RESPONSES TO WELDING FUMES: Lung Injury, Inflammation, and the Release of Tumor Necrosis Factor-α and Interleukin-1β

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Possible mechanisms were examined whereby welding fumes may elicit injury and inflammation in the lungs. The effects of different welding fumes on lung macrophages and on the in vivo production of two inflammatory cytokines, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β), were assessed. Fume was collected during flux-covered manual metal arc welding using a stainless steel consumable electrode (MMA-SS) and gas metal arc welding using a mild steel electrode (GMA-MS). For the in vitro study, bronchoalveolar lavage was performed on untreated rats to recover lung macrophages, and the effects of the welding fumes on macrophage viability and respiratory burst were examined. In vivo, additional rats were intratracheally instilled with the welding fumes at a dose of 1 mg/100 g body weight. These rats were lavaged 1, 14, and 35 days postinstillation, and indicators of lung damage (cellular differential, albumin, TNF-α and IL-1β release, and lactate dehydrogenase and β-n-acetyl glucosaminidase activities) were measured. In vitro, the MMA-SS fume was more cytotoxic to the macrophages and induced a greater release of reactive oxygen species as measured by the respiratory burst compared to the GMA-MS fume. In vivo, evidence of lung damage was observed for both fumes 1 day postinstillation. By 14 days, lung responses to the GMA-MS fume had subsided and were not different from the saline vehicle control group. Significant lung damage was still observed for the MMA-SS group at 14 days, but by 35 days, the responses had returned to control values. One day after the instillations, both welding fumes had detectable levels of TNF-α and IL-1β within the lavage fluid. However, the MMA-SS particles caused a significantly greater release of both cytokines in the lavage fluid than did the GMA-MS group. The results demonstrate that MMA-SS fume caused more pneumotoxicity than GMA-MS. This increased response may reflect enhanced macrophage activation, the increased production of reactive oxygen species, as well as secretion of TNF-α and IL-1β.

Keywords *interleukin-1β, lung inflammation, lung macrophages, occupational health, reactive oxygen species, respiratory burst, tumor necrosis factor-α, welding fumes*

Received 13 June 1996; accepted 11 September 1996.

This work was supported by the New England Welding Consortium, American Welding Society, and NIOSH grant U60/CCU109979. The authors thank Kenneth Brown of the American Welding Society for his gift of the welding fume samples. We also thank Thomas Eagar of MIT for his helpful suggestions in the preparation of this manuscript and Bruce Ekstein for his expert technical assistance.

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0190-2148/97 $12.00 + .00

Electric arc welding joins pieces of metal that have been made liquid by the heat produced as electricity passes from one electrical conductor to another [1]. The high temperatures (4000°C) of this process heat both the base metal pieces to be joined and the consumable electrode, which is fed into the weld. Fumes are generated by the evaporation of metals, including alloying elements and fluxes, primarily at the tip of the electrode. These metal vapors are oxidized on contact with the air (depending on the type of consumable used such as mild steel [MS] or stainless steel [SS]) and form small particulates of different complexes of metal oxides. The hot gases generated by welding are less dense than the surrounding air and rise, carrying with them the fine particulates.

The resulting welding fume, and thus the inhalation exposures of welders, then may vary according to the materials and processes used. It has been estimated that more than 1,000,000 workers worldwide are currently employed full-time as welders [2]. Respiratory effects observed in full-time welders have included bronchitis, airway irritation, metal fume fever, chemical pneumonitis, lung function changes, a possible increase in the incidence of lung cancer, and small opacities on chest radiographs of asymptomatic welders [3].

Animal studies have indicated that differences exist in the degree of lung damage induced by fumes collected from welding processes using different consumable electrodes. In an acute toxicology study by Coate [4], the effects of a single 6-h inhalation exposure of fumes to rats from six welding processes were examined. They reported that SS fumes from flux-covered manual metal arc welding caused the most severe pneumontitis, while MS fumes generated from gas metal arc welding were relatively nontoxic to the lungs.

In a chronic study by Hicks et al. [5], inhalation and intratracheal instillation of welding fumes from SS electrodes caused greater and more prolonged lung damage than did fumes collected from MS welding in rats. However, significant lung inflammation and injury, such as evidence of fibrosis, were also observed in the rats exposed to MS fume. Questions arise concerning this observation since epidemiology studies have reported no incidence of fibrosis among workers exposed to only welding fumes.

The goals of this study were to compare different fumes in regard to their potential to induce lung inflammation and injury and to assess the possible mechanisms responsible. Also, unique to this investigation, we compared the responses of the welding fumes to other occupationally relevant particles whose potential to injure the lungs have been well characterized.

Welding fumes were collected during flux-covered manual metal arc welding using a stainless steel electrode (MMA-SS) and gas metal arc weld-

ing using a mild steel electrode (GMA-MS). Bronchoalveolar lavage was performed on untreated rats, and the effects of the two welding fumes on macrophage viability and the release of reactive oxygen species as measured by the respiratory burst were examined. Activation of lung macrophages and the subsequent release of reactive oxygen species has been shown to be a mechanism by which particles damage the lungs [6–8]. Additional rats were intratracheally instilled with the welding fume samples, and the recovered bronchoalveolar lavage fluid was analyzed to assess lung injury and inflammation. Lindenschmidt et al. [9] have shown that early changes in a variety of biochemical and cellular parameters of the bronachoalveolar lavage fluid after the intratracheal instillation of different mineral particles were predictive of chronic lung responses. To examine possible mechanisms in which welding fumes may injure the lungs, the pulmonary release of two inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), was assessed. Many studies have demonstrated that TNF-α and IL-1β play significant roles in the development of mineral particle-induced lung disease [10–15].

MATERIALS AND METHODS

Welding Process and Particle Collection

The welding fumes tested were received as a gift from the American Welding Society and Lincoln Electric (St. Louis, MO, USA) courtesy of Kenneth Brown. The fumes were generated in a cubical 1-m^3 open-front fume chamber by a skilled welder using a manual or semi-automatic technique appropriate to the electrode. The fumes were collected onto filters during 1–2 min of welding. The fume samples were generated from either manual metal arc welding using a flux-covered, stainless steel (MMA-SS) electrode (E308-16-1) or from gas metal arc welding using a mild steel (GMA-MS) electrode (ER70S-3) with argon and CO_2 shielding gases to protect the weld from oxidation.

Particle Characterization

Bulk analysis of the elemental constituents of the fumes was obtained using energy dispersive spectroscopy (KEVEX Corporation, Model μ7000, Foster City, CA, USA). The surface chemical analysis of the fumes was obtained using X-ray photoelectron spectroscopy (XPS; Perkin–Elmer Model 5100, San Francisco, CA, USA). The depth of the surface analysis has a sensitivity of 1–3 nm.

Particles from the different samples were suspended in sterile saline,

sonicated, dispersed onto glass slides in microwell chambers, and then sized by a Sarastro 2000 (Molecular Dynamics, Sunnyvale, CA, USA) laser scanning confocal microscope (Optiphot-2, Nikon, Inc., Melville, PA, USA) fitted with an argon-ion laser. Using the 488-nm excitation light, polarized images were recorded at wavelengths <510 nm. The diameters of 200 different particles for each sample were measured using Image Space software that accompanies the Sarastro 2000.

Silica and iron oxide were used as positive and negative controls when evaluating the potential of the welding fume samples to induce lung damage. Crystalline Min-U-Sil-5 Silica (U.S. Silica, Berkeley Springs, WV, USA) had a purity of 99.5% alpha-quartz with a mean diameter of 1.362 ± 0.14 μm. Before use, the silica was boiled in 1.0 M HCl for 60 min to remove any surface contaminants. It was then washed with distilled water, dried, and sterilized. Iron oxide particles (gamma-Fe_2O_3) were produced by the combustion of iron pentacarbonyl ($Fe(CO)_5$) vapors as described by Valberg and Brain [16]. The resulting iron oxide agglomerates had a mean diameter of 0.865 ± 0.08 μm.

Animals

Male CD/VAF rats weighing 200–250 g were used for all experiments (Charles River Laboratories, Wilmington, MA, USA). They were given a conventional laboratory diet and tap water ad libitum. They were housed in a clean air, viral- and antigen-free room with restricted access.

In Vitro Lung Macrophages Studies

Bronchoalveolar Lavage. Bronchoalveolar lavage was performed on untreated rats to recover lung macrophages. The rats were deeply anesthetized with an overdose of sodium pentobarbital and exsanguinated by severing the abdominal aorta. Their lungs were lavaged with 15 separate 3-mL aliquots of warm, calcium- and magnesium-free phosphate buffer solution (PBS), pH 7.4. The samples were centrifuged for 7 min at 500*g* and the cell-free lavage fluid discarded. The cell pellets were washed and resuspended in 1 mL of PBS buffer. Total cell number was determined using a hemacytometer. Viability of recovered cells was assessed by trypan blue exclusion and found to be >95%.

Using a cytospin centrifuge (Shandon Southern Products, Cheshire, UK), 1.5×10^5 cells were spun for 5 min at 800 rpm and pelleted onto a slide. Cells (200/rat) were identified on the cytocentrifuge-prepared slides after staining with Wright Giemsa Sure Stain (Fischer Scientific, Pittsburgh, PA, USA). We found that >98% of the cells recovered were macrophages.

Viability Studies. For the viability studies (n = 4 rats/particle group), macrophages were suspended in sterile RPMI 1640 culture medium (pH 7.4) enriched with 10% fetal bovine serum and plated at a concentration of 2×10^5 cells/well. The lung macrophages were then treated with a 25-μg/mL concentration of the silica, iron oxide, MMA-SS, and GMA-MS particles suspended in sterile saline and incubated for 24 h at 37°C and 5% CO_2. Before cell treatment, all particles were sonicated for 1 min using a Sonicor Cell Disruptor (Heat Systems–Ultrasonic, Plainview, NY, USA). The control group received an equal volume of sterile saline. Cell viability was measured on aliquots from each well by trypan blue exclusion.

Respiratory Burst Studies. For the respiratory burst studies (n = 6 rats/particle group), nonactivated macrophages and macrophages primed with lipopolysaccharide (LPS; 0.1 μg/mL) for 4 h at 37°C were suspended in sterile Hanks' Balanced Salt Solution (pH 7.4) and added to a 96-well plate at a concentration of 2×10^5 cells/well. Macrophages undergo a respiratory burst to phagocytic stimuli causing the generation and release of reactive oxygen species, such as superoxide anion and hydrogen peroxide. Kobzik et al. [17] have described a method to measure respiratory burst using dichlorofluorescin (DCFH). The cells were incubated for 15 min with the nonfluorescent precursor, DCFH diacetate (15 μM) at 37°C and 5% CO_2. The lung macrophages were then treated with a 25-μg/mL concentration of the silica, iron oxide, MMA-SS, and GMA-MS particles suspended in sterile saline and incubated for 30 min at 37°C and 5% CO_2. The control group received an equal volume of sterile saline. The oxidation of DCFH to the fluorescent form dichlorofluorescein (DCF) was measured in a Cytofluor 2300 96-well microplate reader (Millipore, Bedford, MA, USA).

In Vivo Welding Fume Treatment

Intratracheal Instillation. Rats (n = 4/treatment group) were intratracheally instilled with 1.0 mg suspended in 150 μL of 0.9% sterile saline/100 g body weight of the MMA-SS and GMA-MS welding fume samples, iron oxide (negative control), and silica (positive control). Animals in the vehicle control group were intratracheally dosed with 150 μL of 0.9% sterile saline/100 g body weight.

This dose of 1.0 mg/100 g body weight was chosen after a preliminary dose–response study was performed with silica, iron oxide, and the welding fumes. When using a 0.2-mg/100-g body wt dose (data not shown), no differences in lung response were observed when the test samples were compared with the saline vehicle control. At a higher dose (5.0 mg/100 g body wt; data not shown), massive lung inflammation and prominent particle burdens were observed for all groups. Pulmonary clearance mecha-

nisms may have been compromised, complicating the results, and making comparisons among the groups difficult.

Before the intratracheal instillation procedure, the 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). Then, the instillations were performed according to the method of Brain et al. [18]. Each rat was placed on a slanted board and was supported by a rubber band under its upper incisors. The tongue of the animal was moved aside, and the larynx was transilluminated by an external light source. Particle suspensions were intratracheally instilled through a 20-gauge, 4-in. needle (Popper and Sons, New Hyde Park, NY, USA). Before instillation, the particles were suspended in 0.9% sterile saline and sonicated for 1 min.

Bronchoalveolar Lavage. Bronchoalveolar lavage was performed on animals from each group 1, 14, and 35 days postinstillation as described earlier. The first two 3-mL aliquots from the lavage sample were centrifuged at 500*g* for 7 min, and the resultant cell-free supernatant was analyzed for various biochemical parameters. The lungs were then lavaged 12 more times with 3-mL aliquots of PBS. These samples were also centrifuged for 7 min at 500*g* and the cell-free lavage fluid discarded. The cell pellets from all washes for each rat were combined, washed, and resuspended in 1 mL of PBS buffer, and evaluated as described below.

Cellular Evaluation. Total cell numbers were determined using a hemacytometer. Cells (200/rat) were differentiated as described in a previous section on the cytocentrifuge-prepared slides after staining with Wright Giemsa Sure Stain.

Biochemical Assays. Within the acellular BAL fluid, three indicators of pulmonary damage were assessed: albumin; the activity of the lysosomal enzyme, β-*n*-acetyl glucosaminidase (β-NAG); and the activity of the cytosolic enzyme, lactate dehydrogenase (LDH). Albumin concentration was determined by the method of Doumas and Biggs [19], and the β-NAG and LDH enzyme activities were assayed by the methods of Sellinger et al. [20] and Pesce et al. [21], respectively. Enzyme reagents were purchased from Sigma Chemical (St. Louis, MO, USA), while other chemicals used in this study were from Fischer Chemical (Pittsburgh, PA, USA).

Inflammatory Cytokines. TNF-α and IL-1β were measured in the acellular bronchoalveolar lavage fluid using the Factor-Test-X TNF-α and the Inter-Test-X IL-1β ELISA kits (Genzyme Immunobiologicals, Cambridge, MA, USA), respectively. In the measurement of both cytokines, samples and standards (recombinant mouse TNF-α and recombinant mouse IL-1β) were measured in duplicate. These kits have been shown to successfully quantitate natural rat TNF-α [22] and IL-1β [23].

Statistical Analysis

All results are expressed as means ± standard error (SE). Statistical analyses were carried out with a Statview statistical program. The significance of the interaction among the different treatment groups in examining macrophage function (Figures 1–3) and parameters of lung injury (Table 2 and Figures 4–8) was assessed by using an analysis of variance (ANOVA). The significance between each of the individual groups at each time point was analyzed using the Fischer's Least Significance Difference post-hoc test. Due to the many different treatment groups and time points, symbols denoting groups of different significance are absent in Figures 4–6. Instead, significant differences among groups are discussed in the Results section. For all analyses, the criterion of significance was $p < .05$.

RESULTS

Particle Characterization

Table 1 shows the sizes and the elemental compositions of the two welding fume samples. No difference was observed in the size of the two fumes. Both were of respirable size and <1 μm. However, the chemical composition of the two welding fumes was quite different. The GMA-MS particles consisted of oxides from the metals used in the manufacture of the consumable wire (~80% iron oxide), and the water solubility was relatively low. Compared to the GMA-MS particles, the core of the MMA-SS particles had decreased levels of iron and manganese, increased levels of silica, as well as potassium, chromium, calcium, sodium, titanium, copper, aluminum, nickel, vandium, and sulfur which were not present in the GMA-MS

Table 1 Size and chemical analysis of the welding fume samples

Sample	Count mean diameter (μM)	Bulk analysis (% wt)	Surface analysis (% wt)
GMA-MS	0.83 ± 0.15	80.1% Fe 12.9%Mn 7.0% Si	88.2% Fe_2O_3 11.8% $(Fe,Si)O_3$
MMA-SS	0.92 ± 0.11	22.3% K,19.4%Fe, 13.1% Cr,12.3% Si, 8.2%Ca,8.0%Mn, 5.0%Na,4.3% Ti, 3.1%Cu,2.1% Al, 1.2% Ni,0.8% V, 0.2% S	>97% complex fluoride compound

Note. Diameter values are means ± SE; n = 200 particles/sample.

sample. A larger component of the MMA-SS sample was soluble in water as compared to the GMA-MS particles.

The surface of the MMA-SS fume consisted almost entirely of complex fluoride compounds (97%), while 88% of the GMA-MS fume surface was Fe_2O_3. The fluoride originates from the flux used during manual metal arc welding. For the MMA-SS fume sample, no metallic elements were detected on the surface. When the sample surface was cleaned by sputtering for 2, 4, 6, and 8 min at a rate of 2.8 nm/min, no new elements were detected.

In Vitro Lung Macrophage Studies

Macrophage Viability. The viability of lung macrophages was assessed after a 24-h incubation with the different particle samples at a concentration of 25 μg/mL (Figure 1). The MMA-SS and silica samples caused a 53 and a 47% loss in cell viability, respectively. These increases in macrophage death were significantly greater than that seen in the other three groups. When treated with GMA-MS, 13% of the cells died as compared to 12% for the iron oxide group. After the 24-h incubation without particles (control group), only 6% of the macrophages had died, which was significantly less than all the other groups.

Respiratory Burst. The respiratory burst of macrophages incubated for 30 min with the different particle samples at a concentration of 25 μg/mL was measured (Figure 2). Increases of 30 and 27% in respiratory burst over control value (macrophages not exposed to particles) were observed for the silica and MMA-SS groups, respectively. These increases in respiratory burst were significantly higher than what was observed with the GMA-MS fume, which increased the burst by 17%. Interestingly, the iron oxide particles caused a depression of the respiratory burst by 7%.

In Figure 3, the particles were incubated with macrophages that had been primed with LPS (0.1 μg/mL) for 4 h. This treatment increased the respiratory burst by 19.6 ± 5.7% compared to control cells shown in Figure 2. All groups increased the respiratory burst as compared to control cells. MMA-SS increased the burst by 47%, while silica showed an increase of 40%. These increases in respiratory burst were significantly greater than the increases of 29 and 12% for GMA-MS and the iron oxide groups, respectively.

In Vivo Analysis of Bronchoalveolar Lavage Fluid

In the above studies, macrophages from normal control animals were recovered and then incubated in vitro. We now present the in vivo responses to particles instilled into the lungs.

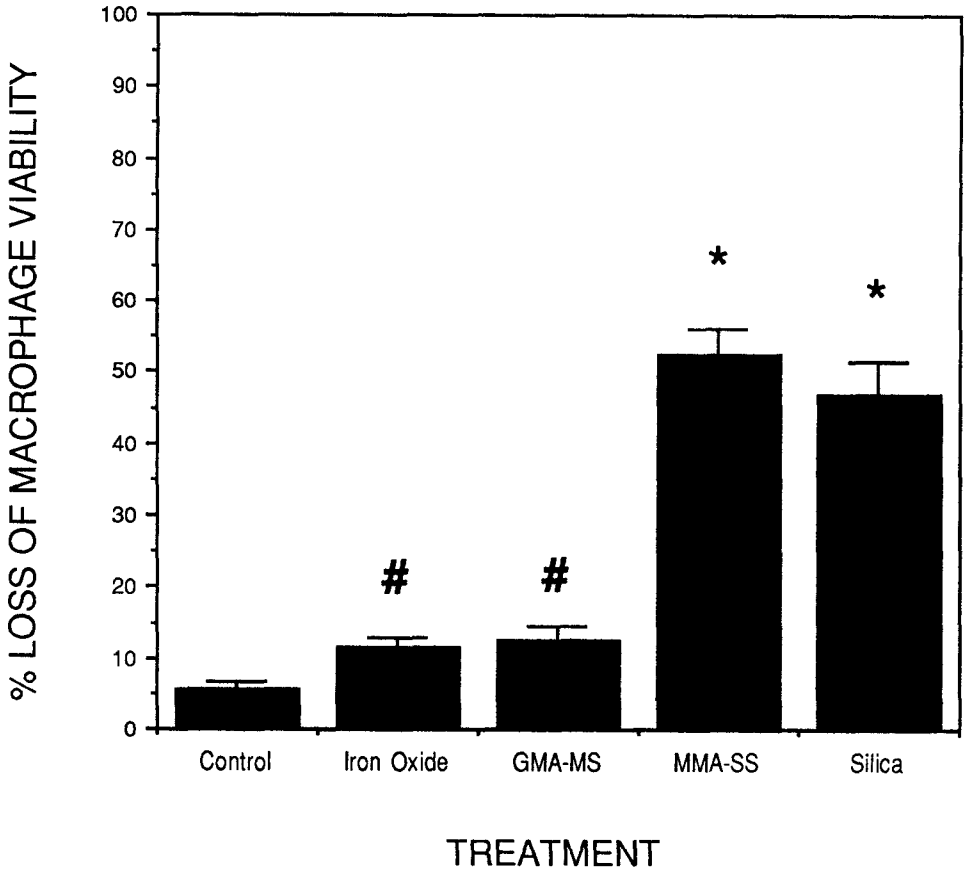

Figure 1. Viability of lung macrophages incubated for 24 h with MMA-SS and GMA-MS welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The final concentration of the particle samples was 25 μg/mL in the incubation. Viability was evaluated by trypan blue exclusion. Values are means ± SE (n = 4; *, significantly greater than all other groups; #, significantly greater than the control group; and the criterion of significance was $p < .05$).

Cellular Parameters of Lung Inflammation. Table 2 shows the cell differentials of the different treatment groups 1, 14, and 35 days postinstillation. At all time points, the instillation of silica induced a dramatic and significant infiltration of cells into the lungs. When compared to the saline control, all treatment groups had significant elevations in the cells recovered from the lung 1 day after the instillations. But at 14 and 35 days, only the silica and MMA-SS groups had significant increases in cells recovered from the lungs.

No significant differences in the number of macrophages recovered from the lungs were observed among any of the groups at 1 day and among the GMA-MS, iron oxide, and saline groups at any of the time points. At 14

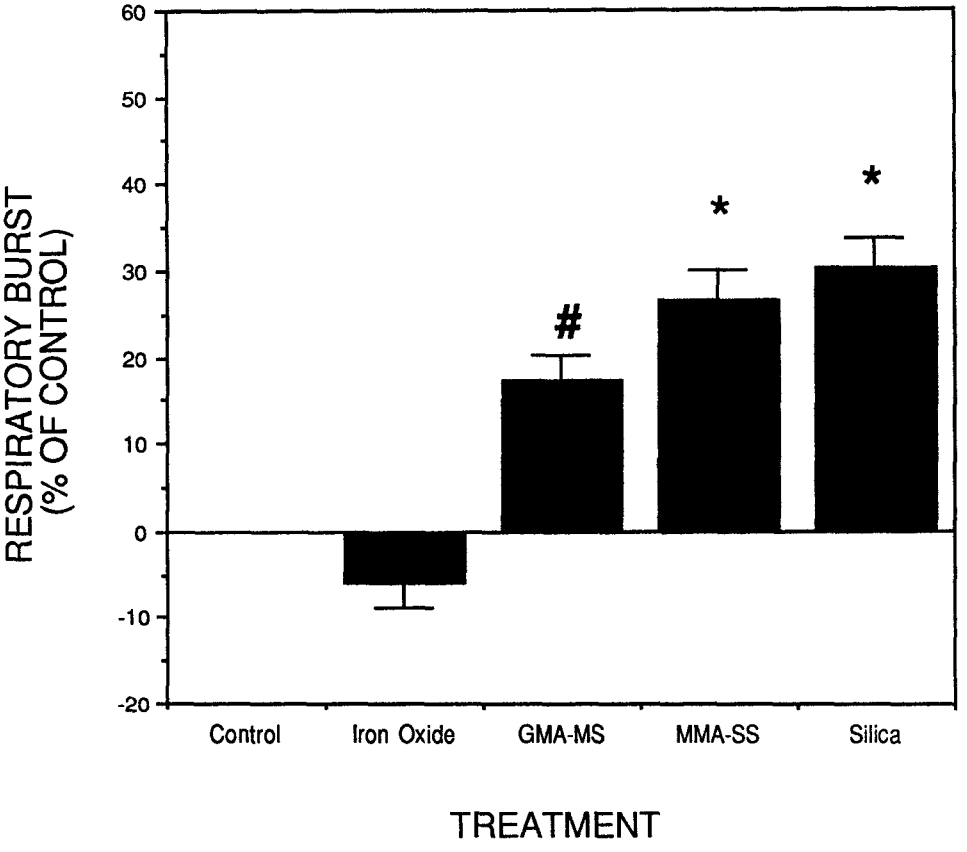

Figure 2. Respiratory burst of nonactivated lung macrophages incubated for 30 min with MMA-SS and GMA-MS welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The final concentration of the particle samples was 25 μg/mL in the incubation. Values, expressed as % difference of respiratory burst of saline control (macrophages not treated with particles), are means ± SE ($n = 6$; *, significantly greater than all other groups; #, significantly greater than the iron oxide and control groups; and the criterion of significance was $p < .05$).

days, a significant increase in macrophage number was observed only for the MMA-SS group. By 35 days, more macrophages were lavaged from both the silica and MMA-SS groups when compared to the other groups, although the silica response was far greater.

Neutrophil numbers were substantially elevated for the silica group at all time points as compared to the other groups. Significant increases in neutrophils were observed for all groups at 1 day compared with the saline control. By 14 days, the number of lavaged neutrophils for the GMA-MS and iron oxide groups returned to the saline control level, while the neutrophil number remained elevated for the MMA-SS group.

Significant increases in lymphocyte number were observed for the silica

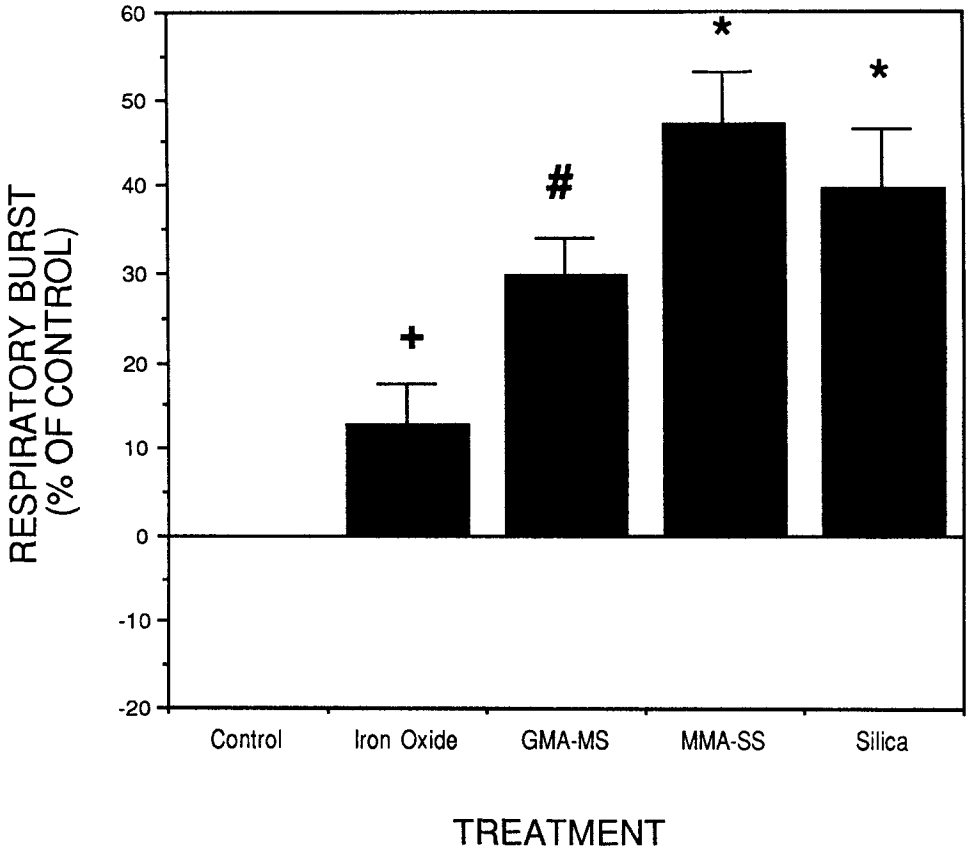

Figure 3. Respiratory burst of lipopolysaccharide (LPS)-primed lung macrophages incubated for 30 min with MMA-SS and GMA-MS welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The final concentration of the particle samples was 25 μg/mL in the incubation. Lung macrophages were primed with LPS (0.1 μg/mL) for 4 h before incubation with the particle samples. Values, expressed as % difference of respiratory burst of saline control (macrophages not treated with particles), are means ± SE (n = 6; *, significantly greater than all other groups; #, significantly greater than the iron oxide and control groups; +, significantly greater than the control group; and the criterion of significance was $p < .05$).

group at all time points when compared to the other groups. At all time points, there were no differences in the number of lymphocytes lavaged from the animals in the other groups except for the MMA-SS group at 14 days.

Biochemical Parameters of Lung Injury. When the albumin content in the acellular bronchoalveolar lavage fluid was measured 1 day postinstillation (Figure 4), all groups had significant elevations when compared to the saline control. Moreover, the silica and MMA-SS groups had significant increases in albumin as compared to the other groups. At 14 days, the silica,

Table 2 Bronchoalveolar lavage cell profiles

Treatment	Total number ($\times 10^6$)			
	Total	Macrophage	Neutrophil	Lymphocyte
1 day				
Silica	18.9 ± 1.9*	4.2 ± 0.2	14.2 ± 2.2*	1.2 ± 0.3*
MMA-SS	10.9 ± 0.3#	4.5 ± 0.5	5.6 ± 0.7#	0.5 ± 0.1
GMA-MS	10.3 ± 0.6#	4.4 ± 0.7	5.8 ± 0.7#	0.4 ± 0.1
Iron oxide	10.7 ± 0.3#	4.5 ± 0.5	5.9 ± 0.3#	0.4 ± 0.1
Saline	5.0 ± 0.5	4.7 ± 0.4	0.2 ± 0.1	0.2 ± 0.1
14 days				
Silica	28.4 ± 3.3*	6.1 ± 0.9	20.4 ± 2.4*	1.8 ± 0.2*
MMA-SS	12.3 ± 0.5‡	9.9 ± 0.5*	1.9 ± 0.3‡	0.6 ± 0.1‡
GMA-MS	5.5 ± 0.8	5.1 ± 0.7	0.2 ± 0.1	0.1 ± 0.0
Iron oxide	4.6 ± 0.3	4.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.0
Saline	4.3 ± 0.7	4.2 ± 0.7	0.1 ± 0.0	0.0 ± 0.0
35 days				
Silica	58.2 ± 4.0*	10.6 ± 2.9‡	45.2 ± 3.7*	4.8 ± 0.5*
MMA-SS	8.5 ± 0.7‡	7.3 ± 0.6‡	0.9 ± 0.1‡	0.3 ± 0.1
GMA-MS	5.2 ± 0.6	4.5 ± 0.3	0.2 ± 0.1	0.1 ± 0.1
Iron oxide	5.7 ± 0.3	5.6 ± 0.3	0.1 ± 0.1	0.0 ± 0.0
Saline	4.9 ± 0.4	4.8 ± 0.3	0.1 ± 0.0	0.1 ± 0.1

Note. Values are means ± SE; $n = 4$. *, significantly greater than all other groups ($p < .05$); #, significantly greater than saline control group ($p < .05$); ‡ significantly greater than ER70S-3, iron oxide, and saline groups ($p < .05$).

MMA-SS, and iron oxide groups had significant elevations as compared to the GMA-MS and saline groups. By 35 days, levels of albumin for the MMA-SS and iron oxide groups returned to the saline control level, while injury induced by silica continued to rise and was significantly higher than all other groups.

LDH activity was significantly elevated for all groups 1 day after the instillations when compared to the saline control group (Figure 5). At this time point, LDH values for the MMA-SS, GMA-MS, and iron oxide groups were not significantly different from each other, were greater than the saline group, but were less than silica group. For the GMA-MS and iron oxide groups, the increases in activity were diminished by 14 days and no different than saline control levels. Both the silica and MMA-SS groups had substantial elevations in LDH activity at this time point when compared to all the other groups. However, at 35 days, LDH levels were still elevated for the silica group, but had returned to the saline control level for the MMA-SS group.

For β-NAG activity (Figure 6), all groups caused significant increases in activity at 1 day. Silica induced the greatest release of β-NAG from the pulmonary phagocytes, followed by the MMA-SS particles, and then the

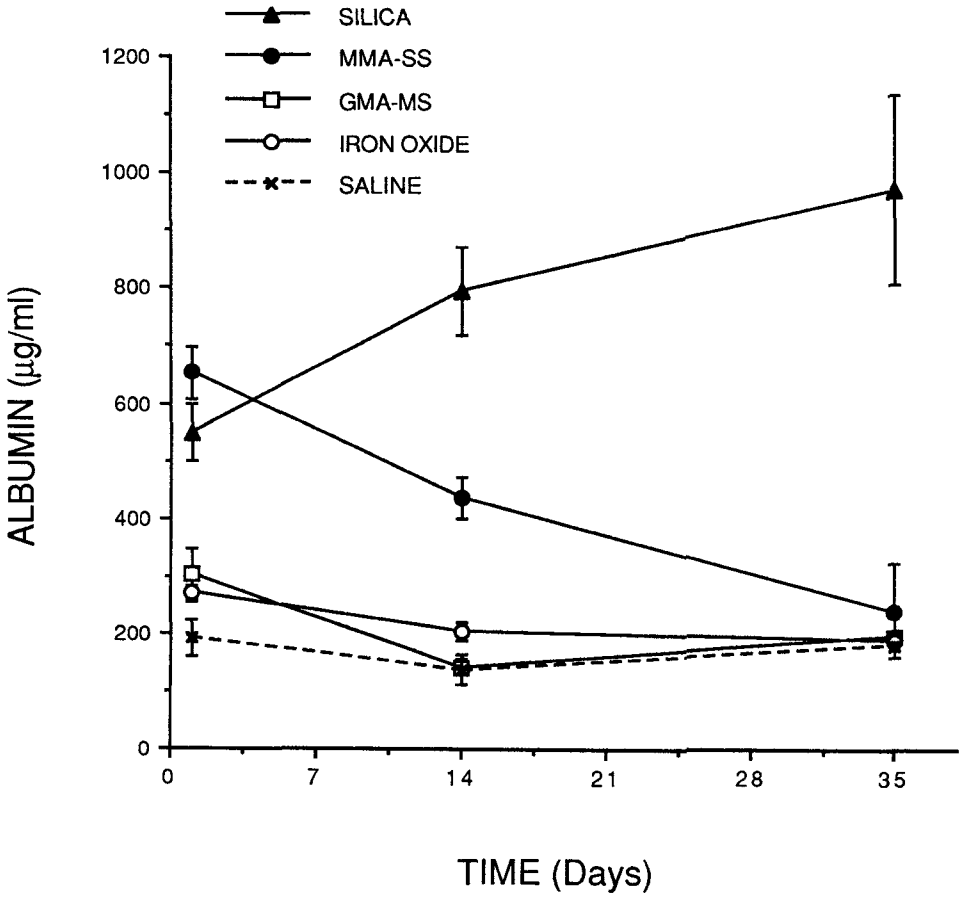

Figure 4. Total albumin of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1, 14, and 35 days after the intratracheal instillation of MMA-SS and GMA-MS welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The animals received a single dose of 1.0 mg in 150 µL of sterile saline/100 g body weight. Values are means ± SE.

GMA-MS and iron oxide samples. At 14 days, the activity of the iron oxide and GMA-MS groups had returned to the saline control level at this time point, while the silica and MMA-SS groups still had significant increases in activity. At 35 days, β-NAG levels were still increasing for the silica group, but were no different from the saline control value for the MMA-SS group.

TNF-α and IL-1β Release. One day after the intratracheal instillation of the different particle samples, significant increases in TNF-α and IL-1β were measured in the acellular bronchoalveolar lavage fluid of the MMA-SS, GMA-MS, and silica groups as compared to the iron oxide and saline groups (Figures 7 and 8). A significantly greater amount of TNF-α was observed for the silica group when compared with all the groups, while significantly

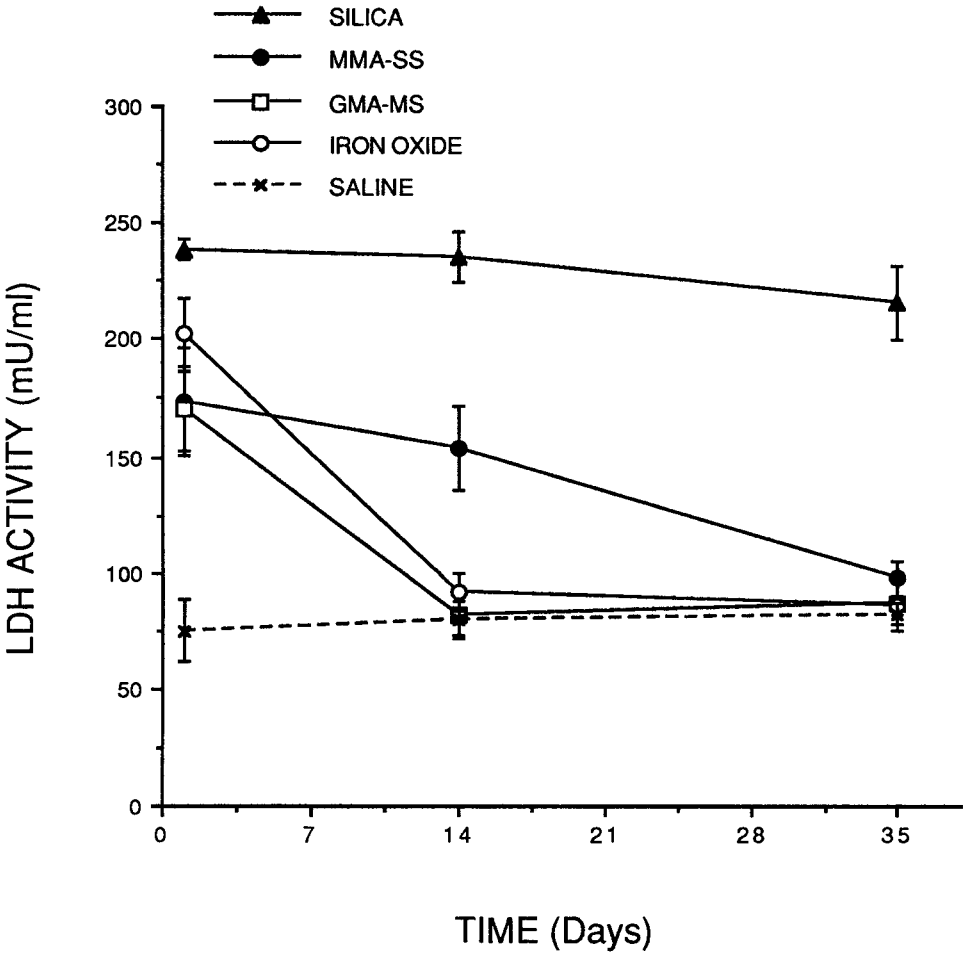

Figure 5. Lactate dehydrogenase (LDH) activity of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1, 14, and 35 days after the intratracheal instillation of MMA-SS and GMA-MS welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The animals received a single dose of 1.0 mg in 150 μL of sterile saline/100 g body weight. Values are means ± SE.

more TNF-α was observed in the MMA-SS group as compared to the GMA-MS group. In the measurement of IL-1β, a significant elevation was observed in the lavage fluid of the MMA-SS group as compared to the other groups, while a greater amount was observed in the silica group compared with the animals exposed to the GMA-MS fume. For both TNF-α and IL-1β, no detectable levels of either cytokine were observed in the lavage fluid of the saline and iron oxide groups. At the other two time points postinstillation (14 and 35 days), no TNF-α and IL-1β were detected in the lavage fluid for any of the groups (data not shown).

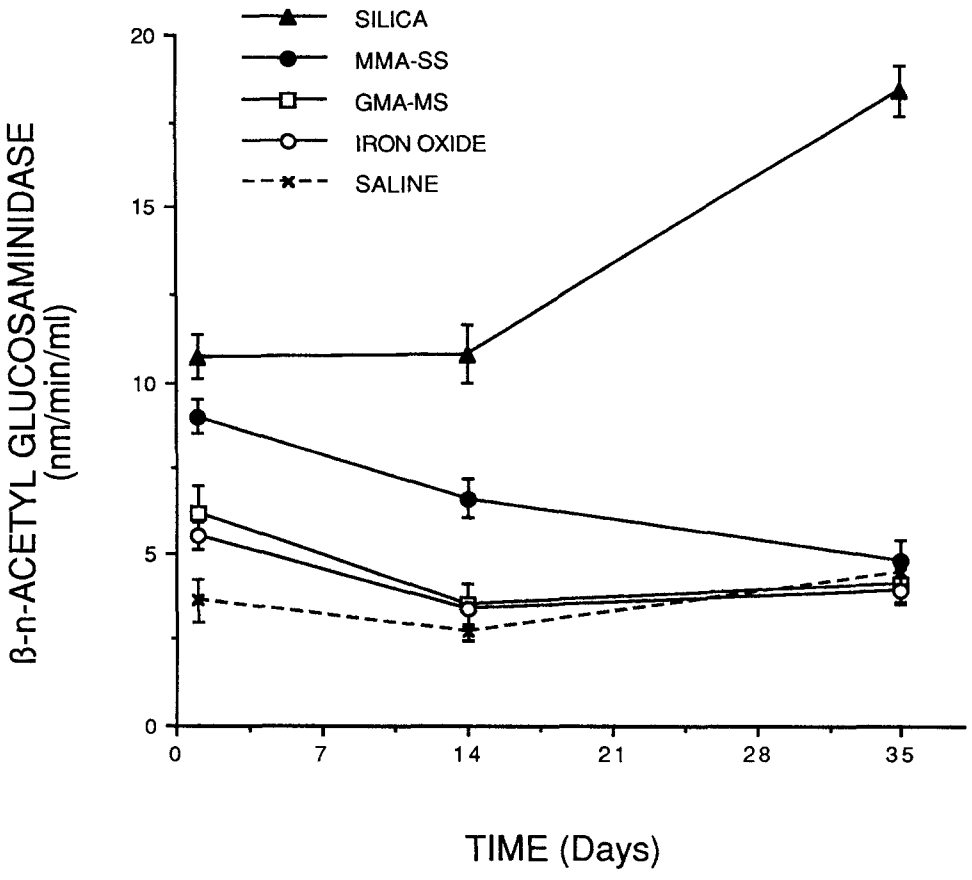

Figure 6. β-*n*-Acetyl glucosaminidase activity of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1, 14, and 35 days after the intratracheal instillation of MMA-SS and GMA-MS welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The animals received a single dose of 1.0 mg in 150 μL of sterile saline/100 g body weight. Values are means ± SE.

DISCUSSION

In this investigation, two different welding fumes, one from flux-covered manual metal arc welding using a SS electrode and one from gas metal arc welding using a MS electrode, were collected and characterized. To study the mechanisms in which welding fumes may damage the lungs, the effect of the fumes on lung macrophage function and the induction of pulmonary injury and inflammation were assessed. The fumes were also compared to two extensively studied dusts, silica and iron oxide.

The collected welding fumes had mean diameters of <1 μM, giving the particles a high probability of being deposited in the deep airspaces of the lungs. Several studies have shown that most particles generated during

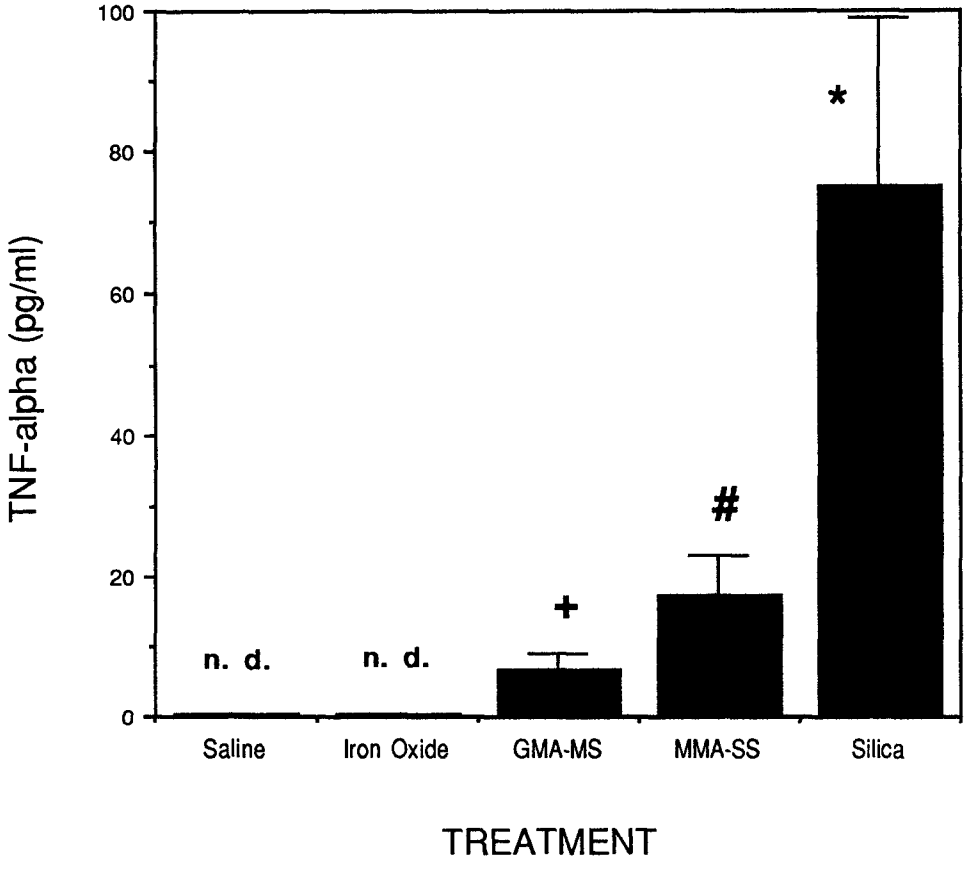

Figure 7. Tumor necrosis factor-alpha (TNF-α) of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1 day after the intratracheal instillation of MMA-SS and GMA-MS welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The animals received a single dose of 1.0 mg in 150 μL of sterile saline/100 g body weight. Values are means ± SE (nd, not detected; $n = 4$; *, significantly greater than all other groups; #, significantly greater than the GMA-MS, iron oxide, and control groups; +, significantly greater than the iron oxide and control groups; and the criterion of significance was $p < .05$).

welding are smaller than 1 μM [1, 24, 25]. Many of the individual fume particles appeared to be <0.1 μM (data not shown) but had aggregated together during welding or the collection of the fumes. Agglomeration has been shown to be enhanced by the turbulent conditions resulting from heat generated in the welding process, which increases particle movement and chances for particle collision. Since the particles of the two welding samples were of similar size, a comparison of their potential to induce lung damage could be examined. In this study, the contribution of smaller ultrafine particles to the toxicity of the fumes that may be inhaled in the workplace was not addressed.

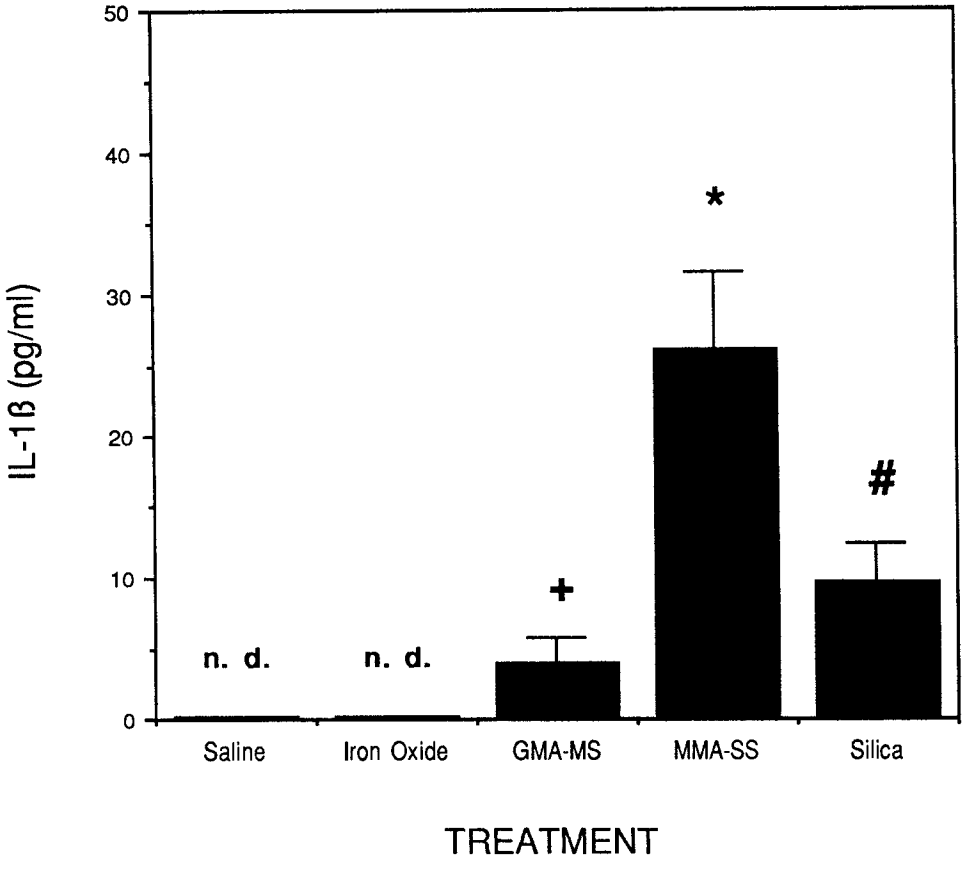

Figure 8. Interleukin-1β (IL-1β) of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1 day after the intratracheal instillation of MMA-SS and GMA-MS welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The animals received a single dose of 1.0 mg in 150 μL of sterile saline/100 g body weight. Values are means ± SE (nd, is not detected; $n = 4$; *, significantly greater than all other groups; #, significantly greater than the GMA-MS, iron oxide, and control groups; +, significantly greater than the iron oxide and control groups; and the criterion of significance was $p < .05$).

The fume generated from the stainless steel electrode, MMA-SS, contains many elements that were not present in the mild steel fume, GMA-MS, which consisted of over 80% of insoluble iron oxides. Differences in composition could lead to differing lung responses to the inhaled fumes. Of interest, chromium was present in the core of the MMA-SS particles but not in the GMA-MS sample. Hexavalent chromium, Cr(VI), has been implicated as a carcinogen [26] and is normally present in fumes generated during welding of SS materials [27, 28]. After the inhalation of chromium or chromium containing compounds, Van Burg and Liu [29] observed impaired lung function, bronchitis, emphysema, and lung inflammation. It

is also important to note that both welding fumes contained silica. The form of silica found in the two welding fumes was of the amorphous, nonpneumotoxic form. This is in contrast to the highly inflammatory and fibrogenic crystalline form of α-quartz used in this investigation as a positive particle control.

Fluxes in consumable electrodes are used to carry away impurities during metal arc welding. Fluorides have been used in the manufacture of the flux-covered, consumable electrodes. For the MMA-SS fume, 97% of the surface of the particles consisted of complex fluoride compounds. The presence of fluorides on fume surfaces could also contribute to the pulmonary responses in welders. Inhalation of gases containing fluorine has been shown to injure the lungs of rats [30], and pulmonary exposures to particulate fluorides in the workplace have been implicated as a contributing factor in the induction of occupational asthma [31].

When comparing the two fumes, the MMA-SS fume was more cytotoxic to lung macrophages and induced a greater respiratory burst than did GMA-MS fume. This increase in oxidative metabolism results in the enhanced generation of reactive oxygen species. These highly, reactive oxidants, such as superoxide anion, hydrogen peroxide, and hydroxyl radical, damage the lung tissue if produced in excess [6–8]. The presence of these reactive oxygen species may be responsible for the elevations in macrophage cytotoxicity observed for the MMA-SS fume when compared with GMA-MS fume. The effect of the MMA-SS on the macrophages was similar to that seen with the highly cytotoxic, silica dust.

In other in vitro studies where macrophages were treated with welding fumes, similar findings were observed. Hooftman et al. [32] have shown that bovine lung macrophage viability and phagocytosis were greatly reduced by particles generated in the manual metal arc welding using SS electrodes as compared to welding using MS materials. Pasanen et al. [33] and Stern and Pigott [34] also reported that SS fumes from manual metal arc welding were far more toxic to rat peritoneal macrophages than fumes from other welding processes. We believe that differences in the elemental composition of different welding fumes affect macrophage viability and function, as well as, the concentration of the particles that macrophages are exposed.

The MMA-SS and silica particles had similar pneumotoxicity profiles when damage was examined early after the intratracheal exposures. However, in the context of chronic lung injury, silica had the much greater response. Since the pulmonary responses to the MMA-SS fume had mostly subsided by 35 days, the lungs may have cleared or solubilized many of the particles. Silica particles have been demonstrated to persist in the lungs [35], and this greater pulmonary persistence may contribute to the chronic lung disease (silicosis) that it causes.

The differences in lung effects between the GMA-MS and MMA-SS fumes are consistent with the conclusions of a chronic study by Hicks et al. [5]. In their investigation, the SS fume also caused a greater level of pneumotoxicity, which persisted longer when compared to the MS fume. However, they also reported significant lung inflammation and injury in the animals exposed to the MS fume, and pulmonary fibrosis was observed in the rats treated with either fume. The intratracheal instillation doses (10 and 50 mg/rat for both groups) used by Hicks et al. [5] were very high. These high particle doses might explain the increase in toxicity and signs of fibrosis induced by the MS fume.

Also in the Hicks et al. [5] study, animals were exposed by a single inhalation dose (1178 $\mu g/m^3$ for the MS and 400 $\mu g/m^3$ for the SS). After 200–300 days, lung nodules were observed in both MS and SS groups, as well as, evidence of low-grade fibrotic changes in the lungs of the rats exposed to the MS fume. In our current acute study, using an intratracheal dose that results in a higher lung burden than the single inhalation dose used by Hicks et al. [5], we saw little lung inflammation or injury in the MS groups as early as 7 days, which then completely subsided by 14 days. For the SS group, most of the lung pneumotoxicity was approaching control values at 35 days. Our results, as compared to the observations of Hicks et al. [5], correlate more closely with the findings of a number of epidemiology studies. While pulmonary accumulation of welding particles and a pneumoconiosis have been observed in welders, no cases of progressive pulmonary fibrosis have been reported.

In an acute study by Coate [4], fume from manual metal arc welding using an MMA-SS electrode was found to be the most pneumotoxic when compared with fumes generated from five different welding process. After a single 6-h aerosol exposure (600 $\mu g/m^3$), MMA-SS induced pulmonary irritation in rats as characterized by pulmonary edema and pneumonitis. After inhalation of fume generated from gas metal arc welding using the GMA-MS electrode, very minimal histopathologic changes were observed in the lungs of exposed rats. In agreement with our investigation, Coate [4] concluded that the GMA-MS was nontoxic at the concentration used in their study, and the MMA-SS fume could be regarded as highly toxic. He also noted that further investigation of the mechanisms of the pneumotoxicity of this fume were needed.

The instillation technique is a nonphysiological method to reliably introduce substances directly into the lungs [18]. However, Lindenschmidt et al. [9] have shown that the intratracheal instillation of mineral particles to rats, with the dose (1 mg/100 g body weight) used in our current investigation, easily differentiated fibrogenic from nonfibrogenic materials. This dose was also still high enough so that a potential adverse effect could be

observed and pneumotoxicity of different mineral particles could be compared. Recently, Henderson et al. [36] conducted a study comparing the inflammatory response of the lungs to particles of high and low toxicity by both instillation or inhalation. Their results indicated that the degree of pulmonary inflammation induced by different materials could be appropriately evaluated using either exposure method. While much higher doses of particles are intratracheally instilled into the lungs of animals as compared to the doses of particles that may be inhaled by workers in an occupational setting, the instillation technique, as demonstrated by this study and others, remains useful in assessing the relative toxicities of different particle samples of similar sizes.

To assess possible mechanisms involved in the responses to welding fumes, the release of the inflammatory cytokines, TNF-α and IL-1β, was studied. They are produced by a number of pulmonary cells and initiate the cascade of cytokines and other factors associated with inflammation [37]. There is also convincing evidence that TNF-α contributes to collagen accumulation and the eventual formation of pulmonary fibrosis following exposure to certain dusts, such as silica [38]. IL-1β has been shown to stimulate fibroblast proliferation [39] and increase the transcription of type I, II, and IV collagen [40]. TNF-α plays an important role in the recruitment of neutrophils to the lungs after exposure to different dusts [12]. Both TNF-α and IL-1β can induce expression of other chemotactic cytokines, such as monocyte chemotactic peptide (MCP) and macrophage inflammatory proteins-1α and 2 (MIP-1α, MIP-2), which contribute to pulmonary increases of neutrophils, lymphocytes, and macrophages observed during inflammation in both rats and humans [15, 41]. Several investigators have provided direct evidence that TNF-α and IL-1β are key to the pathogenesis of granulomatous inflammation [42, 43].

Detectable levels of TNF-α and IL-1β were measured within the acellular bronchoalveolar lavage fluid for the silica and both welding fumes groups. In comparison, silica and MMA-SS caused a significantly greater release of both cytokines in the lavage fluid than did the GMA-MS group. In agreement with our study at the same intratracheal dose, Driscoll et al. [12] found that silica elicited an IL-1 and TNF-α response from recovered macrophages only after 24 h and not at later time points. The relatively inert dust titanium dioxide did not cause a spontaneous release of either cytokine from recovered macrophages at any of the time points. The ability of silica and the MMA-SS welding fume to induce a greater respiratory burst in alveolar macrophages and the presence of significantly more TNF-α and IL-1β in the lavage fluid 24 h after the intratracheal instillations may be responsible, at least in part, for the greater inflammation and pneumotoxicity associated with these two groups at the later time points. The increased

production of pulmonary TNF-α and IL-1β for the MMA-SS group when compared with the GMA-MS group may explain the elevations in macrophages and neutrophils observed at 14 and 35 days, and the elevations seen in lymphocyte number at 14 days.

In this study, we have demonstrated in rats that welding fumes of different composition produce different lung responses. The MMA-SS fume generated a greater release of reactive oxygen species from lung macrophages and was more pneumotoxic after the intratracheal instillation of the particles into the lungs of rats when compared to the GMA-MS fume. Elevated levels of TNF-α and IL-1β were also measured in the acellular bronchoalveolar lavage fluid of the rats exposed to the MMA-SS fume. The presence of these inflammatory cytokines within the lavage fluid may help explain the increases observed in the lung injury and inflammation caused by the MMA-SS fume. Unlike the highly pneumotoxic and fibrogenic dust, silica, it appears that the potential for chronic pneumotoxicity after the exposure to MMA-SS is minimal if exposure to the fume is low and/or intermittent. It is also important to note that intratracheal instillation of the GMA-MS fume induced a similar pulmonary response as iron oxide, which has been characterized as a nuisance dust with little inflammatory and fibrogenic potential. More studies may be needed to assess the role individual elemental components of different fume, such as chromium, manganese, and fluorides, play in the development of lung injury and inflammation after welding.

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