., 2001), as well as compromised cell-mediated immunity by inhibiting AM secretion of IL-12 (Yin et al., 2002). In addition, it was concluded that the depressive effect of diesel exhaust particles on lung defenses was related to the organic components absorbed onto the particulate core (Yang et al., 2001). It would seem possible that the organic compounds associated with asphalt fumes also may modulate lung defense against infection.

Acute asphalt inhalation exposure at a relatively high dose did not induce lung injury or inflammation in rats before infection as assessed by cellular and acellular parameters within the recovered BAL fluid. In a related study, Ma et al. (2000) demonstrated that single and multiple intratracheal instillations of asphalt fume condensate did not result in lung damage or the production of reactive oxygen species and proinflammatory cytokines. Concerns can be raised that the intratracheal instillation administration of the asphalt fume condensate may not be appropriate. However, the results from the current inhalation study corroborate the finding that acute asphalt exposure does not cause acute lung injury in rats. It was only after infection with *L. monocytogenes* that elevations in lung AMs, PMNs, albumin, and LDH were observed. Importantly, no significant differences were seen in lung injury when comparing the asphalt group with the air and nonexposed naive groups, indicating that the increased lung response to pulmonary inoculation with bacteria was not affected by prior asphalt fume inhalation.

In addition, asphalt inhalation exposure did not affect the rate of clearance of *L. monocytogenes* from the lungs. The amount of bacteria in the lungs of the three treatment groups was not significantly different at the time points examined and was almost completely cleared by 7 days. These bacterial clearance results are not surprising, considering that acute asphalt inhalation exposure had no effect on nonspecific lung defense responses. AM production of reactive oxygen and nitrogen species and cytokine secretion measured in the BAL fluid of asphalt-treated rats were not different from air and nonexposed naive groups before or after infection. Unlike the suppressive effect of diesel exhaust particles (Yang et al., 2001; Yin et al., 2002) and fly ash (Antonini et al., 2002) or the stimulatory effect of silica (Antonini et al., 2000) on AM function, asphalt fume exposure did not alter nonspecific innate lung defenses to infection in rats.

In the assessment of adaptive immune responses, acute asphalt inhalation did cause some minor alterations in lymphocyte function. The secretion of specific lymphokines was elevated in some instances after bacterial inoculation in rats preexposed to asphalt fumes, even though the total number of

lymph node cells and the percentage of CD4+ and CD8+ cells were not significantly different among the treatment groups. Asphalt-related increases in lymphocyte IFN-γ, IL-6, and IL-10 were observed at different times after bacterial infection. Presumably, elevations in IFN-γ and IL-6 would likely enhance immune responses and improve the lungs' defense against infection, whereas increases in IL-10 secretion would be suggestive of possible suppressive effects in immune defense responses.

Moreover, it has been clearly documented that the release of IFN-γ plays an important role in regulating bactericidal activity by stimulation of AMs and CD8+ cells (Unanue, 1997). It has been observed that mice lacking the genes for IFN-γ (Dalton et al., 1993) or for the α-chain of the IFN-γ receptor (Huang et al., 1993) were more susceptible to *L. monocytogenes* infection. In addition, the absence of endogenous IL-6 impaired the development of antibacterial immune responses and severely compromised the ability of IL-6-deficient mice to clear *L. monocytogenes* infection (Dalrymple et al., 1995). The increases in lymphocyte IL-6 and IFN-γ observed in the current study would support bacterial clearance from the lungs. On the other hand, IL-10 suppresses immune response by inhibiting IFN-γ and granulocyte–macrophage colony-stimulating factor (GM-CSF) cytokine production by Th1 cells (Fiorentino et al., 1989; Moore et al., 2001). Importantly, increases in lymphocyte IL-2 production at day 7 after infection were observed for all groups in the current study, which is indicative of an activated cell-mediated immune defense and a properly functioning adaptive response.

Despite the alterations observed in lymphokine secretion related to asphalt exposure in the current study, pulmonary clearance of *L. monocytogenes* was found to be nearly identical for the different treatment groups. Because innate nonspecific lung responses were unaffected by asphalt exposure, it can be concluded that nonspecific defenses were sufficient to control the infection. Also, adaptive immune function appeared to function normally, as evidenced by increased lymphocyte IL-2 production at day 7, despite the asphalt-induced changes observed in the secretion of IFN-γ, IL-6, and IL-10. Thus, it was observed that acute inhalation of asphalt fumes at a high concentration had little effect on lung defense mechanisms. The question still remains as to whether lung defense responses to infection are affected differently after more chronic inhalation exposures to asphalt fumes.

REFERENCES

Antonini, J. M., Van Dyke, K., Ye, Z., DiMatteo, M., and Reasor, M. J. 1994. Introduction of luminol-dependent chemiluminescence as a method to study silica inflammation in the tissue and phagocytic cells of rat lung. *Environ. Health Perspect.* 102(suppl. 10):37–42.

Antonini, J. M., Yang, H.-M., Ma, J. Y. C., Roberts, J. R., Barger, M. W., Butterworth, L., Charron, T. G., and Castranova, V. 2000. Subchronic silica exposure enhances respiratory defense mechanisms and the pulmonary clearance of *Listeria monocytogenes* in rats. *Inhal. Toxicol.* 12:1017–1036.

Antonini, J. M., Roberts, J. R., and Clarke, R. W. 2001a. Strain-related differences of non-specific respiratory defense mechanisms in rats using a pulmonary infectivity model. *Inhal. Toxicol.* 13:85–102.

Antonini, J. M., Roberts, J. R., Clarke, R. W., Yang, H.-M., Barger, M. W., Ma J. Y. C., and Weissman, D. N. 2001b. Effect of age on respiratory defense mechanisms: Pulmonary bacterial clearance in Fischer 344 rats after intratracheal instillation of *Listeria monocytogenes*. *Chest* 120:240–249.

Antonini, J. M., Roberts, J. R., Jernigan, M. R., Yang, H.-M., Ma, J. Y. C., and Clarke, R. W. 2002. Residual oil fly ash increases the susceptibility to infection and severely damages the lungs after pulmonary challenge with a bacterial pathogen. *Toxicol. Sci.* 70:110–119.

Asphalt Institute.1989. *United States asphalt usage report.* College Park, MD.

Asphalt Institute. 1990. In *Report to OSHA and NIOSH: Status of Asphalt Industry Steering Committee research program on the health effects of asphalt fumes and recommendation for a worker health standard*. Lexington, KY.

Bonnet, P., Binet, S., Brandt, H., Kriech, A. J., Lafontaine, M., Nunge, H., Morele, Y., DeGroot, P., Wissel, H., and Castegnaro, M. 2000. Inhalation study on exposure to bitumen fumes. Part 1: Development and validation of the equipment. *Ann. Occup. Hyg.* 44:15–29.

Burgaz, S., Erdem, O., Karahalil, B., and Karakaya, A. E. 1998. Cytogenetic biomonitoring of workers exposed to bitumen fumes. *Mutat. Res.* 419:123–130.

Burstyn, I., Kromhout, H., Kauppinen, T., Heikkila, P., and Boffetta, P. 2000. Statistical modeling of the determinants of historical exposure to bitumen and polycyclic aromatic hydrocarbons among paving workers. *Ann. Occup. Hyg.* 44:43–56.

Cohen, M. D., Sisco, M., Li, Y., Zelikoff, J. T., and Schlesinger, R. B. 2001. Ozone-induced modulation of cell-mediated immune responses in the lungs. *Toxicol. Appl. Pharmacol.* 171:71–84.

Cohen, M. D., Sisco, M., Baker, K., Li, Y., Lawrence, D., Van Loveren, H., Zelikoff, J. T., and Schlesinger, R. B. 2002. Effects of inhaled ozone on pulmonary immune cells critical to antibacterial responses in situ. *Inhal. Toxicol.* 14:599–619.

Dalrymple, S. A., Lucian, L. A., Slattery, R., McNeil, T., Aud, D. M., Fichno, S., Lee, F., and Murray, R. 1995. Interleukin-6-deficient mice are highly susceptible to *Listeria monocytogenes* infection: Correlation with inefficient neutrophilia. *Infect. Immun.* 63:2262–2268.

Dalton, D. K., Pitts, M. S., Keshav, S., Figari, I., Bradley, A., and Stewart, T. A. 1993. Multiple defects of immune cell function in mice with disrupted interferon-γ genes. *Science* 259:1739– 1742.

Davila, D. R., Romero, P. L., and Burchiel, D. W. 1996. Human T cells are highly sensitive to suppression of mitogenesis by polycyclic aromatic hydrocarbons and this effect is differentially reversed by alpha-napthoflavone. *Toxicol. Appl. Pharmacol.* 139:333–341.

Diotte, N. M., Munson, A. E., Tomblyn, S., and Meade, B. J. 2001. Asphalt fume-induced immunosuppression in B6C3F1 mice. *Toxicol. Sci.* 60:27 (abstr.).

Fiorentino, D. F., Bond, M. W., and Mosmann, T. R. 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170:2081–2095.

Fuchs, J., Hengstler, J. G., Boettler, G., and Oesch, F. 1996. Primary DNA damage in peripheral mononuclear blood cells of workers exposed to bitumen-based products. *Int. Arch. Occup. Environ. Health* 68:141–146.

Gamble, J. F., Nicolich, M. J., Barone, N. J., and Vincent, W. J. 1999. Exposure-response of asphalt fumes with changes in pulmonary function and symptoms. *Scand. J. Work Environ. Health* 25:186–206.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R. 1982. Analysis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. *Anal. Biochem.* 126:131–138.

Huang, S., Hendriks, W., Althage, A., Hemmi, S., Bluethmann, H., Kamijo, R., Vilcek, J., Zinkernagel, R. M., and Auget, M. 1993. Immune response in mice that lack the interferon-γ receptor. *Science* 259:1742–1745.

Jakab, G. J. 1993. The toxicologic interactions resulting from inhalation of carbon black and acrolein on pulmonary antibacterial and antiviral defenses. *Toxicol. Appl. Pharmacol.* 121:167–175.

Jakab, G. J., and Hemenway, D. R. 1989. Restraint of animals required for nose-only inhalation toxicologic studies induced stress which suppresses pulmonary ant-bacterial defense. *Inhal. Toxicol.* 1: 289–300.

Jarvholm, B., Nordstrom, G., Hogstedt, B., Levin, J. O., Wahlstrom, J., Ostman, C., and Bergendahl, C. 1999. Exposure to polycyclic aromatic hydrocarbons and genotoxic effects on nonsmoking Swedish road pavement workers. *Scand. J. Work Environ. Health* 25:131–136.

Karakaya, A., Yucesoy, B., Turhan, A., Erdem, O., Burgaz, S., and Karakaya, A. E. 1999. Investigation of some immunological functions in a group of asphalt workers exposed to polycyclic aromatic hydrocarbons. *Toxicology* 135:43–47.

King, R. W., Puzinauskas, V. P., and Holdsworth, C. E. 1984. Asphalt composition and health effects: A critical review. In *Technical publication: American Petroleum Institute,* pp. 1–11. Washington, DC.

Lange, R. W., and Karol, M. H. 2000. Immunology of the respiratory tract. In *Pulmonary immunotoxicology,* eds. M. D. Cohen, J. T. Zelikoff, and R. B. Schlesinger, pp. 61–84. Boston: Kluwer Academic.

Ma, J. Y. C., Barger, M. W., Kriech, A. J., and Castranova, V. 2000. Effects of asphalt fume condensate exposure on acute pulmonary responses. *Arch. Toxicol.* 74:452–459.

Ma, J. Y. C., Yang, H.-M., Barger, M. W., Siegel, P. D., Zhong, B.-Z., Kriech, A. J., and Castranova, V. 2002. Alteration of pulmonary cytochrome P-450 system: Effect of asphalt fume condensate exposure. *J. Toxicol. Environ. Health A* 65:1247–1260.

Ma, J. Y. C., Rengamasy, A., Frazer, D., Barger, M. W., Hubbs, A. F., Batteli, L., Tomblyn, S., Stone, S., and Castranova, V. 2003. Inhalation exposure of rats to asphalt fumes generated at paving temperatures alters pulmonary xenobiotic metabolism pathways without lung injury. *Environ. Health Perspect.* 111:1215–1221.

Moore, K. W., de Waal Malefyt, R., Coffman, R. L., and O'Garra, A. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19:683–765.

NIOSH. 2003. Asphalt fume exposures during the application of hot asphalt to roofs. Current practices for reducing exposure. In: Department of Health and Human Services NIOSH Publication No. 2003–112, p. 2. Cincinnati, OH.

Qian, H.-W., Ong, T., and Whong, W.-Z. 1996. Induction of micronuclei in cultured mammalian cells by fume condensates of roofing asphalt. *Am. J. Ind. Med.* 29:554–559.

Qian, H.-W., Ong, T., Nath, J., and Whong, W.-Z. 1998. Induction of DNA adducts *in vivo* in rat lung cells by fume condensates of roofing asphalt. *Teratogen. Carcinogen. Mutagen.* 18:131–140.

Qian, H.-W., Whong, W.-Z., Olsen, L., Nath, J., and Ong, T. 1999. Induction of micronuclei in V79 cells by fractions of roofing asphalt fume condensate. *Mutat. Res.* 441:163–170.

Reasor, M. J., McCloud, C. M., DiMatteo, M., Schafer, R., Ima, A., and Lemaire I. 1996. Effects of amiodarone-induced phospholipidosis on pulmonary host defense functions in rats. *Proc. Soc. Exp. Biol. Med.* 211:346–352.

Seaman, M. S., Pararnau, B., Fischer Lindahl, K., Lemonnier, F. A., and Forman, J. 1999. Response to *Listeria monocytogenes* in mice lacking MHC class Ia molecules. *J. Immunol.* 162:5429– 5436.

Shen, H., Tato, C. M., and Fan, X. 1998. *Listeria monocytogenes* as a probe to study cell-mediated immunity. *Curr. Opinion Immunol.* 10:450–458.

Sivak, A., Niemeier, R., Lynch, D., Beltis, K., Simon, S., Salomon, R., Latta, R., Belinsky, B., Menzies, K., Lunsford, A., Cooper, C., Ross, A., and Bruner, R. 1997. Skin carcinogenicity of condensed asphalt roofing fumes and their fractions following dermal application to mice. *Cancer Lett.* 117:113–123.

Toraason, M., Hayden, C., Marlow, D., Rinehart, R., Mathias, P., Werren, D., DeBord, D. G., and Reid, T. M. 2001. DNA strand breaks, oxidative damage, and 1-OH pyrene in roofers with coal-tar pitch dust and/or asphalt fume exposure. *Int. Arch. Occup. Environ. Health* 74:396–404.

Unanue, E. R. 1997. Studies in listeriosis show the strong symbiosis between the innate cellular system and T-cell response. *Immunol. Rev.* 158:11–25.

Van Loveren, H., Rombout, P. J. A., Wagenaar, S. S., Walvoot, H. C., and Vos, J. G. 1988. Effects of ozone on the defense to a respiratory Listeria monocytogenes infection in the rat. *Toxicol. Appl. Pharmacol.* 94:374–393.

Wang, J., Lewis, D. M., Castranova, V., Frazer, D. G., Goldsmith, T., Tomblyn, S., Simpson, J., Stone, S., Afshari, A., and Siegel, P. D. 2001. Characterization of asphalt fume composition under simulated road paving conditions by GC/MS and microflow LC/quadrapole time-of-flight MS. *Anal. Chem.* 73:3691–3700.

Watts, R. R., Wallingford, K. M., Williams, R. W., House, D. E., and Lewtas, J. 1998. Airborne exposures to PAH and $PM_{2.5}$ particles for road paving workers applying conventional asphalt and crumb rubber modified asphalt. *J. Expos. Anal. Environ. Epidemiol.* 8:213–229.

Yang, H.-M., Antonini, J. M., Barger, M. W., Butterworth, L., Roberts, J. R., Ma, J. K. H., Castranova, V.,

and Ma, J. Y. C. 2001. Diesel exhaust particles suppress macrophage function and slow the pulmonary clearance of *Listeria monocytogenes* in rats. *Environ. Health Perspect.* 109:515–521.

Yin, X.-J., Schafer, R., Ma, J. Y. C., Antonini, J. M., Weissman, D. N., Siegel, P. D., Barger, M. W., Roberts, J. R., and Ma, J. K. H. 2002. Alteration of pulmonary immunity to *Listeria monocytogenes* by diesel exhaust particles (DEPs). I. Effects of DEPs on early pulmonary responses. *Environ. Health Perspect.* 110:1105–1111.