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Pneumotoxicity and Pulmonary Clearance of Different Welding Fumes after Intratracheal Instillation in the Rat

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The objectives of this study were to compare different welding fumes in regard to their potential to elicit lung inflammation or injury and to examine possible mechanisms whereby welding fumes may damage the lungs. Fume was collected on filters from conventional spray [mild steel (MS-SPRAY) or stainless steel (SS-SPRAY) electrode wire] or pulsed current [mild steel (MS-PULSE) electrode wire] gas-shielded metal arc welding. Rats were given one of the three welding fume samples by intratracheal instillation (1.0 mg/100 g body wt). Other rats received a relatively inert dust (iron oxide), a pneumotoxic dust (crystalline silica), or a vehicle control (saline). Bronchoalveolar lavage (BAL) was performed 1, 7, 14, and 35 days postinstillation, and indicators of pulmonary damage [cellular differential, albumin, as well as, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), lactate dehydrogenase, and β-*n*-acetyl glucosaminidase release] were assessed. One day postinstillation, some evidence of lung inflammation (more neutrophils) was observed for all particle groups, while increased BAL TNF-α and IL-1β were observed only in the SS-SPRAY and silica groups. By 14 days, lungs appeared normal among the MS-SPRAY, MS-PULSE, and iron oxide groups. At 14 and 35 days postinstillation, elevated pulmonary responses persisted for the animals exposed to silica and the SS-SPRAY welding fume. By 35 days, however, the SS-SPRAY group approached control levels, while the injury induced by silica increased. Using magnetometric estimates of welding fumes, we observed that MS-SPRAY fume was cleared from the lungs at a faster rate than the SS-SPRAY particles. We have demonstrated that the SS-SPRAY fume has more pneumotoxicity than MS fumes. This difference may reflect a greater retention of the SS-SPRAY particles in the lungs and different elemental composition of the fume. The SS-SPRAY fume also had enhanced release of TNF-α and IL-1β from lung cells soon after fume instillation. In contrast, we saw no influence of the power supply on particle size, composition, or toxicity.

More than 1,000,000 workers worldwide are currently employed full-time as welders, while higher numbers of workers perform welding intermittently as part of their job (Sundin, 1988). A number of epidemiologic studies have reported a higher incidence of respiratory illness in welders. 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 (as reviewed by Sferlazza and Beckett, 1991). Thus it is important to reduce welding fume toxicity and exposure whenever possible.

Electric arc welding joins pieces of metal that have been made liquid by heat produced as electricity passes from one electrical conductor to another (Howden, 1988). Temperatures above 4000°C in the arc heat both the base metal pieces to be joined and a filler metal coming from a consumable, electrode wire, which is fed into the weld. The mixture of hot gases produced during welding is less dense than the surrounding air, causing it to rise and carry upward fine fume particulates.

The nature of the respirable fume depends on the composition of the consumable wire and the type of welding process used. The majority of the fume comes from the electrode wire and is primarily composed of oxides of metals found in the wire. Stainless steel (SS) and mild steel (MS), two of the most common types of wire used in welding, have different elemental compositions and thus produce welding fumes with different chemical constituents. Also, in an attempt to find cleaner and more economic approaches to welding, new welding technologies are being developed. For example, newly commercialized pulsed-current power supplies (PULSE) alter the fume formed during welding (Irving, 1992). PULSE welding reduces the quantity of the welding fume and the size of the particles generated when compared with conventional spray (SPRAY) welding. In turn, the potential of the fume to affect the respiratory health of workers may be altered.

Animal studies have shown that differences can exist in the degree of lung damage induced by different fumes. In a study by Hicks *et al.* (1984) in rats, inhalation or intratracheal instillation of SS particles caused greater and more

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prolonged lung damage than did MS welding fume. Still, the MS fume induced a significant amount of lung inflammation and injury. Evidence of pulmonary fibrosis was also observed in the animals treated by either 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 our study were: (1) to evaluate the potential of different fumes to induce lung inflammation and injury; (2) to assess possible mechanisms whereby welding fumes may injure the lungs; and (3) to compare responses to welding fumes with other occupationally relevant particles whose potential to injure the lungs have been well-characterized. Rats were intratracheally instilled with different welding fumes, a relatively inert dust (iron oxide) and a pneumotoxic, inflammatory dust (crystalline silica). To evaluate pulmonary responses induced by the particle samples, histopathologic analysis was performed and a variety of biochemical and cellular parameters of damage were measured in bronchoalveolar lavage fluid (BALF).

To examine possible mechanisms whereby welding fumes may injure the lungs, two inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were measured in the BALF of exposed rats. Many studies have demonstrated that TNF-α and IL-1β play significant roles in the development of mineral particle-induced lung disease (Hunninghake, 1984; Borm *et al.*, 1988; Driscoll *et al.*, 1990, 1991; Gosset *et al.*, 1991). Particle persistence in the lungs is also considered to be an indicator of increased particle toxicity. Pulmonary retention of SS fume in rats has been shown to be greater when compared with particles from MS welding (Kalliomaki *et al.*, 1983a,b, 1986). Since welding fume contains magnetic constitiuents, the pulmonary clearance of the collected fume samples for this study could be assessed by magnetometry.

MATERIALS AND METHODS

Welding Process and Particle Collection

Welding was performed in the Department of Chemical Engineering at the University of New Hampshire using a gas metal arc welder (Hobart Arc-Master 500, Hobart Brothers Co., Troy, OH) and a Hobart 2410 wire packer. The welder was capable of producing both conventional spray transfer and pulse mode currents. The voltage used for the welding was 26 V at a current level of 220 A. Stainless and mild steel were the two types of consumable electrode wire used. Electrode speed was set at 127 mm/sec while the welding was performed on a circular 12.7-mm-thick plate (0.45 mm in diameter). The plate was set in a circular motion using a motor with a speed of 5.3 mm/sec at the weld site. During the welding process, shielding gases were added to minimize oxidation and other reactions. A combination of 92% argon and 8% CO_2 at a flow rate of 0.275 liters/sec was used.

The electrode and shielding gases entered the top of a metal box with dimensions of $0.88 \times 0.62 \times 0.17$ m where the welding took place. Particles generated within the box were collected for 2 min onto 0.2-μm Nuclepore filters (Nuclepore Co., Pleasanton, CA) during welding. Collection of particles continued for an additional 2 min after the welding had been completed. Approximately 200 mg constituted each sample. More welding runs were performed during PULSE welding since less fume was formed during the 4 min of particle collection than during SPRAY welding.

Particle Characterization

Bulk analysis of the metal constituents of the fume was obtained using energy dispersive spectroscopy (Kevex Corp., Model μ7000, Foster City, CA). The accelerating voltage used for analysis was 20 keV with an incidence angle of 60° and an effective take-off angle of 43.1°. The spectroscope sorts the collected X-rays generated by the interaction of the electron beam with the sample. Peaks appear in the resulting spectra at energies specific to the elements present in the sample.

To determine the size of the instilled particles, the different samples were suspended in sterile saline, sonicated, dispersed onto glass slides in microwell chambers, and then examined using a Sarastro 2000 (Molecular Dynamics, Inc., Sunnyvale, CA) laser scanning confocal microscope (Optiphot-2, Nikon, Inc., Melville, PA) fitted with an argon-ion laser. Micrographs were recorded through a 60× objective using the 488-nm laser line. Polarized light (<510 nm) that reflected from particle surfaces was recorded. Area and diameter measurements of 200 particles from each sample were measured using Image Space software (Molecular Dynamics, Inc.).

Silica and iron oxide were used as positive and negative controls. Crystalline Min-U-Sil-5 Silica (U. S. Silica Corp., Berkeley Springs, WV) was 99.5% alpha-quartz with a mean diameter of 1.36 μm ± 0.14. Before use, the silica was boiled in 1.0 M HCl for 60 min to remove any surface contaminants, 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 (1979). The resulting iron oxide particle agglomerates had a mean diameter of 0.865 μm ± 0.08.

Animals and Welding Fume Treatment

Male CD/VAF rats weighing 250–300 g (Charles River Laboratories, Wilmington, MA) were given a conventional laboratory diet and tap water *ad libitum.* During the study, they were housed in a clean air, virus- and antigen-free room with restricted access.

Rats were intratracheally instilled with 1.0 mg suspended in 150 μl of 0.9% sterile saline/100 g body wt of one of the three welding fume particle samples [stainless steel electrode-spray welding (SS-SPRAY); mild steel electrode-spray welding (MS-SPRAY); mild steel electrode-pulse welding (MS-PULSE)], iron oxide (negative control), and silica (positive control). Animals in the vehicle control group were intratracheally dosed with 150 μl of sterile saline/100 g body wt.

This dose of 1.0 mg/100 g body wt was chosen after a preliminary dose–response study was performed (data not shown). When using a 0.2 mg/100 g body wt dose, no significant inflammatory responses were observed 1 day postinstillation when the animals receiving the test samples were compared with the saline vehicle control. At a higher dose (5.0 mg/100 g body wt; data not shown), dramatic pulmonary inflammation and injury were observed. Pulmonary clearance mechanisms may have been significantly compromised at this higher concentration, 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 Co., Indianapolis, IN). Then, the instillations were performed according to the method of Brain *et al.* (1976). 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. Particle suspensions were instilled into the trachea through a No. 20 gauge, 4 in. needle (Popper and Sons, Inc., New Hyde Park, NY). Before instillation, the particles were suspended in 0.9%

sterile saline and sonicated for 1 min using a Sonicor Cell Disruptor (Heat Systems–Ultrasonic, Inc., Plainview, NY).

Bronchoalveolar Lavage

BAL was performed on animals from each group 1, 7, 14, and 35 days postinstillation. Four animals were used for each treatment group at each time point. The rats were deeply anesthetized with an overdose of sodium pentobarbital and then exsanguinated by severing the abdominal aorta. Their lungs were first lavaged with two separate 3-ml aliquots of warm, calcium- and magnesium-free phosphate buffer solution (PBS), pH 7.4, which was left in the lungs for 30 sec, withdrawn, reinstilled for an additional 30 sec, and then withdrawn again. These two BALF samples were centrifuged at 500*g* for 7 min, and the resultant cell-free supernatant was analyzed for various biochemical parameters. Then, the lungs were 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 BALF was 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. Using a Cytospin centrifuge (Shandon Southern Products, Ltd., Ceshire, England), 1.5×10^5 cells were spun for 5 min at 800 rpm and pelleted onto a slide. Cells (200/rat) were evaluated on the slides after staining with Wright Giemsa Sure Stain (Fischer Scientific, Pittsburgh, PA).

Biochemical Assays

Within the acellular supernatant BAL fluid, three indicators of pulmonary damage were assessed: (1) albumin to quantitate increased permeability of the bronchoalveolar–capillary barrier; (2) the activity of the lysosomal enzyme, β-NAG, to detect the release of enzymes from activated or lysed phagocytes; and (3) the activity of the cytosolic enzyme, LDH, to detect general cytotoxicity. Albumin content was determined by the method of Doumas and Biggs (1972), and the β-NAG and LDH activities were assayed by the methods of Sellinger *et al.* (1960) and Pesce *et al.* (1964), respectively. Enzyme reagents were from Sigma Chemical Co. (St. Louis, MO), while the other chemicals used were from Fischer Chemical Co. (Pittsburgh, PA).

Inflammatory Cytokines

TNF-α and IL-1β were measured in the acellular BALF using the Factor-Test-X TNF-α and the Inter-Test-X IL-1β ELISA kits (Genzyme Immunobiologicals, Cambridge, MA), 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 (Pizzaro *et al.*, 1993) and IL-1β (Wu *et al.*, 1994).

Histopathology

On Days 14 and 35, additional rats from each group were euthanized and the lungs preserved with 2.5% glutaraldehyde by airway fixation at a pressure of 30 cm water. The lobes of the lungs were removed, reproducibly transected, and divided into an equal number of parts. The lung pieces were dehydrated, embedded in parafin, sectioned, and stained with hematoxylin and eosin. Histopathological analysis was performed by Dr. Lester Kobzik who was unaware of the experimental design and blinded to the treatment groups of the study.

Images were recorded using a Sarastro 2000 laser scanning confocal microscope fitted with an argon-ion laser as described previously. Emission spectra >510 nm was diverted to a separate photodetector and used to image lung tissue and cells. Reflected light <510 nm was simultaneously passed to a separate optical path, and provided images of welding fume. Images of lung and welding fume were combined to reveal the position of particles among lung structures.

Pulmonary Clearance of Welding Fume

Histological assessment. Images of particles in lung tissue for microscopic examination of pulmonary clearance were generated by confocal microscopy as described in the previous section. Blocks of fixed lung not used for histopathology were dehydrated in a graded series of ethanol washes, fluorescently labeled with a 0.1 mg/ml concentration of Lucifer Yellow CH, incubated for 24 hr, and then embedded in Spurr's epoxy (Rogers *et al.*, 1992).

Magnetometric assessment. Welding fume contains iron, part of which is in a magnetic form (magnetite). Endogenous lung iron is nonmagnetic. Thus, it is possible to estimate the amount of exogenous lung iron by measuring the magnetic signal from the lungs at different times after instillation. This procedure has been used in our laboratory (Brain *et al.*, 1984) and others (Kalliomaki *et al.*, 1983a,b) to assess the pulmonary clearance of magnetic particles.

For these measurements, additional rats were intratracheally instilled (1 mg/100 g body wt) with SS-SPRAY and MS-SPRAY samples as described above. We omitted the MS-PULSE sample since its toxicity profile was the same as MS-SPRAY. For each of the two groups, the lungs were removed 1 hr and 1, 7, 14, and 35 days postinstillation ($n = 4$ or 5). The lungs were weighed and subdivided into three pieces of approximately equal weight. Each piece of lung tissue was rotated about a vertical axis at 12 Hz and magnetized by a brief pulse (10 μsec) of a strong external magnetic field (~0.1 T). The magnetic field from particles within the the tissue was sensed by four flux gate probes as described by Valberg (1984). For each animal, the magnetic field of the three lung pieces was summed. The total magnetic field from animals euthanized at 1 hr was used as the initial instilled dose of the particles since alveolar clearance is negligible during this time period. The magnetic signals from measured amounts (0.5, 1, 2, and 5 mg) of SS-SPRAY and MS-SPRAY particles were used as standards to generate two calibration curves. The MS-SPRAY particles had the higher iron content, and thus generated a magnetic signal per gram higher than the SS-SPRAY particles. For the tissue samples, the amount of particles (mg) per gram of lung could then be estimated. For each time point, the percentage of the initial instilled dose of the particles remaining in the lung was calculated.

Statistical Analysis

Results are expressed as means ± standard error of measurement (SE). Statistical analyses were carried out with a Statview statistical program. For all parameters, an analysis of variance (ANOVA) was performed. If a significant interaction was present, 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 Figs. 1–3. Instead, significant differences among groups are discussed in the Results section. For all analyses, the criterion of significance was $p < 0.05$.

RESULTS

Characterization of the Particles

Table 1 shows the size and elemental composition of the samples. The samples had mean diameters that were of respirable size <2μm, and no significant differences were observed in the sizes of the particles of the three welding

TABLE 1
Size and Composition of the Welding Fume Samples

Sample	Count mean diameter (μm)	Metal composition (% weight)
SS-SPRAY	1.38 ± 0.16	53.3% Fe, 2.3% Si, 18.3% Mn, 22.2% Cr, 4.9% Ni
MS-SPRAY	1.22 ± 0.11	89.2% Fe, 2.6% Si, 8.2% Mn
MS-PULSE	1.12 ± 0.12	88.6% Fe, 3.8% Si, 7.6% Mn

Note. Diameter values are means ± SE; n = 200 particles/sample. No significant differences were observed in the size of the three welding fume samples; stainless steel-spray (SS-SPRAY), mild steel-spray (MS-SPRAY), and mild steel-pulse (MS-PULSE).

fume samples. However, the composition of the samples was different. All collected fume samples were comprised of oxides from the metals used in the manufacture of the consumable wire, and the water solubility of the generated particles was relatively low. Compared to the MS-SPRAY and MS-PULSE groups, the fume of the SS-SPRAY group had decreased levels of iron and increased levels of manganese. There was also chromium and nickel which were absent in the MS samples.

Analysis of Bronchoalveolar Lavage Fluid

Cellular parameters of lung inflammation. Table 2 shows the number of neutrophils, macrophages, and lymphocytes recovered from the lungs of the different treatment groups 1, 7, 14, and 35 days postinstillation. At all time points, the instillation of silica induced a dramatic infiltration of neutrophils into the lungs that was significantly elevated compared to all the other groups. When compared to the saline control, all particle groups had significant elevations in the number of neutrophils recovered from the lung 1 and 7 days after the instillations. At 14 and 35 days, only the silica and SS-SPRAY groups had significant increases in neutrophil influx into the lungs.

No significant differences in the number of lavaged macrophages were observed among the MS-SPRAY, MS-PULSE, iron oxide, and saline groups at any of the time points. Significant elevations in macrophage numbers were observed at 7, 14, and 35 days for the silica and SS-SPRAY groups. At 7 and 14 days, more macrophages were recovered from the lungs of the SS-SPRAY group than the silica group, but at 35 days, significantly more macrophages were observed in the silica group.

Increases in lymphocyte numbers were observed for all particle groups at 1 day postinstillation when compared to the saline control. By 7 days, all the groups had elevated lymphocytes in the lungs except the MS-PULSE group when compared to the saline control. By 14 days, only the SS-SPRAY and silica groups had increases of lymphocytes in the lungs. By 35 days, lung lymphocyte numbers for the SS-SPRAY group returned to normal but were still increasing for the silica group.

Biochemical parameters of lung damage. When the albumin content and β-NAG activity in the acellular BALF was measured 1 day postinstillation (Figs. 1 and 2), all groups had significant elevations in albumin levels when compared to the saline control, but only the silica and MS-PULSE groups had significant increases in β-NAG activity. Animals from the SS-SPRAY and silica groups had significant elevations in these two parameters at 7 and 14 days when compared to all the other groups. By 14 days, there were no differences in response among the MS-SPRAY, MS-PULSE, iron oxide, and saline groups. By 35 days, lev-

TABLE 2
Bronchoalveolar Lavage Cell Profiles

Treatment	Macrophage	Neutrophil	Lymphocyte
	Total number (10^6)		
1 Day			
Saline	5.1 ± 1.0	0.3 ± 0.1	0.1 ± 0.0
Silica	5.7 ± 0.5	13.4 ± 1.8*	0.6 ± 0.2#
SS-SPRAY	4.7 ± 0.3	6.2 ± 0.4#	0.3 ± 0.1#
MS-SPRAY	4.5 ± 0.3	8.3 ± 1.1#	0.6 ± 0.3#
MS-PULSE	4.6 ± 0.7	8.6 ± 0.7#	0.5 ± 0.2#
Iron oxide	3.8 ± 1.0	6.6 ± 0.7#	0.3 ± 0.1#
7 Days			
Saline	4.7 ± 0.6	0.1 ± 0.0	0.0 ± 0.0
Silica	7.2 ± 0.4†	10.3 ± 0.6*	0.7 ± 0.1‡
SS-SPRAY	9.3 ± 0.3*	4.0 ± 0.7†	1.1 ± 0.3‡
MS-SPRAY	5.7 ± 0.7	1.5 ± 0.7#	1.4 ± 0.7‡
MS-PULSE	5.6 ± 0.3	1.6 ± 0.6#	0.2 ± 0.1
Iron oxide	5.5 ± 0.8	1.6 ± 0.6#	1.3 ± 0.3‡
14 Days			
Saline	4.8 ± 0.2	0.2 ± 0.0	0.2 ± 0.0
Silica	6.8 ± 1.1†	20.4 ± 1.4*	1.6 ± 0.2*
SS-SPRAY	9.8 ± 0.5*	7.3 ± 1.3†	1.0 ± 0.1†
MS-SPRAY	4.7 ± 0.5	0.3 ± 0.1	0.1 ± 0.0
MS-PULSE	4.2 ± 0.8	0.3 ± 0.1	0.1 ± 0.0
Iron oxide	4.1 ± 0.3	1.0 ± 0.6	0.2 ± 0.1
35 Days			
Saline	3.7 ± 0.2	0.2 ± 0.1	0.1 ± 0.0
Silica	8.7 ± 0.7*	41.4 ± 5.2*	2.8 ± 0.5*
SS-SPRAY	5.5 ± 1.2†	1.8 ± 0.7†	0.2 ± 0.1
MS-SPRAY	3.0 ± 0.3	0.3 ± 0.1	0.1 ± 0.1
MS-PULSE	3.4 ± 0.4	0.2 ± 0.1	0.1 ± 0.1
Iron oxide	3.3 ± 0.5	0.2 ± 0.1	0.1 ± 0.0

Note. Values are means ± SE: n = 4.
* Significantly greater than all other groups ($p < 0.05$).
# Significantly greater than saline control group ($p < 0.05$).
† Significantly greater than MS-SPRAY, MS-PULSE, iron oxide, and saline groups ($p < 0.05$).
‡ Significantly greater than MS-PULSE and saline groups ($p < 0.05$).

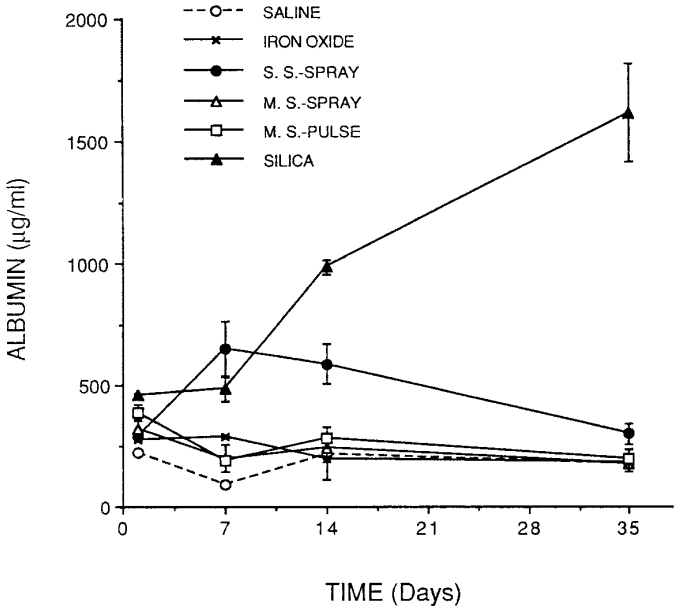

FIG. 1. Albumin in the cell-free bronchoalveolar lavage fluid from the lungs of rats 1, 7, 14, and 35 days after the intratracheal instillation of different welding fumes: stainless steel-spray (SS-SPRAY), mild steel-spray (MS-SPRAY), and mild steel-pulse (MS-PULSE). Silica (positive), iron oxide (negative), 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 wt. Values are means ± SE (n = 4). The criterion of significance was $p < 0.05$.

els of albumin and β-NAG for the SS-SPRAY group were still significantly elevated compared to the other groups but were approaching control levels. Injury induced by silica, however, continued to rise at 35 days and was significantly increased compared to all other groups.

Significant elevations in LDH activity were observed for all groups 1 day postinstillation when compared to the saline control group (Fig. 3). At this time point, LDH values for the silica and MS-PULSE groups were significantly higher than the SS-SPRAY, MS-SPRAY, and iron oxide groups. For the MS-SPRAY, MS-PULSE, and iron oxide groups, the increases in activity were diminished by 7 days and no different than saline control levels by 14 days. Both the silica and SS-SPRAY groups had substantial elevations in LDH activity at both 14 and 35 days when compared to all the other groups. However, LDH levels were still rising for the silica group, but falling for the SS-SPRAY group at 35 days.

TNF-α and IL-1β release. One day after the intratracheal instillation of the different particle samples, TNF-α and IL-1β were measured in the acellular BALF (Figs. 4 and 5). For both cytokines, no detectable levels of either cytokine were measured in the BALF of the saline, iron oxide, and MS-SPRAY groups. Significant increases in TNF-α and IL-1β were observed for the SS-SPRAY and silica groups. A significantly greater amount of TNF-α was observed for the silica group compared to the SS-SPRAY group, while no differences were seen in the release of IL-1β into the BALF. At all other time points postinstillation (7, 14, and 35 days), no TNF-α and no IL-1β were detected in the BALF for any of the groups (data not shown).

Histopathology

Histopathological analyses were performed on lungs 14 and 35 days after the intratracheal instillation of each of the particle samples. At both time points for all treatment groups, the lungs demonstrated localized areas where the particles had accumulated. The dusts were often present in phagocytic vacuoles within lung macrophages.

At 14 days (data not shown), a mild pneumonitis, characterized by a peribronchiolar accumulation of neutrophils and macrophages, was observed in the terminal bronchiolar regions of the lungs from the animals exposed to silica and SS-SPRAY. Minimal changes were observed in the lungs of the iron oxide and both MS groups.

At 35 days (Fig. 6), lung changes from iron oxide and three welding fumes were minimal. Particle-containing macrophages were observed in the terminal bronchioles and alveolar ducts. In contrast, lung changes due to silica were seen both within the alveolar ducts and in perivascular regions. Silica caused an accumulation of alveolar macrophages and

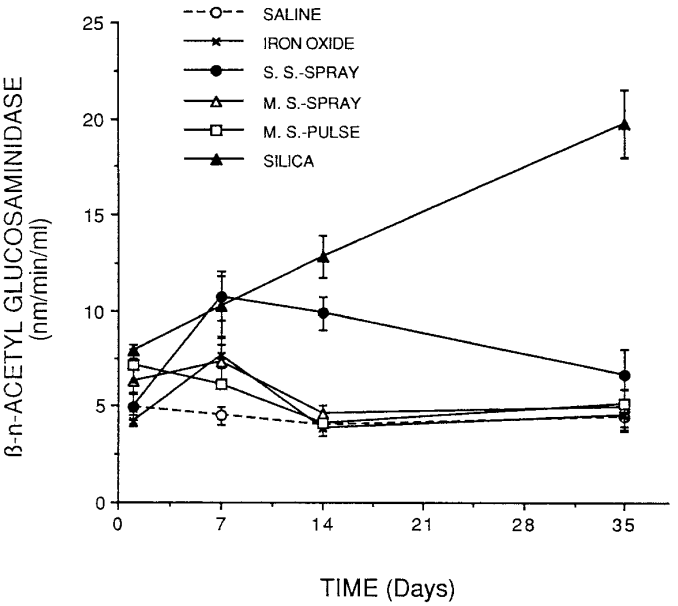

FIG. 2. β-n-Acetyl glucosaminidase activity of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1, 7, 14, and 35 days after the intratracheal instillation of different welding fumes: stainless steel-spray (SS-SPRAY), mild steel-spray (MS-SPRAY), and mild steel-pulse (MS-PULSE). Silica (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 wt. Values are means ± SE (n = 4). The criterion of significance was $p < 0.05$.

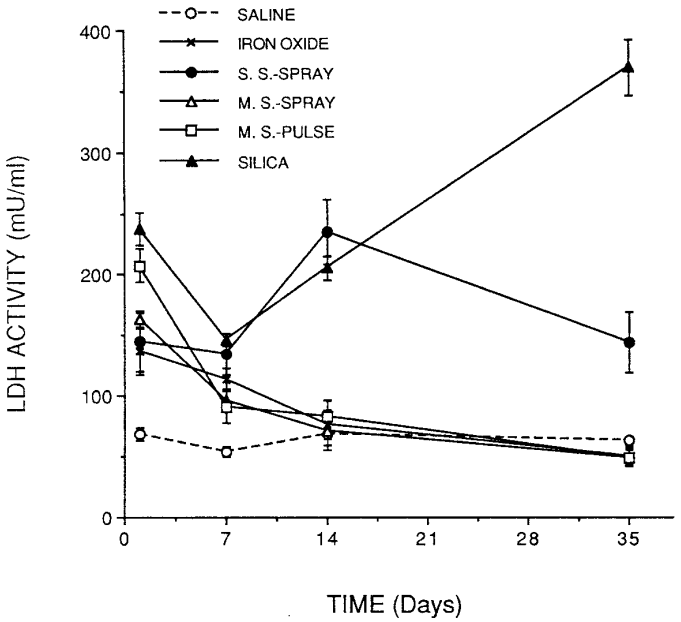

FIG. 3. Lactate dehydrogenase (LDH) activity of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1, 7, 14, and 35 days after the intratracheal instillation of different welding fumes: stainless steel-spray (SS-SPRAY), mild steel-spray (MS-SPRAY), and mild steel-pulse (MS-PULSE). Silica (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 wt. Values are means ± SE ($n = 4$). The criterion of significance was $p < 0.05$.

neutrophils, cellular debris, and extracellular phospholipid in alveoli and an infiltration of lymphocytes in the perivascular areas. Foamy alveolar macrophages were also present (Fig. 6D, inset).

Pulmonary Clearance of Welding Fume Particles

Histological analyses showed that the particle burden in the lungs decreased over time for both the SS-SPRAY (Fig. 7) and MS-SPRAY fumes. One day postinstillations, aggregates of particles were observed throughout the alveolar spaces, frequently internalized by alveolar macrophages. At 35 days, almost all particles remaining in the lungs were contained within alveolar macrophages. By this time point, most of the particles had been cleared from the lungs.

To quantitate the pulmonary clearance of the MS-SPRAY and SS-SPRAY welding fumes, the magnetic signal of the lungs was measured at different times after intratracheal instillation of the particles (Table 3). At each time point, the lung burden was measured and the percentage of the initial dose of particles remaining in the lung calculated. For the MS-SPRAY group, particles were cleared quickly. At 1 day, 92% of the MS-SPRAY particles still remained in the lungs. At 7, 14, and 35 days, 82, 65, and 26% of the particles were present, respectively. For the SS-SPRAY group, very little pulmonary clearance of the particles had occurred after 14 days. Eighty-six percent of the particles were still present in the lungs. By 35 days, 56% of the SS-SPRAY welding fume remained in the lungs. When comparing the two welding fumes, a significantly greater amount of particles had been cleared from the lungs of the MS-SPRAY group at 7, 14, and 35 days. When the data were fitted to an exponential decay model, the half-life was 47 days for the SS-SPRAY and 18 days for the MS-SPRAY.

DISCUSSION

We evaluated the relative ability of welding fumes generated from different materials to elicit lung inflammation or injury. We also examined the impact of two different power supplies on fume size, composition, and toxicity. An *in vivo* rat bioassay was used that measures a variety of markers of pulmonary damage within the BALF. Previous studies have shown that analysis of the BALF is a means of characterizing lung inflammation and lung injury (Beck *et al.*, 1982; Henderson, 1984; Brain and Beck, 1985; Beck *et al.*, 1987; Khan and Gupta, 1991). Early changes in the cellular and biochemical constituents of the lavage fluid have also been

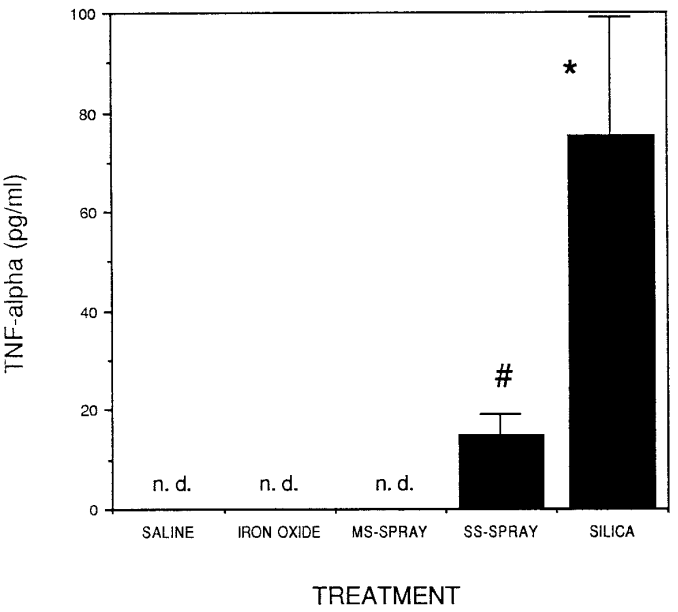

FIG. 4. Tumor necrosis factor-alpha (TNF-alpha) of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1 day after the intratracheal instillation of stainless steel-spray (SS-SPRAY) and mild steel-spray (MS-SPRAY) welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The animals received a single dose of 1.0 mg in 150 μl of sterile saline/100 g body wt. Values are means ± SE ($n = 4$; n.d. is nondetectable). Mean value of the silica group was significantly greater than the values of all other groups (*$p < 0.05$). Mean value of the SS-SPRAY group was significantly greater than the saline, iron oxide, and MS-SPRAY groups (#$p < 0.05$).

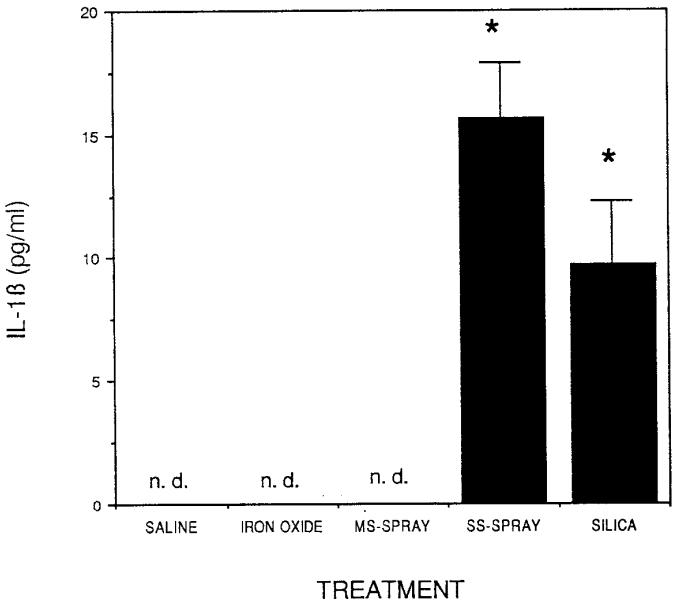

FIG. 5. Interleukin-1β (IL-1β) of the cell-free bronchoalveolar lavage fluid from the lungs of rats 1 day after the intratracheal instillation of stainless steel-spray (SS-SPRAY) and mild steel-spray (MS-SPRAY) welding fumes. Silica (positive), iron oxide (negative), and saline (vehicle) were used as controls. The animals received a single dose of 1.0 mg in 150 μl of sterile saline/100 g body wt. Values are means $\pm$ SE ($n = 4$; n.d. is nondetectable). Mean values of the silica and stainless steel groups were significantly greater than the values of the other groups ($^*p < 0.05$).

shown to be predictive of chronic lung responses (Lindenschmidt *et al.*, 1990).

The pulmonary responses observed after the intratracheal instillation of the welding fumes to animals were compared to the effects of two other dusts studied in humans and animals, pure iron oxide and silica. Intratracheal instillation has been used extensively to examine the potential of respirable size dusts to injure the lungs (Beck *et al.*, 1982, 1987; Lindenschmidt *et al.*, 1990). Even though this exposure route is less physiologic than the inhalation of the particles, there are advantages to this method. The actual dose delivered to each animal is very uniform and can be measured accurately. The procedure is simple and far less expensive when compared to the complicated technology needed for aerosol generation and inhalation chambers. The intratracheal instillation technique also permits the introduction of large, and therefore, effective, doses of materials in a short time (Brain *et al.*, 1976).

One concern about intratracheal instillation is that much higher doses of particles are frequently instilled into the lungs of animals as compared to the doses of particles that may be deposited in the lungs of workers over periods of weeks and months. However, a study by Lindenschmidt *et al.* (1990) showed that the particle dose (1 mg/100 g body wt) used in our study easily differentiated fibrogenic from nonfibrogenic materials in rats. This dose was high enough to permit comparisons of potential adverse effects of different mineral particles.

Another concern of this procedure is that the intratracheal instillation of a large bolus of particles into the lungs could cause an inflammatory response that would not be observed if the same amount of particles accumulated gradually during inhalation. Recently, Henderson *et al.* (1995) conducted a study comparing the inflammatory response of the lungs to particles of high and low toxicity by either instillation or inhalation. Their results indicated that the degree of pulmonary inflammation induced by different doses of the different materials studied could be appropriately evaluated using either exposure method.

A third concern is that the precollected particle samples instilled into the lungs of the animals may not be representative of the actual fume generated in the workplace. With most freshly generated fumes, the presence of reactive free radicals on the surface of the particles, which can be toxic to biological tissues, have been observed (Pryor, 1992). Since the welding procedures and parameters were consistent for each of the samples in this current study, the formation of reactive radicals on the surface of the fumes would likely be the same for each of the different samples collected. The contribution then of any free radical on the surface of the particles would be equal for each of the fume samples. Since all the fume samples were of the same size, and generated, collected, and stored in the same manner, we believe the intratracheal instillation technique is an adequate method to compare the pneumotoxicity of different welding fumes.

Since the mean diameters were slightly >1.0 μm, the welding fumes were of respirable size; moreover we observed no differences in the size of the three samples collected. Many of the individual fume particles had aggregated together. The formation of these aggregates occurred both during generation of the fume and collection of the particles onto the filters. Although it has been shown that most particles generated during welding are smaller then 1.0 μm (Jarnuszkiewicz *et al.*, 1966; Akselsson *et al.*, 1976; American Welding Society, 1979), the particles grow in size with increased time. This agglomeration is enhanced by the turbulent conditions resulting from heat generated in the welding process, thus increasing particle movement and chances for particle collision. A study by Clapp and Owen (1977) concluded that approximately 2 min after welding ceases the particles less than 1.0 μm had grown in size to form particles greater than 1.0 μm. This may explain the particle sizes observed for the three welding fume samples used in our study since the fume was collected during 2 min of welding and then for an additional 2 min.

Each of the particle samples used in this investigation caused increases in neutrophils, albumin, and LDH release in the BALF 1 day postinstillation, while only the silica and MS-PULSE groups had significant increases in β-NAG

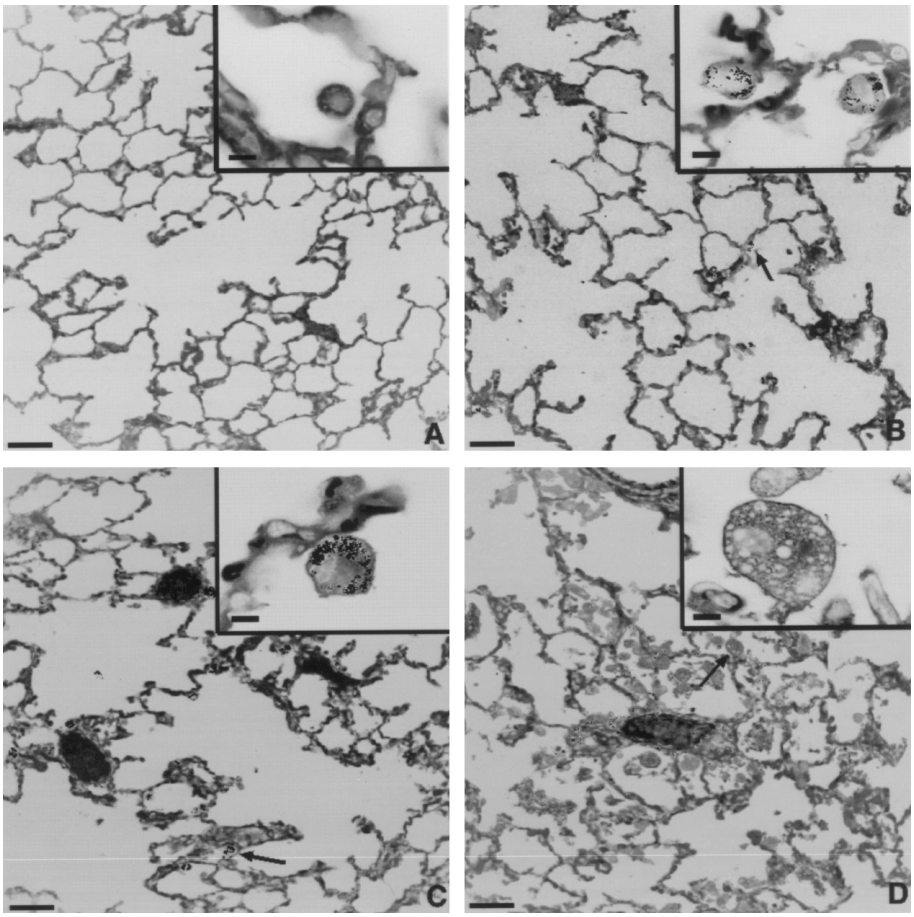

FIG. 6. Confocal micrographs of rat lung 35 days after the intratracheal instillation of (A) saline, (B) MS-SPRAY fume, (C) SS-SPRAY fume, and (D) silica. The animals received a single dose of 1.0 mg in 150 μl of sterile saline/100 g body wt. Saline was used as vehicle control. Particles are present within alveolar macrophages (arrows) in alveolar spaces. Bar is 50 μm. (Inset) Alveolar macrophages 35 days postintratracheal instillation of (A) saline, (B) MS-SPRAY fume, (C) SS-SPRAY fume, and (D) silica. Bar is 5 μm.

activity. The elevations in pulmonary inflammation and injury observed for the two MS and iron oxide groups quickly subsided and were not different from the saline vehicle control group at 14 days. Morever, only minimal changes were observed on histopathological analysis for these groups at this time point. These similar results were not surprising since approximately 90% of the MS fumes was comprised of a relatively insoluble form of iron oxide. The pulmonary responses seen in the animals exposed to the SS-SPRAY and silica particles persisted longer. At 14 and even 35 days postinstillation, significant lung inflammation and injury were observed.

The difference between the MS groups and SS-SPRAY fume is consistent with the conclusions of Hicks *et al.* (1984). In their study, 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 in the Hicks *et al.* study were very high. Their use of these high particle doses could then explain the increase in toxicity and signs of fibrosis induced by the MS fume.

In the Hicks *et al.* study, animals were also 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, they observed the presence of lung nodules 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 study, using an intratracheal dose that results in a higher lung burden than the single inhalation dose used by Hicks *et al.*, 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.*, correlate more closely with the findings of a number of epidemiology studies. While

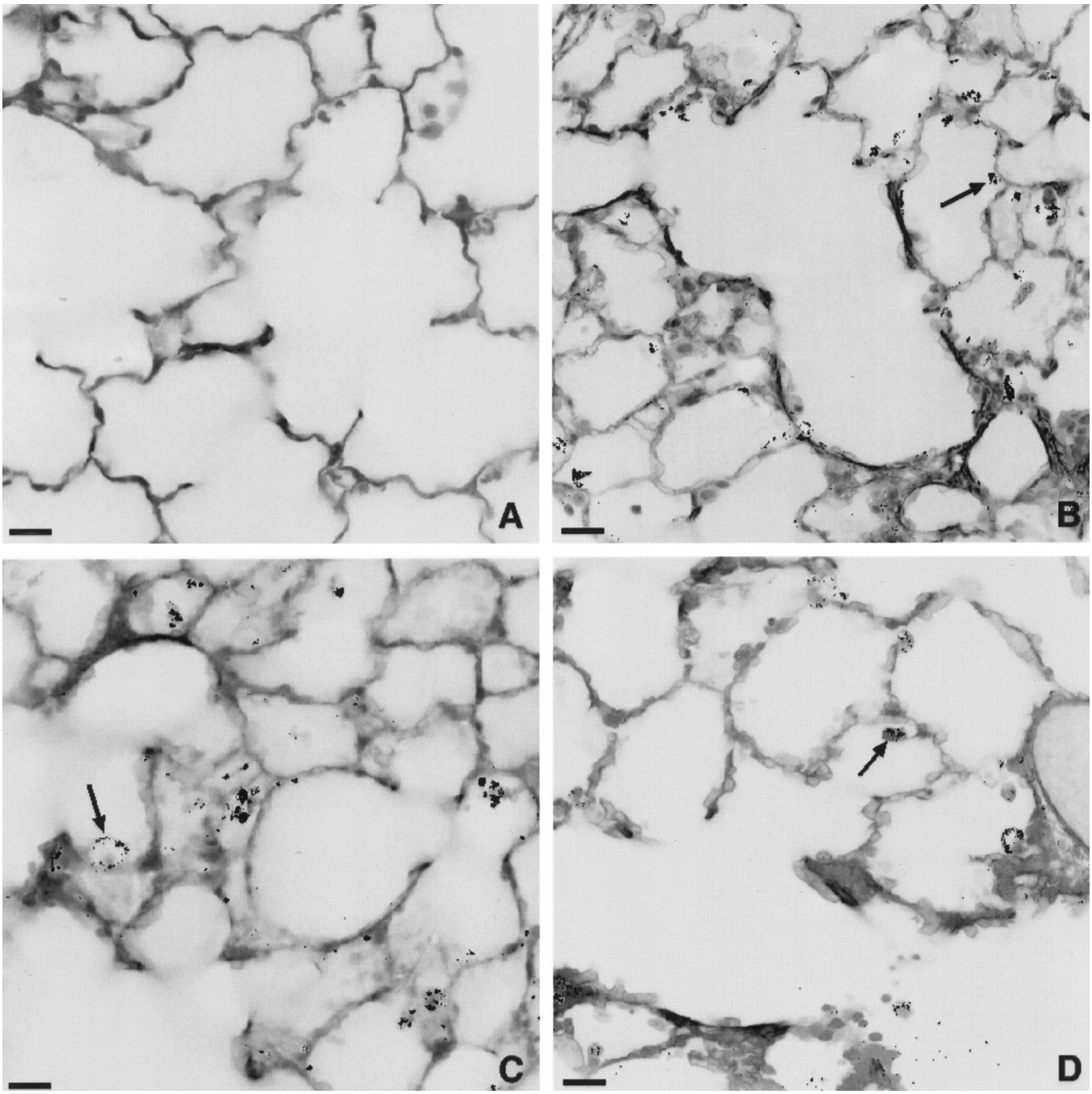

FIG. 7. Histopathologic evaluation of particle clearance. Confocal micrograph of SS-SPRAY welding fume in rat lung (A) 0, saline (vehicle control), (B) 1, (C) 14, and (D) 35 days after the intratracheal instillation. The animals received a single dose of 1.0 mg in 150 μl of sterile saline/100 g body wt. At 1 and 14 days, particles are present in alveolar macrophages (arrows) and on epithelial surfaces. At 35 days, particles are observed inside alveolar macrophages. Bar is 20 μm.

pulmonary accumulation of welding particles and a pneumoconiosis have been observed in welders, no cases of progressive pulmonary fibrosis have been reported.

When the responses to silica and SS-SPRAY were compared, no differences were observed in the parameters at 7 days except for a greater number of neutrophils in airspaces caused by silica and a greater number of macrophages after the instillation of the SS-SPRAY particles. By 14 days, the silica response was the same in regard to LDH activity, significantly less in regard to numbers of macrophages re-

TABLE 3
Pulmonary Clearance of Welding Fumes

Sample	Lung burden (mg/lung)	% Initial dose
SS-SPRAY		
0 days	1.95 ± 0.46	100
1 day	1.74 ± 0.19	89.2
7 days	2.06 ± 0.26	105.6*
14 days	1.68 ± 0.31	86.2*
35 days	1.09 ± 0.07	55.9*
MS-SPRAY		
0 days	2.84 ± 0.31	100
1 day	2.60 ± 0.13	91.6
7 days	2.34 ± 0.17	82.3
14 days	1.83 ± 0.37	64.5
35 days	0.73 ± 0.07	25.6

Note. Lung burden values are means ± SE, $n = 4–5$. The animals received a single dose of 1.0 mg/100 g body wt of the stainless steel-spray (SS-SPRAY) and the mild steel-spray (MS-SPRAY) welding fumes. The initial dose (0 days) of welding fumes delivered to the lungs was measured 1 hr after instillation. The % initial dose values in the lung of the SS-SPRAY group were significantly different from the values for the MS-SPRAY group (*$p < 0.05$).

covered from the lungs, and significantly greater in regard to other parameters when compared to the lung responses to SS-SPRAY fume. But after 35 days, the lung injury seen in the animals exposed to silica continued to increase, while the responses in the SS-SPRAY group were significantly less and appeared to be returning to control levels. It appears that the SS-SPRAY and silica particles had similar pneumtoxicity profiles when damage was examined at earlier time points. However, in the context of chronic lung injury, silica produced much greater responses. Since the lung responses to the SS-SPRAY fume had started to subside, the particles are being cleared from the lung. Silica particles have been demonstrated to persist in the lung (Brody *et al.*, 1982), and this slow clearance may contribute to the fibrosis it causes.

The silica and SS-SPRAY groups also had significant levels of TNF-α and IL-1β within the acellular BALF. These cytokines have been shown to help initiate the cascade of cytokines and other factors associated with inflammatory responses (Le and Vilcek, 1987). These two cytokines produced by pulmonary cells, predominantly the alveolar macrophage, participate in numerous inflammatory processes, such as recruitment and activation of neutrophils and lymphocytes, stimulation of fibroblast proliferation and collagen synthesis, and increased oxygen radical production (Schmidt *et al.*, 1982; Goldring and Krane, 1986; Tsujimoto *et al.*, 1986; Driscoll *et al.*, 1990, 1991). Thus, it is likely that the presence of the these two cytokines in the lung may contribute to the inflammatory response associated with exposure to certain environmental and occupational dusts. The ability of silica and SS-SPRAY welding fume to activate pulmonary cells to release TNF-α and IL-1β is likely responsible, at least in part, for the greater inflammation and pneumotoxicity associated with these two groups.

Lung responses to deposited particles may also depend on the persistence of the particles within the lungs. Using magnetometry, we found that the SS-SPRAY particles were cleared more slowly from the lungs than the MS-SPRAY particles. Significantly elevated amounts of the SS-SPRAY particles were retained in the lungs of the animals 7, 14, and 35 days postinstillation when compared to animals given the mild steel fume. Studies by Kalliomaki *et al.* (1983a,b), using a similar magnetometric procedure, have also demonstrated an increase in retention of SS particles as compared to MS fumes in rat lung.

Since the macrophage plays an important role in clearing particles from the lungs, the increase in pulmonary persistence of the SS-SPRAY particles may be due to its effect on this cell type. In some *in vitro* studies where SS and MS welding fumes were incubated with alveolar macrophages, differences in cytoxicity were observed. Hooftman *et al.* (1988) have shown that bovine alveolar macrophage viability and phagocytosis were greatly reduced by particles generated in the welding of SS. Similar findings have been reported by Pasanen *et al.* (1986) and by Stern and Pigott (1983) in their studies on welding fumes with rat peritoneal macrophages.

Lung responses to welding fumes also depend on the physiochemical properties of the particles. Since the metal compostion of the SS-SPRAY particles was vastly different from the MS fume, the differences observed in the pneumotoxicity between the groups were not surprising. Compared to the MS fumes, the SS-SPRAY fumes had elevated levels of manganese as well as nickel and chromium, absent from the MS fumes. All three of these metals alone or in combination with other materials have been shown to be cytotoxic to pulmonary cells and to be associated with lung disease (Ulrich *et al.*, 1979; Lees, 1991; Camner and Johansson, 1992).

In this study in rats, we have demonstrated that welding fumes generated from different electrode wires produce different lung responses and are cleared from the lungs at different rates. The SS-SPRAY fume was more pneumotoxic and was retained in the lung longer when compared to the MS fumes. Detectable levels of TNF-α and IL-1β were measured in the acellular BALF of the rats intratracheally instilled with the SS-SPRAY fume. The increased pulmonary persistence and the presence of these inflammatory cytokines within the BALF may help explain the increases observed in the lung injury and inflammation caused by SS-SPRAY welding fumes. However, unlike the highly pneumotoxic and fibrogenic dust, silica, it appears that the SS-SPRAY particles are eventually cleared from the lungs and thus, the potential for chronic pneumotoxicity is low if exposure to the fume is ceased. It is also important to note that intratracheal

instillation of the MS fumes induced a similar pulmonary response as iron oxide, a mineral particle which has been characterized as a nuisance dust with little inflammatory and fibrogenic potential. Additional animal studies, focusing on the water solubility of the particles and the role played by components of SS welding fumes, such as chromium, nickel, and manganese, may be needed to further elucidate the mechanisms by which different welding fumes may affect the lung of welders. We also conclude that altering the power supply (SPRAY versus PULSE), at least for the MS fume, had a negligible effect on the size and elemental composition of fume as well as its ability to elicit injury and inflammation. If PULSE welding is associated with a reduction in fume production (Irving, 1992), this new technology should reduce hazards to welders.

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