Effect of Metal-Containing Environmental and Occupational Particulates on Lung Defense Mechanisms

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Abstract

Epidemiological studies have demonstrated that inhalation of polluted air containing increased levels of metal-containing particulates may exacerbate preexisting health conditions and augment pulmonary infection. The objective of this study was to examine the effect of metal-containing particulates found in environmental and occupational settings on lung defense responses. Animal infectivity models have been developed as a means to determine the mechanisms by which inhaled toxicants may affect lung defenses against infection. Due to the prevalence of different metal-containing particulates in the environment and workplace, numerous animal studies have evaluated the effects of agents, such as fly ash, concentrated ambient air particulates, and welding fumes, on lung defense mechanisms. The alveolar macrophage is the primary lung cell responsible for non-specific innate pulmonary host defenses. Toxicological evidence indicates that metals associated with different occupational and environmental particulates may alter macrophage function and increase the susceptibility to lung infection. Changes in macrophage phagocytosis, bactericidal activity, and the production of reactive oxygen and nitrogen species have been observed. In addition, the secretion of cytokines by macrophages, which are important in immune cell responses, has been shown to be affected by metal-containing particulates. Evidence suggests that the soluble metal fraction of specific environmental particulates is responsible for the alterations observed in lung immune responses. Studies are ongoing in an attempt to assess the role that individual metals (e. g. Cr, Fe, Ni, V, and Mn) may play in suppressing lung defense against infection. With the use of animal infectivity models, it

may be possible to determine the mechanisms by which metal-containing particulates may suppress lung defenses in order to develop a better understanding of how to prevent adverse health effects and protect susceptible populations at risk.

Key Words

lung defense; residual oil fly ash; welding fumes; metals; alveolar macrophage; *Listeria monocytogenes*

Introduction

Epidemiology has demonstrated that inhalation of air containing elevated levels of particulates is associated with increased morbidity and mortality (Dockery and Pope, 1994; Pope et al., 1995; Schwartz, 1994). Workers in certain industries (e. g. boilermakers, welders, miners) who are exposed to particulates also may be at a greater risk to adverse pulmonary effects compared to the general population (Antonini, 2003a; Hauser et al., 1995; Palmer et al., 2003; Sferlazza and Beckett, 1991). Pulmonary effects associated with inhalation of environmental and occupational particulates include an increased incidence, severity, and duration of upper and lower respiratory tract infections (American Thoracic Society, 2000; Howden et al., 1988). The goal of the current study was to determine whether or not pulmonary exposure to specific environmental and occupational particulates increases the susceptibility to infection using an animal infectivity model. In addition, an attempt was made to determine which component of these particulates may be responsible for the possible suppressive effects on lung defense responses.

Particulates generated in the environment and workplace can be quite complex. Particle size and chemical composition as well as physical and biological properties may contribute to the pulmonary effects observed after inhalation. Environmental particulates, such as residual oil fly ash (ROFA), may contain metals, sulfates, acids, fuel contaminants, and other unknown materials complexed with an insoluble, particulate carbon core (Ghio et al., 2002). Animal studies have suggested that soluble transition metals associated with environmental and occupational particulates contribute to adverse pulmonary effects (Dreher et al., 1997; Kodavanti et al., 1998; Lewis et al., 2003). Transition metals are potentially toxic to cells and tissues because of their capacity to support electron exchange and catalyze free radical production.

Animal infectivity models have been developed to determine the mechanisms by which inhaled particles and gases may increase the susceptibility to lung infection. The gram-positive, facultative intracellular bacterial pathogen, *Listeria monocytogenes*, has been extensively used

by our group and others to study lung defense mechanisms before and after treatment with different pneumotoxic substances (Antonini et al., 2000, 2001a; Cohen et al., 2001; Jakab, 1993; Van Loveren et al., 1988; Yang et al., 2001). The initial response to inoculation with *L. monocytogenes* is characterized by the rapid activation of non-specific immune responses (e. g. alveolar macrophage (AM) stimulation and neutrophil recruitment) to limit the spread of infection (Seaman et al., 1999). In addition, the clearance of the *L. monocytogenes* also is dependent on the later development of acquired T-cell responses (Shen et al., 1998; Unanue, 1997).

In the present study, male Sprague-Dawley rats were pretreated with metal-containing environmental and occupational particles (ROFA and welding fumes) by intratracheal instillation prior to pulmonary inoculation with *L. monocytogenes*. The development of lung injury and inflammation, alterations in AM function, and the effects on the pulmonary clearance of *L. monocytogenes* after ROFA exposure were assessed.

Materials and Methods

Experimental Design

ROFA and welding fume samples were collected and suspended in sterile saline. At day 0, male Sprague-Dawley rats were pretreated by intratracheal instillation of the welding fumes, ROFA, or saline (vehicle control). At day 3, the pretreated animals were intratracheally inoculated with the bacterial pathogen, *L. monocytogenes*. At days 6, 8, and 10, lungs were removed, the left lung was homogenized, and the number of bacteria colony-forming units (CFUs) was determined as an indication of pulmonary clearance. Right lungs were preserved with 10 % buffered formalin, removed, sectioned, embedded in paraffin, and stained with hematoxylin and eosin for histopathological analysis. AMs were recovered from animals pretreated with ROFA and the welding fume samples, and bacterial killing was determined as an index of macrophage function.

Particle Samples

ROFA was collected from a precipitator at Boston Edison Co., Mystic Power Plant #4, Everett, MA. Particle size of the ROFA sample was characterized by scanning electron microscopy (JSM-#5600 SEM, JEOL Ltd., Peabody, MA). The ROFA particles were of respirable size with a count mean diameter of 2.2 μm.

Welding fume samples were kindly provided by Kenneth Brown of Lincoln Electric Co. (Cleveland, OH). The fumes were generated in a cubical

open front fume chamber (volume = 1 m^3) by a skilled welder using a manual or semi-automatic technique appropriate to the electrode and collected on 0.2 μm filters. The fume samples were generated in three different ways: 1) gas metal arc welding using a mild steel E70S-3 electrode (GMA-MS); 2) gas metal arc welding using a stainless steel ER308L Si electrode (GMA-SS) with argon and CO_2 shielding gases to protect the weld from oxidation; and 3) manual metal arc welding using a flux-covered stainless steel E308-16 electrode (MMA-SS). Particle size of the welding samples was characterized by scanning electron microscopy. Particles were of respirable size with count mean diameters for the GMA-MS, GMA-SS, and MMA-SS fumes measuring 1.22 μm, 1.38 μm, and 0.92 μm, respectively.

Elemental Characterization

ROFA and welding fume samples (MMA-SS, GMA-SS, and GMA-MS) were suspended in sterile phosphate-buffered saline (PBS), pH 7.4, and sonicated for 1 min. The particle suspensions (Total) were further divided into soluble and insoluble components by incubating the suspension for 24 hr at 37 °C, and then centrifuging at 12,000 g for 30 min. The supernatant of the sample (Sol) was recovered and filtered with 0.22 μm filters. The pellet (Insol) was resuspended in PBS. Analysis of the metal constituents of the total particle samples as well as its soluble and insoluble fractions was obtained using inductively coupled argon plasma atomic emission spectroscopy (NIOSH, 1994).

Animals

Male Sprague-Dawley [Hla: (SD) CVF] rats (Hilltop Lab Animals, Scottdale, PA) weighing ~250 g were used. The animals were housed in an AAALAC-approved animal facility in a room with restricted access and HEPA-filtered air, monitored free of pathogens, and allowed to acclimate for one week before use. The rats were maintained on ProLab 3500 diet and tap water *ad libitum*. Alpha-Dri virgin cellulose chips and hardwood Beta-chips were used as bedding.

Particle Treatment

Rats were lightly anesthetized by an intraperitoneal injection of 0.6 ml of a 1 % solution of sodium methohexital (Brevital, Eli Lilly, Indianapolis, IN, USA) and intratracheally instilled with 1.0 mg/100 g body weight of ROFA or the welding fume samples in 300 μl of saline. Animals in the vehicle control group were intratracheally dosed with 300 μl of sterile saline as previously described (Reasor and Antonini, 2001).

Bacterial Inoculation

L. monocytogenes (strain 10403S, serotype 1) was obtained as a gift from Rosana Schafer of the Department of Microbiology and Immunology at West Virginia University (Morgantown, WV) and was cultured overnight in brain heart infusion broth at 37 °C in a shaking incubator. Following incubation, the bacteria concentration was determined by a spectrophotometric method using optical density at a wavelength of 600 nm and diluted with sterile saline to the desired concentrations. At day 3, the rats pretreated with ROFA were inoculated intratracheally with 5×10^5 of *L. monocytogenes* in 500 µl of sterile saline, whereas the rats pretreated with the welding fumes were inoculated with 5×10^3 of *L. monocytogenes* in 500 µl of sterile saline. In a previous pilot study, these bacteria doses gave a uniform infection and did not kill untreated naive Sprague-Dawley rats (Antonini et al., 2001b).

Morbidity/Histopathology

Animal weights and morbidity were monitored over the course of the treatment period. Histopathological analysis was performed on the right lungs of rats from each group. Rats were euthanized with sodium pentobarbital, and the right lungs were preserved with 10 % buffered formalin by airway fixation at total lung capacity. The lobes of the lungs were removed, sectioned, embedded in paraffin, and stained with hematoxylin and eosin.

Pulmonary Clearance of *L. monocytogenes*

At days 6, 8, and 10, the left lungs were removed from all rats in each treatment group. The excised lungs were suspended in 10 ml of sterile water, homogenized using a Polytron 2100 homogenizer (Brinkmann Instruments, Westbury, NY, USA), and cultured quantitatively on brain heart infusion agar plates. The number of viable CFUs was counted after an overnight incubation at 37 °C.

Bacterial Killing by Alveolar Macrophage

Bacterial killing by AMs was determined by a method modified from Ohya et al. (1998). AMs were recovered by bronchoalveolar lavage from rats 3 days after the intratracheal instillation of ROFA, welding fumes, or saline. The recovered AMs from each animal were allowed to attach in 24-well plates for 3 hr at 37 °C in RPMI-1640 media with 10 % fetal bovine serum at a concentration of 5×10^5 cells/well. After the incubation period, the wells were washed four times with media to remove non-adherent cells.

The adherent cells (found to be > 90 % AMs) were treated with 1 x10^7 *L. monocytogenes* at 37 °C for 1.5 hr. After this second incubation, one set of AMs from each animal was washed six times with media and lysed in distilled H_2O using sonication. This first lysate was diluted, cultured overnight at 37 °C, and the number of viable CFUs were counted to determine AM phagocytosis. The remaining AMs from each animal were incubated for an additional 4 and 18 hr at 37 °C. The AMs were continually washed every 2 hr during the incubation period with media containing 10 μg/ml of chloramphenicol to kill any extracellular bacteria that had not been taken up by the AMs after the first incubation. After the incubations, the AMs were lysed, diluted, cultured overnight at 37 °C, and the number of viable CFUs were counted.

Statistical Analysis

Results are expressed as means ± standard error of measurement (SE) and assessed using an analysis of variance (ANOVA) followed by Tukey's-Kramer post-hoc test. For all analyses, the criterion of significance was set at $p < 0.05$.

Results

When suspended in PBS, both the ROFA-total (pH 5.0) and ROFA-sol (pH 4.1) samples were found to be acidic, whereas the ROFA-insol sample was neutral (pH 7.04). Elemental analysis of the ROFA samples is shown in Table 1. Significant amounts of Ni, Al, Ca, Fe, and Zn were present in ROFA-sol, whereas ROFA-insol was mostly comprised of Fe, V, and Al. When the ROFA-sol sample was treated with the metal-binding resin, Chelex, almost all of the metals were removed except for small amounts of Fe and V.

Table 1: Elemental Composition of ROFA (μg/mg).

Elements	Total	Insoluble	Soluble	Soluble+Chelex
Fe	122	93.0	18.6	3.38
Al	60.5	32.1	23.3	n. d.
V	46.0	41.6	0.585	0.287
Ni	38.5	5.50	27.9	n. d.
Ca	30.6	3.58	22.6	n. d.
Zn	5.35	0.565	4.35	n. d.

Trace Elements: Ba, Ca, Co, Cr, Cu, Mn, Pb; n. d. = not detected.

The effect of ROFA pretreatment on pulmonary bacterial clearance after infection with 5 x 10^5 *L. monocytogenes* was examined. Rats pretreated with ROFA-Total and ROFA-Sol were not able to clear the bacteria from the lungs as readily as rats pretreated with saline and ROFA-Insol (Table 2). There was significantly more bacteria in the lungs of the ROFA-Total and ROFA-Sol groups at all three time points compared to the saline and ROFA-Insol groups. When the rats were pre-exposed to the ROFA-Sol sample that had been treated with Chelex, no difference in bacterial clearance from the lungs was observed compared to the saline control.

Table 2: Bacterial CFUs per Left Lung (Log_{10} units).

Time (Days)	Saline	ROFA-Total	ROFA-Insol	ROFA-Sol	ROFA-Sol + Chelex
6	7.00 ± 0.12	8.24 ± 0.13*	7.17 ± 0.23	8.58 ± 0.13*	7.01 ± 0.10
8	4.37 ± 0.15	6.28 ± 0.48*	4.68 ± 0.31	7.97 ± 0.59*	4.86 ± 0.23
10	2.53 ± 0.56	4.84 ± 0.31*	3.19 ± 0.21	5.35 ± 0.06*	2.48 ± 0.45

Values are means ± SE; *significantly greater than saline within a time point ($P < 0.05$).

In the assessment of morbidity and lung histopathology, rats pretreated with ROFA-Total and ROFA-Sol before pulmonary inoculation with *L. monocytogenes* demonstrated a significant decline in body weight and survival at day 8 compared to the saline and ROFA-Insol groups (data not shown). By day 10, 40 % of the ROFA-Total rats had unexpectedly expired, whereas 90 % of the ROFA-Sol group had died. None of the rats expired that were exposed to the ROFA-Sol treated with Chelex. Pulmonary inoculation with 5 x 10^5 *L. monocytogenes* led to edema, inflammation, and the formation of granulomatous lesions characterized by the presence of amorphous tissue debris in the lungs of the saline and ROFA-Sol + Chelex groups at day 6 (data not shown). The lesions that were observed in the lungs of the rats that were pretreated with ROFA-Sol and ROFA-Total were more extensive and significantly more pronounced than those observed in the other groups. Lung injury and inflammation induced by ROFA-Sol were still progressing at day 10, whereas the lung response of the other groups had subsided by this time point.

To determine the mechanism that may be involved in the observed reduction in bacterial clearance associated with ROFA exposure, the bactericidal activity of AMs recovered from rats exposed to the ROFA-Total sample was measured. At 4 and 18 hours after incubation with *L. monocytogenes*, there were significantly more viable bacteria in the AMs recovered from ROFA-treated rats as compared to the saline group (Figure 1), indicating a reduction in the ability of the AMs to kill the bacteria.

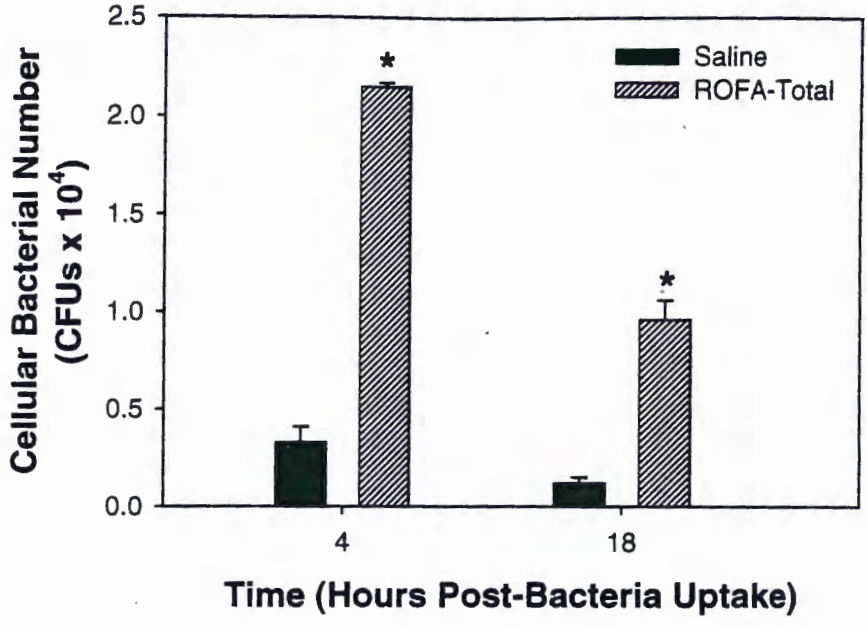

Figure 1: Bactericidal activity of alveolar macrophages recovered from rats pretreated with ROFA-Total and saline 4 and 18 hr after uptake of *L. monocytogenes*. Values are means ± SE; *significantly different from saline at each time point.

Table 3: Welding Fume Characterization.

Sample	Metal Analysis (weight %)	Soluble/Insoluble Ratio	pH
MMA-SS	Fe 41 % Mn 17 % Cr 29 % Ni 3 %	0.345 Soluble metals: Cr 87 % Mn 11 %	Tot 6.92 Sol 7.05 Insol 7.09
GMA-SS	Fe 53 % Mn 23 % Cr 19 % Ni 5 %	0.006	Tot 6.94 Sol 6.97 Insol 7.01
GMA-MS	Fe 85 % Mn 15 %	0.020	Tot 7.02 Sol 7.44 Insol 7.03

The welding fume samples were suspended in saline and the relative amounts of different metals were determined as weight %. The elemental composition of the three fumes was found to be quite different (Table 3). The GMA-MS sample was comprised almost entirely of Fe (85 %) and Mn (14 %). The two SS samples (MMA-SS and GMA-SS) were composed of similar Mn levels but with much less Fe as compared to the GMA-MS fume. Cr and Ni were also present in the two SS samples, which were absent in the GMA-MS sample. The GMA-SS and GMA-MS samples were found to be relatively insoluble with soluble-to-insoluble ratios of 0.006 and 0.020, respectively. The flux-covered MMA-SS sample was much more soluble than the other two samples with a soluble-to-insoluble ratio of 0.345. The majority of the soluble fraction of the MMA-SS fume was comprised of mostly Cr (87 %) with some Mn (11 %). The pH of the welding fume samples and their soluble and insoluble fractions were mostly neutral.

Pretreatment with the MMA-SS fume before infection with 5×10^3 *L. monocytogenes* caused a significant delay in the pulmonary clearance of *L. mono cytogenes* (Table 4). Significant increases of 407-, 1511-, and 24-fold were observed in viable lung CFUs for the MMA-SS group compared with the saline group at days 6, 8, and 10, respectively. Pretreatment with the GMA-SS and GMA-MS fumes caused no significant difference in viable lung CFUs at days 8 and 10 compared with the saline group. Significantly more bacteria was present in the lungs of the MMA-SS group compared with the GMA-SS and GMA-MS groups at days 6 and 8.

Table 4: Bacterial CFUs per Left Lung (Log_{10} units).

Time (Days)	Saline	MMA-SS	GMA-SS	GMA-MS
6	5.32 ± 0.04	7.93 ± 0.17*	5.42 ± 0.07	5.32 ± 0.11
8	4.11 ± 0.15	7.29 ± 0.40*	4.93 ± 0.26	5.00 ± 0.27
10	2.08 ± 0.44	3.45 ± 0.32*	3.00 ± 0.23	3.08 ± 0.14

Values are means $\pm$ SE; *significantly greater than saline within a time point ($P < 0.05$).

In examining the effect of the different fractions of the MMA-SS fume on bacterial clearance after infection with 5×10^3 *L. monocytogenes*, significant increases in viable lung CFUs were observed for the MMA-SS-Total sample compared with the saline group at all three time points (Table 5). Bacterial clearance of rats pretreated to the MMA-SS-Sol and MMA-SS-Insol fractions before infection was not significantly different from saline controls.

Table 5: Bacterial CFUs per Left Lung (Log_{10} units).

Time (Days)	Saline	MMA-SS-Total	MMA-SS-Sol	MMA-SS-Insol
6	5.52 ± 0.15	7.08 ± 0.16*	5.70 ± 0.06	6.14 ± 0.08
8	4.17 ± 0.30	5.90 ± 0.60*	3.78 ± 0.31	4.16 ± 0.21
10	2.41 ± 0.60	4.54 ± 0.73*	1.84 ± 0.78	2.57 ± 0.66

Values are means ± SE; *, significantly greater than saline within a time point ($P<0.05$).

Pretreatment with MMA-SS-Total fume before infection with 5 x 10^3 *L: monocytogenes* had a significant effect on animal survival (data not shown). By day 10, 30 % of the rats from the MMA-SS-Total groups had expired. All rats from the saline, GMA-SS, and GMA-MS groups survived during the 10-day period. In the assessment of body weight change, rats from the MMA-SS-Total group lost significantly more weight at each time point after infection compared to the other groups (data not shown). Of the animals from the MMA-SS-Total group which had survived at day 10, body weights returned to control values. Pretreatment with the MMA-SS-Insol and MMA-SS-Sol fractions before infection did not cause a loss in body weight or affect animal survival. In addition, histopathological analysis was performed on lungs from all treatment groups (data not shown). Lungs from the saline group appeared mostly normal at each time point after infection with 5 x 10^3 *L. monocytogenes*. A few localized areas of mild inflammation were observed for the saline group at the earlier time points after infection. Mild to moderate areas of inflammatory lesions were observed in the lungs of the groups treated with GMA-SS, GMA-MS, MMA-SS-Insol, and MMA-SS-Sol groups after infection on day 6 and 8. By day 10, most of pulmonary inflammation observed for these groups had cleared. Severe pneumonitis, characterized by a peribronchiolar accumulation of neutrophils, consolidation, edema, and the appearance of multiple granulomatous lesions were observed throughout the lungs at each time point for the MMA-SS-Total group.

To examine the mechanism which may be involved in the reduction in bacterial clearance associated with MMA-SS-Total pretreatment, the bactericidal activity of AMs recovered from rats exposed to the MMA-SS-Total sample was measured. At 4 and 18 hours after incubation with *L. monocytogenes*, there were significantly more viable bacteria in the AMs recovered from MMA-SS-treated rats as compared to the saline group (Figure 2), indicating a suppression in the ability of the AMs recovered from the MMA-SS-Total treated rats to kill the bacteria.

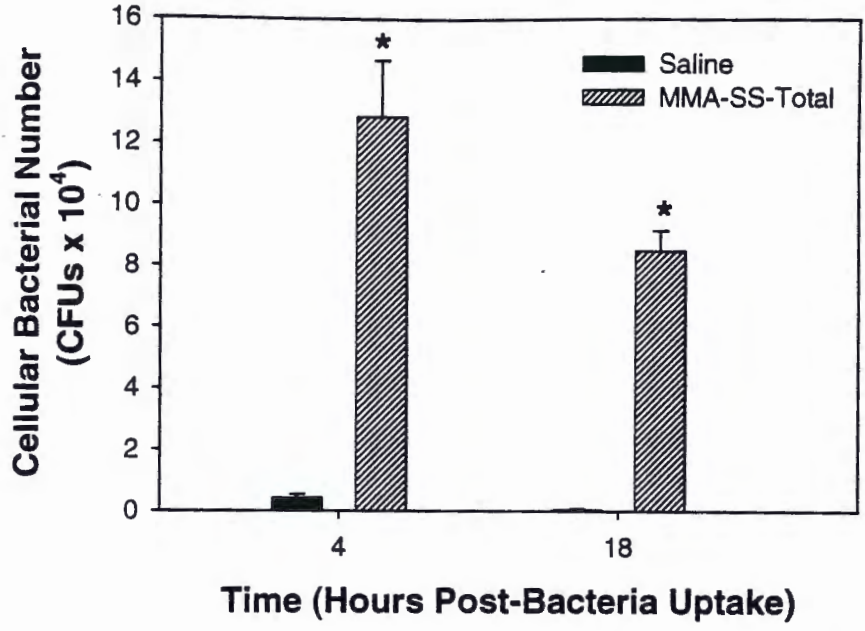

Figure 2: Bactericidal activity of alveolar macrophages recovered from rats pretreated with MMA-SS-Total and saline 4 and 18 hr after uptake of *L. monocytogenes*. Values are means ± SE; *significantly different from saline at each time point.

Discussion

Laboratory animals were pretreated with metal-containing particulates obtained from different environmental and occupational settings prior to bacterial inoculation, to assess the mechanisms by which different metals may suppress local pulmonary defense function. One particulate used in these studies was ROFA. Because of its chemical likeness with ambient air particulate matter and its significant contribution to air pollution burden, ROFA is commonly used in studies that evaluate the pulmonary responses to particulate matter exposure (Ghio et al., 2002). In the current study, it was observed that the pulmonary clearance of *L. monocytogenes* by rats pretreated with ROFA was dramatically slowed compared with control animals pretreated with saline. In addition, ROFA pretreatment caused a significant loss in body weight and a substantial elevation in lung inflammation and morbidity. In earlier studies, an excess in mortality was observed in mice that had been intratracheally instilled with ROFA prior to exposure to aerosolized *Streptococcus zooepidemicus* (Hatch et al., 1985; Pritchard et al., 1996).

Because of the central role of AMs in lung defense against infection, it was hypothesized that phagocytosis of ROFA particles may alter AM function. A previous study observed that ozone inhalation delayed the clearance of *L. monocytogenes* from the lungs, likely due to a suppression in AM phagocytosis (Van Loveren et al., 1988) and an alteration in the production of bactericidal reactive oxygen species (Cohen et al., 2001). Similarly, diesel exhaust particles decreased the ability of AMs to produce antimicrobial oxidants and the pro-inflammatory cytokines, tumor necrosis factor-α and interleukin-1β (Yang et al., 2001), as well as compromised cell-mediated immunity by inhibiting AM secretion of interleukin-12 (Yin et al., 2002) in response to infection with *L. monocytogenes*. Corroborating these findings, a decrease in bactericidal activity was observed in AMs recovered from ROFA-treated animals in the current study. Recent evidence indicated that the AM production of nitric oxide, a highly reactive nitrogen intermediate which has an important role in AM-mediated defense against infection, was decreased by ROFA treatment (Antonini et al., 2002). In addition, treatment with the soluble fraction of ROFA caused a reduction in pulmonary interleukin-2, a cytokine involved in T cell growth and proliferation, as well as enhanced interleukin-10, a cytokine shown to inhibit AM function, after infection with *L. monocytogenes* (Roberts et al., 2003).

The bioavailability of soluble transition metals has been implicated as one of the mechanisms by which ROFA may affect pulmonary responses (Dreher et al., 1997; Kodavanti et al., 1998; Lewis et al., 2003). In an attempt to determine which component of ROFA may suppress lung defense mechanisms, laboratory animals were pretreated with the water-soluble and insoluble fractions of ROFA before pulmonary inoculation with bacteria. Indeed, the soluble fraction of the ROFA sample dramatically slowed the pulmonary clearance of the *L. monocytogenes*, whereas the insoluble fraction had no effect on the clearance of the bacteria from the lungs. The lung defense response after treatment with the insoluble fraction was not significantly different compared to the saline control group. By removing the metals from the soluble fraction with the metal-binding resin Chelex, the inhibitory effect was reversed.

The soluble metals present in significant quantities in the ROFA sample included Ni, Al, and Fe. Interestingly, very little soluble V was present in the sample used in the current study. It has been suggested that soluble V may play an important role in the toxic lung responses associated with ROFA (Dreher et al., 1997). Previous *in vitro* studies have indicated that ROFA may be a strong activator of AMs (Kodavanti et al., 1998; Ghio et al., 1997). However, the total ROFA sample as well as the soluble and insoluble fractions which were used in the current study, had a minimal effect on the *ex vivo* production of AM chemiluminescence, a measure of AM activation (Lewis et al., 2003). Kodavanti et al. (1998) showed that a ROFA sample that did not contain water-soluble V (as was the case with the ROFA samples used in the current study) did not cause an elevation in AM chemiluminescence. In addition, Kodavanti et al. (1998) indicated

that soluble Ni appeared to have a suppressive effect on *in vitro* AM activation. Because the ROFA sample used in the current study contained a significant level of soluble Ni, the AM activation associated with the other soluble metals present in the ROFA sample may have been suppressed by the presence of Ni.

In a related *in vivo* study, Zelikoff et al. (2002) exposed rats that had been infected with *Streptococcus pneumoniae* to a single inhalation of soluble Fe or Ni. Results indicated that inhalation of Fe altered non-specific innate and adaptive immune responses in uninfected rats, whereas both Fe and Ni reduced the clearance of the bacteria from the lungs of the infected animals. It was observed that the effects on clearance in the infected Fe-exposed rats were similar to those seen in infected rats exposed to ambient particulate matter, indicating that Fe inhalation exposure may play an important role in altered immune response. Additional *in vivo* studies are needed to assess the effect of individual metals on pulmonary host defenses.

The second metal-containing particulate used in our studies was welding fume. The effect of three different welding fumes on lung defense was assessed after infection. Pretreatment with two mostly insoluble welding fumes (GMA-SS and GMA-MS) had no effect on lung bacterial clearance after infection. Conversely, pretreatment with a highly water-soluble stainless steel welding fume (MMA-SS) dramatically slowed the clearance of *L. monocytogenes* from the lungs, caused a significant loss in body weight, and elevated lung inflammation and morbidity after infection. However, neither the soluble nor insoluble component of the MMA-SS fume had any significant effect on lung defense responses, which was unlike what was observed for the ROFA soluble fraction. It appears that both the soluble and insoluble fractions of the MMA-SS fume are required to produce the observed lung responses. The suppression of lung defenses associated with MMA-SS welding fume are not dependent exclusively on water-soluble metals. It is important to note that lung responses associated with certain metal-containing particulate samples can be dependent on insoluble metals. Imrich et al. (2000) observed that the effect of concentrated ambient air particles, collected in Boston, MA, on AM function, cytokine production, and phagocytosis was predominantly influenced by the insoluble components.

The mechanism involved in the alteration of bacterial clearance from the lungs caused by MMA-SS treatment is likely due to a suppression of AM function. A reduction in bactericidal activity was observed for the AMs recovered from animals treated with the MMA-SS fume. New evidence suggests that MMA-SS pretreatment before infection caused an elevation in lung interleukin-10 production, indicating a likely attenuation of AM activity (Antonini et al., 2003b). In addition, lung interleukin-2 secretion was reduced, which suggests an alteration of Th1 immune responses.

In summary, the present study demonstrated that rats pre-exposed to ROFA or a stainless steel welding fume have altered lung defenses, making them more susceptible to lung injury, inflammation, and infection after bacterial challenge. A dramatic alteration in the pulmonary clearance of a bacterial pathogen as well as a reduction in AM activity were observed. Thus, exposure to metal-containing particulates in the workplace or from air pollution may suppress the lungs' ability to defend against infection. Studies are ongoing in an attempt to determine which individual metal or combination of metals may be responsible for the alterations observed in lung defenses after infection.

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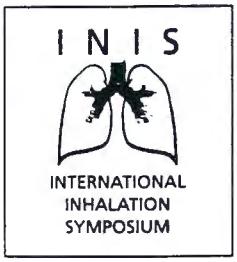
Effects of Air Contaminants on the Respiratory Tract – Interpretations from Molecules to Meta Analysis



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