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Effects of asphalt fume condensate exposure on acute pulmonary responses

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Abstract *Objective:* The present study was carried out to characterize the effects of in vitro exposure to paving asphalt fume condensate (AFC) on alveolar macrophage (AM) functions and to monitor acute pulmonary responses to in vivo AFC exposure in rats. *Methods:* For in vitro studies, rat primary AM cultures were incubated with various concentrations of AFC for 24 h at 37 °C. AM-conditioned medium was collected and assayed for lactate dehydrogenase (LDH) as a marker of cytotoxicity. Tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) production were assayed in AM-conditioned medium to monitor AM function. The effect of AFC on chemiluminescence (CL) generated by resting AM or AM in response to zymosan or PMA stimulation was also determined as a marker of AM activity. For in vivo studies, rats received either (1) a single intratracheal (IT) instillation of saline, or 0.1 mg or 0.5 mg AFC and were killed 1 or 3 days later; or (2) IT instillation of saline, or 0.1, 0.5, or 2 mg AFC for three consecutive days and were killed the following day. Differential counts of cells harvested by bronchoalveolar lavage were measured to monitor inflammation. Acellular LDH and protein content in the first lavage fluid were measured to monitor damage. CL generation, TNF- α and IL-1 production by AM were assayed to monitor AM function. *Results:* In vitro AFC exposure at <200 $\mu\text{g/ml}$ did not induce cytotoxicity, oxidant generation, or IL-1 production by AM, but it did cause a small but significant increase in

TNF- α release from AM. In vitro exposure of AM to AFC resulted in a significant decline of CL in response to zymosan or PMA stimulation. The in vivo studies showed that AFC exposure did not induce significant neutrophil infiltration or alter LDH or protein content in acellular lavage samples. Macrophages obtained from AFC-exposed rats did not show significant differences in oxidant production or cytokine secretion at rest or in response to LPS in comparison with control macrophages. *Conclusions:* These results suggest that: (1) in vitro AFC exposure did not adversely affect cell viability or induce the release of high levels of inflammatory cytokines or oxidants; and (2) exposure of rats to AFC did not cause acute pulmonary inflammation or injury, and did not significantly alter AM functions.

Key words Paving asphalt · Asphalt fume condensate · Alveolar macrophage · Pulmonary inflammation · Lung injury

Introduction

Asphalt fumes are complex mixtures of particulate and organic compounds and may pose a potential health risk to exposed workers. Approximately 300,000 workers are currently employed in the asphalt paving industry in the U.S. (Miller and Burr 1996). The current concern for these workers includes both dermal exposure to asphalts and inhalation exposure to asphalt fumes. These fumes consist of an inorganic part, dust from the mineral aggregates, and a highly complex mixture of paraffinic and polycyclic aromatic hydrocarbons (PAHs) and heteroatomic compounds containing sulfur, nitrogen, and oxygen (King et al. 1984). Due to the presence of PAHs in asphalt, most studies of the health hazards for asphalt exposure have been centered on carcinogenicity (Machado et al. 1993; Qian et al. 1998, 1999; Sivak et al. 1997), while very few studies have been devoted to characterization of the effects of asphalt fumes on non-cancerous responses in the lung.

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Asphalt workers (road workers and roof layers) have reported symptoms of mucous membrane and skin irritations, but decreased respiratory function has not been demonstrated (Nyqvist 1978; Waage 1987). Norseth et al. (1991) have reported a correlation between subjective symptoms and asphalt temperature or asphalt fume concentration among road repair and construction asphalt workers. Other nonmalignant pulmonary effects, such as bronchitis, emphysema, and asthma have been reported among roofers (Hammond et al. 1976), mastic asphalt workers (Hansen 1991), and California highway workers (Maizlish et al. 1988). Recently NIOSH have conducted health assessment evaluations at several locations and showed an increased incidence of mucous membrane irritation among road pavers (Almaguer et al. 1995; Hanley and Miller 1994a, b; Kinnes et al. 1996; Miller and Burr 1995, 1996), with a few pavers exposed to a crumb rubber asphalt mix exhibiting a work-shift decline in peak expiratory flow rate (Kinnes et al. 1996; Miller and Burr 1996). However, Gamble et al. (1999) have reported that there is no consistent association between an acute reduction in lung function or the incidence of symptoms among workers exposed to asphalt fumes. The few studies reported suggest that paving asphalt fumes may exhibit pulmonary toxicity. At present, there is a serious lack of biochemical and physiological information related to the mechanisms involved in such asphalt fume-induced lung injury.

The respiratory system is considered a prime target for potential adverse effects of exposure to asphalt fumes. Alveolar macrophages (AM) play an important role in the response of the lung to inhaled environmental particles or fumes. AM are responsible for the clearance of particles and microorganisms from the distal airways and the alveolar spaces. Activated AM are known to release reactive oxygen species (ROS) and a wide variety of mediators. The ROS produced by AM are highly reactive and can interact with biological membranes to cause cell damage. Proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) are produced by activated AM and promote the inflammatory process by recruiting polymorphonuclear leukocytes (PMN) into the airspaces and stimulating these phagocytes to release ROS and enzymes (Laskin and Pendino 1995; Le and Vileck 1987). Activation of AM is believed to play a key role in the development and progression of pulmonary disease in response to particles such as silica or asbestos. Inhalation exposure to organic substances, such as diesel exhaust particles (DEP), has also been shown to induce pulmonary inflammation and proinflammatory cytokine production (Yang et al. 1999b). However, the depressive effect of DEP on AM responsiveness to microbial products differs from that of carbon black, particles which have a carbonaceous core similar to that of DEP but contain a markedly lower amount of adsorbed organic compounds, suggesting that the organic components adsorbed on DEP may play an important role in modulating macrophage antimicrobial function (Yang et al. 1997, 1999a, b). These studies have shown that DEP

exposure suppresses proinflammatory cytokine secretion by AM in responses to LPS stimulation, which may be a contributing factor to the increased susceptibility to pulmonary infection after prolonged DEP exposure (Castranova et al. 1985; Hahon et al. 1985).

It has been shown in our laboratory as well as others that the inflammatory response of the lung on exposure to occupational or environmental agents can be readily demonstrated by analysis of bronchoalveolar lavage fluid (BALF) of the exposed animals (Driscoll et al. 1990; Ma et al. 1999; Yang et al. 1999b). Cellular changes in BALF during acute inflammation include an influx of PMN and activation of AM. The protein content and cytoplasmic enzyme activity, e.g. lactate dehydrogenase (LDH), in acellular BALF provide important information concerning the degree of acute lung injury. Secretion of oxidants and cytokines from harvested AM are important markers of macrophage functions. To date the effects of exposure to asphalt fumes on pulmonary inflammation and lung injury have not been elucidated. The present study was designed to evaluate the acute pulmonary responses to paving asphalt fume condensate (AFC) exposure. The objectives of this study were to investigate: (1) the effects of *in vitro* exposure to AFC on AM functions; and (2) the effects of *in vivo* exposure of rats to AFC on acute pulmonary inflammation and lung damage.

Materials and methods

Asphalt fume condensate

AFC was collected at the top of a paving storage tank (at Asphalt Materials Indianapolis, Ind.) by cold trap using the same pumps and traps as used in the laboratory fume generator described by Sivak et al. (1997). The paving asphalt was a PG 64-22 used on the I-65 (1997) project collected at 160 °C. AFC stock solution was made up in DMSO and subsequently diluted with medium or buffer to the desired final concentration with a final DMSO concentration of <1%.

Treatment of animals

Male Sprague-Dawley rats (about 250 g) were obtained from Hilltop Laboratories (Scottsdale, Pa.). Rats were acclimatized for 1 week in an AAALAC approved animal facility before use. For *in vivo* AFC exposure studies, animals were lightly anesthetized with sodium methohexital (Brevitol; Eli Lilly Co., Indianapolis, Ind.). Once the rats were anesthetized, intratracheal (IT) instillation was performed. The experimental design consisted of three treatment groups: (1) a saline-treated group that received an IT instillation of 0.25 ml sterile saline (control); (2) an AFC-treated group that received an IT instillation of 0.1 mg, 0.5 mg, or 2 mg AFC in 0.25 ml sterile saline; and (3) a solvent control group that received an IT instillation of 0.25 ml 1% DMSO which was equivalent to the final DMSO concentration contained in a 2-mg AFC sample. Rats from each treatment group were killed either 1 day or 3 days after exposure. To determine the cumulative exposure effects on animals, another set of rats received IT instillations for three consecutive days and were killed the following day.

Isolation of alveolar macrophages

Animals were anesthetized with sodium pentobarbital (0.2 g/kg body weight) and exsanguinated by cutting the renal artery.

Alveolar macrophages were obtained by pulmonary lavage with a Ca^{++} -, Mg^{++} -free phosphate-buffered medium (145 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4 , 9.35 mM Na_2HPO_4 , and 5.5 mM glucose; pH 7.4). A total of 80 ml BALF was collected from each animal and centrifuged at 500 *g* for 5 min. Cell pellets were combined, washed, and resuspended in a HEPES-buffered medium (145 mM NaCl, 5 mM KCl, 10 mM HEPES, 5.5 mM glucose, and 1.0 mM CaCl_2 ; pH 7.4). For *in vivo* studies, the acellular supernate from the first lavage (6 ml) was saved separately from the following lavages for further analysis. Cell counts and purity were measured using an electronic cell counter equipped with a cell sizing attachment (Coulter model Multisizer II with a 256C channelizer; Coulter Electronics, Hialeah, FL).

Alveolar macrophage cultures

Lavage cells were resuspended in Eagle's minimum essential medium (EMEM; BioWhittaker, Walkersville, Md.) containing 1 mM glutamine, 100 $\mu\text{g}/\text{ml}$ streptomycin, 100 U/ml penicillin, and 10% heat-inactivated bovine serum. Aliquots of 1 ml containing 1×10^6 AM were added to a 24-well tissue culture plate and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 2 h. The nonadherent cells were then removed with three washes of EMEM and the adherent macrophage-enriched cells were used for all AM culture studies. For *in vitro* studies AM were incubated in fresh EMEM with various amounts of AFC for an additional 24 h. The macrophage-conditioned medium was collected, centrifuged, and the supernatant collected. An aliquot of this conditioned medium was used for LDH analysis and the rest was stored in aliquots at -80 °C for further analysis of TNF- α and IL-1. For the *in vivo* studies the acellular LDH, protein and nitric oxide (NO) content in the first lavage fluid were determined. AM were cultured as mentioned above and NO, TNF- α , and IL-1 were determined in the culture medium.

Chemiluminescence determination

Chemiluminescence (CL) generated by AM was determined using an automated luminometer (Berthold Autolumat LB 953; Wallac, Gaithersburg, Md.). Rat AM ($0.75 \times 10^6/0.75$ ml) were preincubated at 37 °C for 10 min in HEPES-buffered medium containing 0.08 $\mu\text{g}/\text{ml}$ luminol in the absence or presence of AFC or vehicle. Zymosan (2 mg/ml) or PMA (3 μM) was added to the preincubated AM samples and CL production was immediately measured for 15 min. NO-dependent CL was determined by preincubation of AM with 1.0 mM *N*-nitro-*L*-arginine methyl ester (L-NAME), an inhibitor of NO synthase, for 10 min before the addition of stimulant. CL generated from AM under four different conditions was determined: (1) resting CL generated from AM as baseline control; (2) resting NO-dependent CL determined by measuring L-NAME-inhibitable CL, i.e. resting CL minus CL generated from AM in the presence of 1 mM L-NAME; (3) stimulant-induced CL determined from AM in the presence of zymosan or PMA, i.e. stimulated minus resting; and (4) stimulant-induced NO-dependent CL in AM determined by measuring CL generated from AM stimulated by zymosan or PMA in the absence minus in the presence of L-NAME. Data were plotted as CL vs time and the area under the curve integrated to give the total CL in counts per minute per 0.75×10^6 AM per 15 min.

Measurement of protein and lactate dehydrogenase

Total acellular protein and LDH in BALF were determined using an automated Cobas FARA II. The protein content was measured using biuret reagent (Layne 1957) with Sigma Diagnostic reagents and procedures (Sigma Chemical Company, St. Louis, Mo.). LDH was measured by monitoring the formation of NADH spectrophotometrically at 340 nm using Roche Diagnostic reagents and procedures (Roche Diagnostic Systems, Indianapolis, Ind.) according to the method described by Gay et al. (1968).

Cytokine assays

TNF- α in macrophage-conditioned medium was determined using an enzyme-linked immunosorbent assay (ELISA; Biosource International, Camarillo, Calif.) according to the manufacturer's instructions. ELISA was first described by Engvall and Perlmann (1971) and the biotin-avidin system was described by Bayer and Wilchek (1978). The IL-1 activity in the macrophage-conditioned medium was determined according to a previously described method (Kang et al. 1992). Briefly, thymocytes were obtained from male CD-1 mice (6–10 weeks of age) and suspended in RPMI-1640 medium with 2 mM glutamine, 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 10% heat-inactivated fetal bovine serum, and 2×10^{-5} M mercaptoethanol. Cells ($1 \times 10^6/0.1$ ml per well) and aliquots (0.1 ml) of macrophage-conditioned medium were added in quadruplicate to 96-well microculture plates. Cell cultures were incubated at 37 °C in an atmosphere containing 5% CO_2 for 30 h. The cultures were pulsed for 22 h with [^3H]thymidine (1.0 $\mu\text{Ci}/\text{well}$, activity 11.3 Ci/mmol; Dupont NEN Products, Boston, Mass.), and the thymocytes harvested using a Harvester 96-cell harvester (Tomtech, Orange, Conn.). The cell radioactivity in the collecting glass fiber filters was measured using a liquid scintillation counter (1214 Rackbeta, Wallac, Finland). The amount of IL-1-like activity in the tested macrophage supernatants was expressed as disintegrations per minute. Antibodies of anti-rat IL-1 α and anti-rat IL-1 β (Endogen, Cambridge, Mass.) were used to confirm IL-1 activity measured by the thymocyte proliferation assay. These antibodies completely neutralized the activity in the macrophage-conditioned supernatant, thus indicating that the increase in thymocyte proliferation was due to the presence of IL-1 in the macrophage-conditioned culture medium.

Statistical analysis

All data were expressed as the means \pm standard error of means (SE) of experimental values from at least five measurements from different animals. All data were analyzed by one-way analysis of variance (ANOVA) to analyze the differences among the various treatment groups, followed by comparison with the saline control group using Dunnett's method. The significance level was set at $P < 0.05$.

Results

In vitro effects of AFC exposure on macrophage functions

The cytoplasmic enzyme, LDH, released from macrophages cultured in the absence and presence of various amounts of AFC was determined as an indicator of AFC-induced cell damage. Figure 1 shows that AFC at concentrations lower than 200 $\mu\text{g}/\text{ml}$ did not affect the amount of LDH released from AFC-exposed AM in comparison to the amount released from control macrophages. AFC at concentrations ≥ 200 $\mu\text{g}/\text{ml}$ caused significant cytotoxicity. These results also show that solvent alone at the corresponding concentration, i.e. DMSO in the absence of AFC, did not affect cell integrity.

The effects of exposure of AM to AFC *in vitro* on the production of reactive oxygen species were monitored by measuring the generation of chemiluminescence (CL). Figure 2 shows that AFC exposure did not markedly affect the CL generation from resting AM. However, when macrophages were challenged with zymosan, a

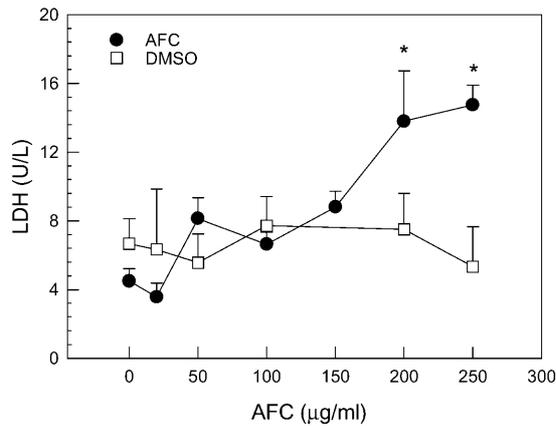


Fig. 1 The LDH release from AM when the cells were exposed to AFC in vitro for 24 h. Various AFC concentrations were obtained by the addition of different volumes of a 100-mg/ml stock solution in DMSO. The solvent control (□) samples contained the corresponding amount of DMSO in the absence of AFC. The points are the mean values obtained from at least six different animals, and the bars represent SE (* $P < 0.05$ vs control)

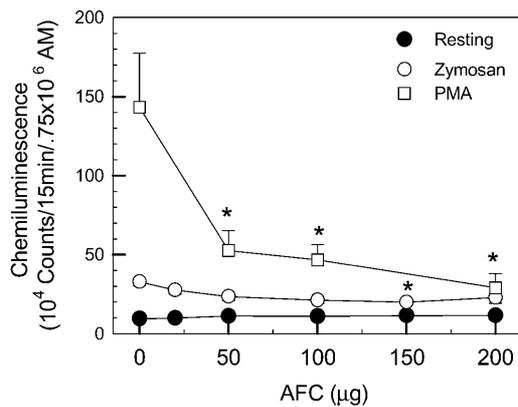


Fig. 2 The effects of in vitro AFC exposure on cellular CL generation from resting (●), zymosan-stimulated (○) and PMA-stimulated (□) AM. AM were exposed to AFC or vehicle for 10 min at 37 °C prior to determination of luminol-enhanced CL for 15 min at 37 °C. The points are the mean values obtained from at least six different animals, and bars represent SE (* $P < 0.05$ vs control)

fungal product, AFC exposure resulted in a small but significant decline in the generation of CL at AFC concentrations ≥ 150 $\mu\text{g/ml}$. When AM were challenged with PMA, a tumor promoter, significant attenuation of the CL generated was observed at ≥ 50 $\mu\text{g/ml}$ AFC.

Figure 3A, B shows the production of the proinflammatory cytokines IL-1 and TNF- α by AM incubated in the absence or presence of various concentrations of AFC. AFC exposure did not affect resting IL-1 release, while it induced a small but significant increase in resting TNF- α production from macrophages at AFC concentrations ≥ 50 $\mu\text{g/ml}$. In a comparative study, AM were pre-exposed to AFC for 2 h and then challenged with 0.1 $\mu\text{g/ml}$ lipopolysaccharide (LPS; *E. coli* 026:B6; DIFCO Laboratories, Detroit, Mich.) and incubated for a total of 24 h. The supernatant from macrophage-

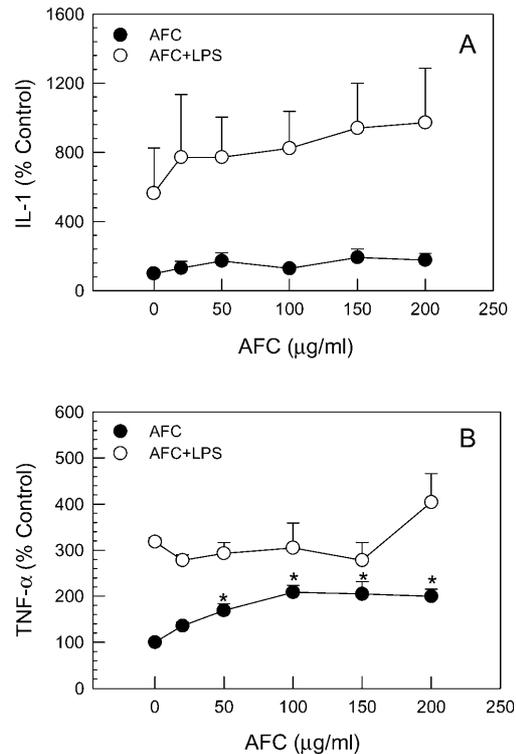


Fig. 3A, B Effects of in vitro AFC exposure on the release of IL-1 (A) and TNF- α (B) by AM at rest or in response to LPS (0.1 $\mu\text{g/ml}$) challenge (● AM exposed to AFC only for 24 h, ○ AM exposed to AFC for 2 h prior to a 22-h treatment with LPS). The points are the mean values obtained from at least six different animals, and bars represent SE (* $P < 0.05$ vs control AM)

conditioned medium was collected and analyzed. AFC exposure did not alter the release of TNF- α in response to LPS challenge. Similarly, AFC did not significantly affect LPS-stimulated IL-1 release. Thus, AFC stimulated resting AM production of TNF- α , whereas resting IL-1 production and LPS-stimulated cytokine secretion were not significantly affected.

Effects of exposure of rats to AFC on acute inflammatory responses

The solvent control group, i.e. rats exposed to 1% DMSO, did not exhibit marked alterations in any of the parameters measured in comparison with saline control animals (data not shown). Differential cell counts from animals exposed to AFC by IT instillation were determined as shown in Table 1. For saline control rats differential cell analysis showed that the BAL cells consisted mostly of macrophages with very few PMN. There was no significant difference in cell distribution among control and various AFC exposure groups, i.e. (1) after a single IT instillation of 0.1 or 0.5 mg AFC per rat killed 1 or 3 days later; or (2) after three consecutive IT exposures to 0.1, 0.5, or 2 mg AFC per rat killed the following day. Under the present treatment regimen, AFC exposure did not induce significant infiltration of

Table 1 Bronchoalveolar lavage cell differentials of rats exposed to saline or AFC. Values are mean \pm SE cell numbers $\times 10^{-6}$ from at least six different animals in each treatment group

Treatment	Measurement day after treatment	AM	PMN	Red blood cells	Lymphocytes
Single IT					
Saline	Day 1	8.45 \pm 1.00	1.88 \pm 0.29	9.64 \pm 0.99	4.28 \pm 0.48
AFC (0.1 mg)		7.68 \pm 0.71	1.94 \pm 0.25	11.82 \pm 2.96	4.36 \pm 0.89
AFC (0.5 mg)		8.50 \pm 0.38	1.83 \pm 0.26	6.71 \pm 0.41	2.81 \pm 0.22
Single IT					
Saline	Day 3	7.14 \pm 0.23	1.34 \pm 0.16	9.21 \pm 1.60	3.11 \pm 0.35
AFC (0.1 mg)		8.71 \pm 0.72	1.14 \pm 0.11	6.53 \pm 0.93	2.41 \pm 0.31
AFC (0.5 mg)		8.34 \pm 0.74	1.25 \pm 0.07	9.03 \pm 2.49	3.30 \pm 0.90
Three daily IT					
Saline	Day 1	10.34 \pm 0.41	1.96 \pm 0.22	10.79 \pm 1.45	3.60 \pm 0.47
AFC (0.1 mg)		9.27 \pm 0.62	1.74 \pm 0.18	9.99 \pm 0.53	3.53 \pm 0.26
AFC (0.5 mg)		8.70 \pm 0.55	1.61 \pm 0.21	8.03 \pm 0.59	2.61 \pm 0.19
AFC (2 mg)		8.95 \pm 0.56	2.25 \pm 0.77	7.10 \pm 0.79	2.60 \pm 0.33

AM, red blood cells, PMN, or lymphocytes into the alveolar space at any AFC concentration tested after one or three exposures. These results suggest that exposure of rats to AFC did not induce marked damage at the alveolar/blood barrier (i.e. an increase in red blood cells) or pulmonary inflammation (i.e. an increase in PMN). There was no significant difference in the LDH or protein content of the acellular lavage fluid among different treatment groups (data not shown). These results suggest that AFC exposure did not induce significant cytotoxicity (marked by leakage of LDH into the alveolar space) or cause significant damage of the alveolar/blood barrier (leading to increased protein content in the lavage fluid).

Release of reactive species from AM after AFC exposure was determined by monitoring the generation of CL as a measure of macrophage activity. Figure 4 shows that CL generated by AM isolated from AFC-exposed rats was not significantly different from the CL generated by AM from the saline control group either at rest or after zymosan stimulation. Furthermore, AFC exposure did not alter nitric oxide-related CL generation. These results suggest that AFC exposure did not affect the production of reactive species by AM.

The proinflammatory cytokines, IL-1 and TNF- α , produced by AM harvested from rats after AFC exposure were evaluated, at rest or in response to LPS, as

another measure of macrophage activity. Figure 5 shows that IL-1 and TNF- α release from either resting or LPS-stimulated AM from AFC-exposed animals were not significantly different from the AM from control animals. These results suggest that exposure of rats to AFC did not significantly affect macrophage function.

Discussion

Road workers are subject to asphalt fume exposure during paving operations. Several field studies have shown that the level of such exposure is low (Brandt et al. 1985; Monarca et al. 1987) and that asphalt fumes contain only trace levels of PAH (Monarca et al. 1987). However, an increased incidence of mucous membrane irritation and a decline in peak expiratory flow rate among road pavers have been noted and these symptoms are positively correlated with increased exposure time (Nygqvist 1978; Waage 1987), asphalt temperature, and asphalt concentration (Norseth et al. 1991). Thus asphalt fumes may have adverse effects on the respiratory system. The objective of the present study was to investigate the effect of asphalt fume exposure on the function and activity of alveolar macrophages through both in vitro and in vivo experiments using a rat model. AFC used for this study was collected from a paving

Fig. 4A–C Effects of in vivo exposure of rats to AFC on CL generated from AM (A) 1 day after a single IT exposure, (B) 3 days after a single IT exposure, and (C) 1 day after three consecutive single IT exposures. The points are the mean values obtained from at least six different animals, and bars represent SE

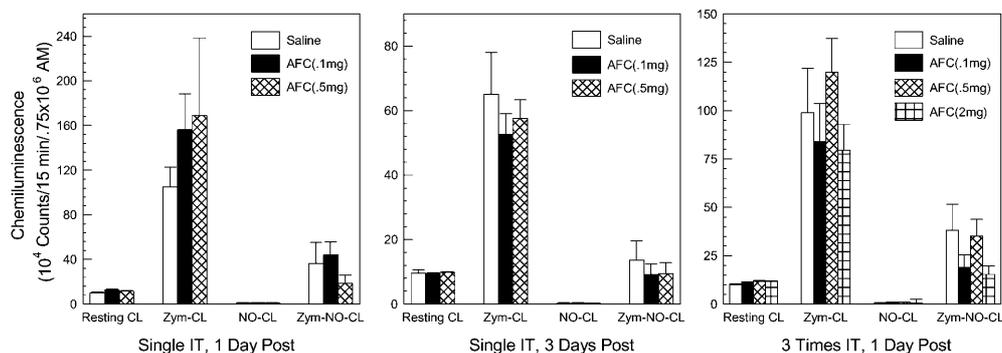
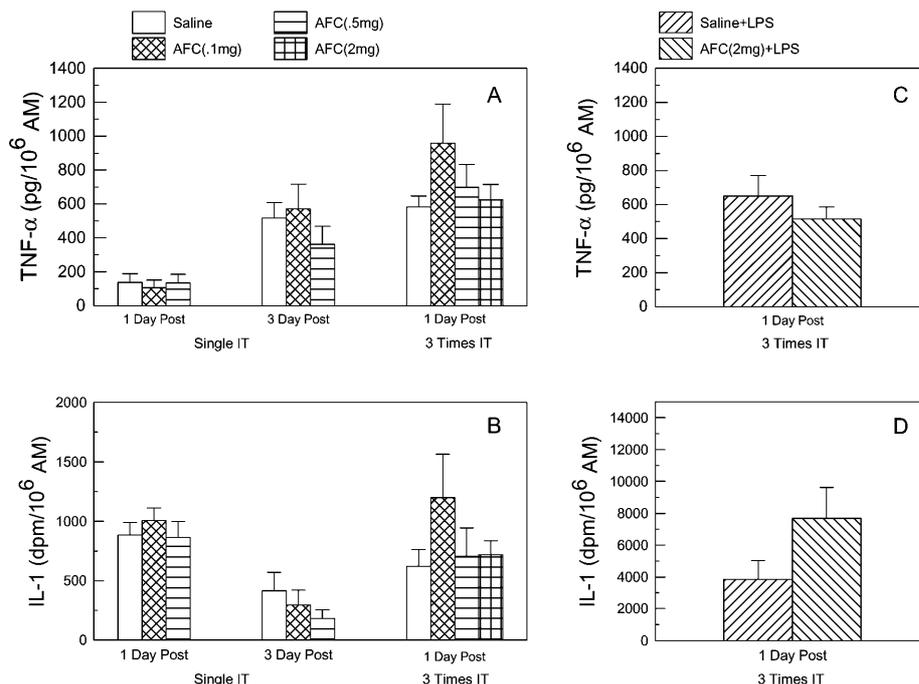


Fig. 5A–D Effects of exposure of rats to AFC on TNF- α and IL-1 production by AM at rest (**A,B**) or in response to LPS stimulation (**C,D**). AM were collected from the AFC-exposed rats 1 day after IT exposure, 3 days after IT exposure, or 1 day after IT exposure for three consecutive days. The points are the mean values obtained from at least six different animals, and bars represent SE



asphalt storage tank by cold trap condensation. The composition of the condensate was found to be similar to asphalt fume collected on personnel monitoring cassettes used by the asphalt workers (Kriech et al. 1999).

Alveolar macrophages play an important role in host defense against a wide range of foreign substances including particles, fumes, infectious agents, and chemical irritants. These cells can destroy invading substances by phagocytosis and by releasing reactive chemical species through respiratory burst activity. However, excess production of these reactive species by activated AM is also known to be one of the mechanisms by which inhaled substances induce pulmonary injury and inflammatory diseases. The data from this *in vitro* study showed that AFC can induce lung injury, as evidenced by the elevated LDH release, but only at relatively high levels of exposure (>200 $\mu\text{g}/\text{ml}$). *In vitro* AFC exposure, however, did not induce macrophage production of reactive species monitored as the generation of CL from resting AM. These results contrast with those obtained in studies of dusts such as silica and DEP exposure, which have been shown to induce macrophage respiratory burst manifested by increased ROS generation (Dong 1997; Ma et al. 1992). DEP consists of a particulate component and particle-adsorbed organic compounds. The effects of DEP on macrophage respiratory burst has been attributed to particle stimulation, since the organic extract of DEP has little effect on AM production of ROS (Dong 1997). Since AFC contains mostly organic compounds, it is not surprising that *in vitro* exposure to AFC has little or no effect on AM generation of CL at rest. Zymosan and PMA are well-known particle and soluble stimulants, respectively, for macrophage respiratory burst activity. The present study

showed that *in vitro* AFC exposure induced a slight decline in production of oxidants by AM in response to zymosan challenge, while significantly inhibiting PMA-stimulated oxidant production. Thus the effects of AFC exposure on AM function may involve action through the PMA-stimulatory site.

In vitro exposure of AM to AFC resulted in a small but significant stimulation of TNF- α release, whereas the secretion of IL-1 by AM was not affected by AFC. In addition, AFC appeared to have no significant effect on LPS-stimulated secretion of these proinflammatory cytokines. The action of *in vitro* exposure to AFC on AM secretion of cytokines is significantly different from that of silica or DEP. *In vitro* exposure of AM to silica enhances AM production of both IL-1 (Kang et al. 1992) and TNF- α (Ma et al. 1996). DEP, on the other hand, stimulates AM production of IL-1 but not TNF- α , and significantly suppresses AM response to subsequent LPS stimulation in the production of IL-1 and TNF- α (Yang et al. 1999b). AFC collected from cold trap condensation consists of organic compounds that are analogous to those organic components adsorbed onto the particles of DEP but contains no carbon particulate core. The fact that AFC did not suppress LPS-stimulated cytokine production by AM suggests that the carbon particles of DEP may facilitate the uptake of adsorbed chemicals and play an important part in potentiating the adverse effects of the organic compounds on AM function.

The *in vitro* data were further substantiated through *in vivo* experiments carried out at various doses and durations of exposure. The AFC exposures did not result in excess infiltration of PMN, a hallmark of pulmonary inflammation, alveolar capillary damage, as indicated by red blood cell counts or BALF protein, or significant

cytotoxicity, as indicated by the LDH content in the acellular lavage fluid. The *in vivo* AFC exposure also did not result in any significant changes in AM production of IL-1 or TNF- α , or in cytokine secretion by AM in response to *ex vivo* challenge by LPS. This lack of acute pulmonary inflammatory injury and cytokine production by AFC exposure is, again, in significant contrast to the effects induced by the particulate systems such as silica (Ma et al. 1999; Yang et al. 1999b), carbon black, or DEP (Yang et al. 1999b). These results suggest that AFC exposure did not cause significant pulmonary inflammation, lung injury, or alter AM functions. However, due to the PAH content of asphalt, the toxic and carcinogenic effects of asphalt fume exposure is a concern. PAHs require metabolic activation to exert their toxic effects. In the lung, the cytochrome P-450 monooxygenase system plays an important role in PAH metabolism. The effect of asphalt fume exposure on pulmonary metabolic activities, which should provide more insight into the mechanisms involved in PAH activation, is under investigation in our laboratory.

In summary, data from the present study show that neither *in vitro* nor *in vivo* exposure to AFC causes significant or consistent pulmonary inflammation or lung damage. Likewise, data concerning the production of oxidants and proinflammatory cytokines do not support the view that alveolar macrophages are either hyperactivated or that their responsiveness to microbial products is dramatically decreased. Therefore, results of the present study do not support an increase in oxidant-induced lung injury or a decreased resistance to microbial infection in lungs resulting from asphalt exposure. It is possible that the lack of effect of asphalt exposure is due to the form of exposure, i.e. intratracheal instillation of condensate rather than inhalation of asphalt fume. To resolve this issue an investigation of the effects of inhalation exposure of rats to asphalt fume on markers of nasal irritation, pulmonary function, lung inflammation and damage, and immune status is underway in our laboratory.

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