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Pulmonary Toxicity of Simulated Lunar and Martian Dusts Intratracheally Instilled into Mice[1]

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ABSTRACT

The National Aeronautics and Space Administration (NASA) is contemplating sending humans to Mars and to the Moon for further exploration. Equipment designated for these extraterrestrial bases will require testing in simulated Martian or lunar environments. The properties of Hawaiian and San Francisco Mountain volcanic ashes make them suitable to be used in these test environments as Martian and lunar dust simulants, respectively. The present toxicity study was conducted to address NASA's concern about the health risk of dust exposures in the test facilities. In addition, the results obtained on these simulants can be used to design a toxicity study of actual moon dust and Martian dust, which will probably be available in a few years. Respirable portions of lunar soil simulant (LSS) and Martian soil simulant (MSS) were separated from their respective raw materials. These soil simulants, together with fine titanium dioxide (negative control for fibrogenesis in mice), and crystalline silica (positive control) were each intratracheally instilled in saline to groups of 4 male mice (C57BL/6J, 2-3 months old) at 0.1 mg/mouse (LD) or 1mg/mouse (HD). The lungs were harvested 7 or 90 days after the single dust treatment for histopathological examination. Lungs of the LSS-LD groups on either the 7- or 90-day study showed no evidence of inflammation, edema, or fibrosis. Clumps of particles and an increased number of macrophages, visible in the lungs examined after 7 days, were absent after 90 days. The LSS-HD-7d group showed mild to moderate alveolitis with neutrophilic and lymphocytic infiltration, and mild perivascular and peribronchiolar inflammation. The LSS-HD-90d group showed signs of chronic inflammation: septal thickening, mild perivascular and peribronchiolar inflammation, mild alveolitis and some fibrosis. Foci of particle-laden macrophages (PLMs) were still visible. Lungs of the MSS-LD-7d group revealed mild focal intraalveolar inflammation with neutrophilic and lymphocytic infiltration, and mild perivascular and peribronchiolar inflammation. The MSS-LD-90d group showed PLMs and scattered foci of mild fibrosis. The MSS-HD-7d group showed large foci of PLMs, intraalveolar debris, mild to moderate focal alveolitis, and mild to moderate perivascular and peribronchiolar inflammation. The MSS-HD-90d group showed focal chronic mild to moderate alveolitis and fibrosis. To mimic the oxidative and reactive properties of Martian surface dust in the test animals, groups of 4 mice were exposed to ozone (0.5 ppm for 3 hours) prior to instillation of the MSS. Lung lesions in the MSS groups were more severe with the ozone pretreatment. The O_3-MSS-HD-90d group had wide spread intraalveolar debris, focal moderate alveolitis and fibrosis. The results for the titanium dioxide and quartz controls were consistent with the known pulmonary toxicity of these compounds. The overall severity of toxic injury to the lungs was TiO_2< LSS<MSS<MSS+O_3<quartz. In general, the toxic responses increased with the increase of dust burden in the lung. Except for TiO_2, the increased duration of dust presence in the lung from 7 to 90 days transformed the acute inflammatory response to a chronic inflammatory lesion.

Key Words: Pulmonary toxicity, intratracheal instillation, dust toxicity, Martian dust, lunar dust, quartz, titanium dioxide, volcanic ash

INTRODUCTION

NASA is considering returning to the Moon to establish a lunar outpost and sending humans to explore Mars (Hoffman and Kaplan, 1997). Instruments and hardware destined for these extraterrestrial bases must be tested in simulated Martian and lunar environments in NASA laboratories. Hawaiian volcanic ash [designated as JSC Mars-1 (Allen et al., 1998)] and San Francisco Mountain volcanic ash [designated as JSC-1 (McKay et al., 1994)], which have mineral properties that resemble to those of Martian and lunar soils, respectively, are used for the extraterrestrial environmental simulations (Glaser, 1992). Since the inhalation toxicity of the extraterrestrial soils and the simulants has not been evaluated, NASA is concerned about the health risk of dust exposures to workers in the Earth-based test facilities. Furthermore, toxicity testing with simulants can provide guidance for designing experiments with actual lunar dust and eventually Martian dust.

During the Apollo lunar landing, the moon dust particles encountered were described by the crew as extremely fine (NASA, 1969). It stuck to spacesuits despite attempts to brush it off and was brought into the lunar landing vehicles. After the spacecraft lifted off from the lunar surface and zero gravity was reestablished, a large quantity of floating dust made breathing difficult and impaired the visual acuity of the crew (NASA, 1970). Shortly after lunar soil samples were brought back to the Earth, NASA conducted comprehensive studies to determine whether the soil contained extraterrestrial, biologically-active agents that could produce disease or possessed "replicating agents" (Taylor et al., 1975; Benschoter et al., 1970; Holland and Simmonds, 1973). Few investigations were done on toxicological properties, and the results failed to reveal whether the dust is toxicologically hazardous (Holland and Simmonds, 1973).

The toxicity of lunar soil was also investigated by the Russians in mice (Kustov et al., 1974) and rats (Belkin et al., 1983). These efforts have been summarized in a tome with the conclusion that lunar soil caused fibrosis and other signs of pneumoconiosis (Kustov et al. 1989). However, the studies contained considerable experimental deficiencies and unsupported interpretations (see Discussion for details).

The present toxicity study was conducted on a simulated lunar soil (JSC-1) because test-facility workers can be exposed to this material during earth-based engineering studies and because NASA requires a successful study on a simulant before it will release a lunar dust sample for a study. The material was mined from volcanic ash deposited in the San Francisco volcano field near Flagstaff, AZ. The oxide composition of the simulant is a good match for mare soils derived from lunar basalts. Both materials contain about 50% SiO_2; other common soil oxides (Al_2O_3, FeO, MgO, and CaO) account for another 42-45% (McKay et al., 1994). The particle size distribution (average about 100 μm) of the simulant fits within the range of lunar samples returned by Apollo missions (McKay et al., 1994). Dust particle fractionation conducted by Lovelace Inhalation Research Institute (Albuquerque, NM) associated with the present study showed that JSC-1 contained 1.1% dust with particle sizes at the respirable range.

Martian regolith is amorphous and chemically similar to Hawaiian volcanic ash. Martian soil and the simulant both contain more than 30% silica (Allen et al., 1998). Besides silica, Fe_2O_3, which imparts the brown coloration to both the regolith and its simulant, together with Al_2O_3 account for another 30% of the total weight. Only 1% of the simulant is smaller than 5 μm. All

compounds identified in the sample are in the form of oxides; none of them are heavy metals. Gas-exchange experiments conducted by the Viking Martian lander biological instrument showed evolution of oxygen when the Martian surface samples were humidified (Oyama and Berdahl, 1977). According to these authors, the test results indicate that the reaction would involve one or more reactive species of ozonides, superoxides, or peroxides; the most likely candidates proposed were potassium, calcium, and sodium superoxides. More recent data led investigators at the Jet Propulsion Laboratory (Pasadena, CA) to conclude that the highly oxidizing substance that releases oxygen is an iron rich clay containing potassium iron tetroxide ($K_2Fe^{++++}0_4$) (Tsapin, 2000). All these species are extremely reactive and oxidative; thus, inhalation exposures to Martian regolith would be expected to produce lesions in the lung partially resembling those caused by powerful oxidants. Since oxidatively-activated Hawaiian volcanic ash or the reactive iron oxide is not available, an ozone exposure of the mice followed by an intratracheal instillation with the unactivated ash was included in the present study to simulate the effects of the oxidative properties of Martian soil in the lung. Ozone was chosen because its effects in the lung have been well characterized and that it was feasible to select a dose that would produce no more than minimal tissue injury.

MATERIALS AND METHODS

Simulated Martian and Lunar Dusts

The lunar soil simulant (JSC-1) was mined from a volcanic ash deposit in the San Francisco volcano field near Flagstaff, AZ (McKay, 1994). The Martian soil simulant (JSC-Mars-1) was obtained from the Mauna Kea volcano in Hawaii (Allen et al., 1998). About 1.1 kg LSS and 1.3 kg of MSS, provided by C. Allen of the NASA Office of Curator at the Johnson Space Center (Houston, TX), were size-fractionated by Lovelace Respiratory Research Institute. The raw material was placed in a DeVilbiss dry powder generator operated with compressed air at 20 psig; the output was then delivered to a 3-stage cyclone operated at 31 L/min (Smith et al. 1979). The median cut-off diameters were 5, 1.95, and 0.28 μm for the 3 stages. The particles from second and third stages and the backup filter were collected and pooled for the present study. The yields of these fine simulated lunar and Martian soil samples were 12.2 g (1.1%) and 5.2 g (0.4%), respectively; the mass median aerodynamic diameter (MMAD) of both soils was less than 5 μm (Cheng, personal communication, 1998).

Particle Size Distribution Determination

Approximately 50 mg of the LSS or MSS was suspended in ca. 200 cc of distilled water and placed into a reservoir chamber of a Microtrac X-100 Particle size analyzer (Microtrac, Inc. Montgomeryville, PA). This tri-laser analytical unit was connected to a computer unit for data acquisition and analysis. The instrument has a particle-size analytical range of 0.04 – 700 μm separated into 120 channels. Timed ultrasonication, which is controlled by the computer, can be generated in the reservoir chamber to ensure that the particles remain homogeneously suspended. Particle size distributions of the LSS and MSS are shown in Figures 1A and 1B, respectively. The ultrasonicated lunar dust simulant showed a bimodal size distribution with peaks at 1.1 and 3.0 microns. The ultrasonicated Martian dust simulant showed peaks at 3.0 and 108 microns, with the majority in the lower size range.

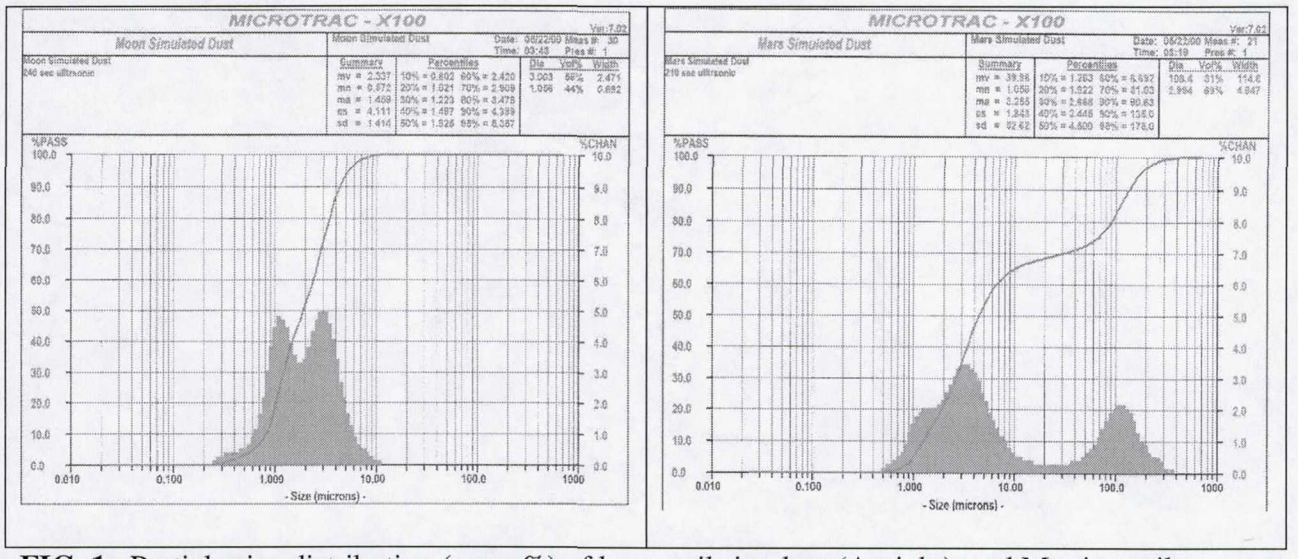

FIG. 1. Particle size distribution (mass %) of lunar soil simulant (A, right), and Martian soil simulant (B, left) after ultrasonication in water.

Titanium Dioxide and Crystalline Silica

The titanium dioxide sample, a product of Particle Information Services (Kingston, MA), had an average particle diameter of 0.45 μm. Crystalline silica (quartz) (acid-washed Min-U-Sil-5) of average size 5 μm was obtained from Pennsylvania Glass and Sand (Pittsburgh, PA).

Animals and Animal Husbandry

Male mice (C57BL/6J, 2 months old, ca. 20g), free of known rodent pathogens, were obtained from Jackson Laboratory (Bar Harbor, ME). The animals were housed in groups of 4 in polycarbonate cages (with HEPA air filters) in the AAALAC-accredited vivarium at the Johnson Space Center (JSC). Animals were allowed to acclimatize at this facility (with a 12-hour light-dark cycle) for at least one week before being used in the study. The mice were freely provided with tap water and Purina Formulab Chow No. 50008 (Ralston Purina Co., St. Louis, MO). They were cared for and used humanely according to NASA animal care and use program guidelines.

Inhalation Exposure to Ozone

Those animals in the MSS study that required an ozone exposure were transported from JSC to the University of Texas School of Public Health, a local university equipped with a 0.75 m^3 Laskin-type stainless steel exposure chamber (Wahman, MD). The exposure procedure of Kirichenko et al. (1996) was adopted. In brief, ozone was generated by passing house air through an electrical ozone generator (Model V5AR-237 ozonator, Ozone Research Equipment Corp, Phoenix, AZ); it was then metered and mixed with house air before entering the chamber. A small air stream was pulled (2 L/min) from the chamber through Teflon tubing into a calibrated ozone monitor (Model 1003AH, Dasibi Environmental Corporation, Glendale, CA). The monitor was calibrated shortly before the present study by a laboratory at the US Environmental Protection Agency (Houston Regional Office). After the chamber ozone concentration had been stabilized at about 0.5 ppm, six groups of mice (4 mice/group) were placed in the chamber. This exposure level was selected to produce no more than minimal tissue injury. An ozone concentration reading was recorded every 10 minutes. After a 3- hour exposure to 0.51 ± 0.02 ppm of ozone, the animals were immediately returned to JSC for dust instillation. This order of treatments was chosen because it allowed the dust instillation to be carried out within 2-4 hours after the ozone exposure. If the order were to reverse, both treatments could not be conducted on the same day because it would take a considerable amount of time for the animals to recover from anesthesia, surgery, and fluid in lung, which was instilled with the dust.

Intratracheal Instillation of Dust Samples

The mice were each anesthetized intraperitoneally with a cocktail of 150 μl of a ketamine (Ketaset, Dodge Laboratory, Ford Dodge, IO) and xylazine (Rompun, Bayer, Shawnee Mission, KS) at a dosage of approximately 80 and 16 mg/kg, respectively. After the animal was tagged with an ear tag, the body weight was determined and the animal was secured on a slant plastic platform (about 45°). The trachea was exposed by a 1-cm incision on the ventral neck skin. An aliquot of 50-μl of

freshly suspended and ultrasonicated dust in normal saline, containing 0.1 mg (LD) or 1 mg (HD) dust, was intratracheally instilled (using 26-g needles) into the lung. For the saline and ozone-only control animals, only saline was given. The incision was then stitched and swabbed with antiseptic (povidone iodine). After the animals recovered from anesthesia, they were returned to the viviarium and were observed daily until scheduled termination.

Collection of Lungs from Animals for Histopathological Study

Seven or 90 days after the dust treatment, the mice were each euthanized intraperitoneally with an over dose of 30 µl of Nembutal sodium solution (ca. 400 mg/kg); body weights were determined. The trachea was exposed and a catheter (a blunt 26-g needle) was inserted into and tied to the trachea. Formalin (10%) in a neutral phosphate buffer (Fisher Scientific, Pittsburgh, PA) was allowed to drip slowly into the lung in situ of the animal lying in supine position on a 75° platform. After perfusing for about 10 to 15 min, the trachea was tied and the lung was removed from the chest cavity. The lung was placed in a glass vial containing 5 to 10 ml of the same fixative solution. Each glass vial was assigned a number blind to the pathologists. The lungs were fixed for at least 7 days before further processing.

Preparation of Lung Tissue Slides for Histopathological Examination

The formalin-fixed mouse lungs were embedded in paraffin, thin-sectioned coronally, and mounted on glass microscope slides using standard histopathologic techniques. Sections were stained with hematoxylin, eosin, van Gieson for elastin, and trichrome dyes for connective tissue. One slide per section per stain for each mouse was examined independently by three pathologists. A formatted score sheet was used for grading intraalveolar, interstitial, vascular, and bronchiolar particles, fibrosis, edema, and inflammation. The scores from the three pathologists were averaged.

Collection of Blood for Cellular Autoimmunity Assay

For those mice in the 90-day study, before the lung was perfused with formalin, the abdominal cavity of the animals was opened and a blood sample was collected from the descending aorta. The blood was centrifuged at 1000 rpm and serum was collected and frozen. Antinuclear antibodies (ANA) were analyzed according to the procedures described by Fritzler and Tan (1985). The procedure involved indirect immunofluorescence microscopy on Hep-2 cells (Immuno Concepts, Inc., Sacramento, CA) grown on Teflon-masked slides. The intensity of the fluorescence was graded 0-4 and the results were analyzed by ANOVA followed by a non-parametric Kruskal-Wallis test followed by a Nunn's test (to compare all columns). $P < 0.05$ was considered significant.

RESULTS

Effects of Instilled Titanium Dioxide on the Lungs of Mice

After receiving a single intratracheal instillation of TiO_2 (0.1mg/mouse, LD), the lungs of the mice killed 7 days after the treatment showed focal clumps of particles; some of the particles were inside macrophages. There was no evidence of inflammation, edema, or fibrosis. For those animals killed 90 days after the dust treatment, the lungs had no identifiable particles and were completely normal (Fig. 2A). This slide, which is indistinguishable from those of saline-treated controls, is used in this manuscript for illustrating normal lung tissue histology.

When compared to the low-dose groups, the lungs of the high-dose (1 mg/mouse) groups killed 7 day after the treatment showed larger focal clumps of particles; the HD-90d group had increased numbers of alveolar macrophages but minimal tissue reaction (Fig. 2B).

Effects of Instilled Quartz on the Lungs of Mice

The lungs of the SiO_2-LD-7d group showed focal clumps of particles and an increased number of alveolar macrophages. Mild to moderate focal alveolar inflammation, mild focal perivascular and peribronchiolar inflammation, neutrophilic and lymphocytic infiltration, and edema also were observed. For those SiO_2-LD mice killed 90 days after the dust treatment, the lungs still had clumps of particles and particle-laden macrophages (PLMs) in addition to mild inflammation and mild focal fibrosis (Fig. 2C).

The lungs of the HD-7d group contained larger focal clumps of particles and more macrophages when they were compared with the lungs of the LD-7d group. In addition, the lung lesions were more severe showing moderate to severe alveolitis, moderate focal perivascular and peribronchiolar inflammation, and edema. For the high-dose group in the 90-day study, large clumps of PLMs were still abundant in the lungs. The lungs also showed moderate alveolitis, moderate to severe focal bronchiolar, perivascular, and peribronchiolar inflammation, necrosis, edema, and focally severe fibrosis, strongly resembling bronchiolitis obliterans organizing pneumonia (Fig. 2D).

Effects of Instilled Lunar Soil Simulant (LSS) on the Lungs of Mice

The lungs of the LSS-LD-7d group showed the presence of focal clumps of particles and, occasionally, macrophages (Fig. 3A). There was no evidence of inflammation, edema, or fibrosis. At 90 days, particles were not observed and the tissue was also completely normal (Fig. 3B).

For the HD-7d group, the lungs showed clumps of particles and macrophages, mild to moderate alveolitis with neutrophilic and lymphocytic infiltration, and mild perivascular and peribronchiolar inflammation (Fig. 3C). When examined 90 days after the dust treatment, foci of PLMs, mild alveolitis, mild focal perivascular and peribronchiolar chronic inflammation, and septal thickening were visible (Fig. 3D).

Effects of Instilled Martian Soil Simulant (MSS) on the Lungs of Mice

The lungs of MSS-LD-7d group revealed the presence of particles and macrophages; there was mild focal intraalveolar debris, infiltration of neutrophils and lymphocytes, and perivascular and peribronchiolar inflammation (Fig. 4A). At 90 days, PLMs and scattered foci of mild fibrosis were detected (Fig. 4B).

Mice of the HD-7d group showed that the lungs had large foci of PLMs, mild focal alveolitis, intraalveolar debris, and mild to moderate perivascular and peribronchiolar inflammation (Fig. 4C). At 90 days, there were particle-laden macrophages, mild alveolitis, focal moderate inflammation, and fibrosis (Fig. 4D).

Effects of Instilled MSS in the lungs of Ozone-pretreated Mice

A single 3-hour inhalation exposure to 0.51 ± 0.02 ppm ozone (followed by saline instillation) produced no overt lung injury when examined 7 days after the exposure, but focal mild alveolitis and peribronchiolar inflammation were noted 90 days after the exposure. When an ozone exposure was followed by an intratracheal installation of MSS (0.1 mg or 1 mg/mouse), the lungs of the LD-7d group showed the presence of foci of particles and an increase of macrophages. Focal mild peribronchiolar inflammation and alveolitis were also visible (Fig. 5A). At 90 days, the lungs of the O_3+MSS-LD mice revealed focal mild peribronchiolar inflammation, alveolitis, and fibrosis (Fig. 5B).

The O_3+MSS-HD-7d group showed large foci of PLMs, focal moderate peribronchiolar inflammation, alveolitis, intraalveolar debris, and early focal moderate to severe fibrosis (Fig. 5C). The HD-90d group had wide spread intraalveolar debris, alveolitis, and focal moderate inflammation and fibrosis (Fig. 5D).

Body Weight Changes after Instillation of the Dusts

There were no statistically significant differences in body weight gains between the exposed groups and control groups terminated either on 7 or 90 days after instillation of the dusts. After 7 days the group average body weight gains ranged from a low of –0.3 g (0.1 mg MSS) to +2.2 g (ozone control group). After 90 days the weight gains ranged from 4.0 g to 6.4 g. The groups with the lowest average body weight gains were the O_3+MSS-HD (4.0 ±1.6 g), SiO_2-LD (4.6 ± 1.3 g), and SiO_2-HD (4.9 ±0.7 g). These are among the groups that showed the most histopathological effects in the lungs exposed to the dust for 90 days.

Determination of Antinuclear Antibodies in Sera of Dust-Treated Mice

In order to assess LSS and the other dusts for the potential of inducing cellular autoimmunity, ANA fluorescence intensity of cells in sera collected from control (saline) mice and dust-treated mice were determined and compared. Results of this screening showed that all dusts tested were capable of increasing ANA (Figs. 6 and 7). However, the results from only those treated with low-dose LSS

and high-dose silica were statistically significantly different from controls. Ozone-pretreatment statistically significantly enhanced the ANA response in the 0.1-mg MMS group; the increase was not significant in the 1-mg group. The reasons for the apparent lack of dose-response in some of these findings are not known. However, the lack of dose response in ANA studies also has been observed by others.

DISCUSSION

Appropriate intratracheal instillation doses were chosen based on information from Henderson et al. (1995). Henderson reported that, in rats exposed by inhalation to 10 mg/m^3 of quartz or TiO_2 for one week, the lung contained 0.76 mg quartz or 0.44 mg TiO_2. Information compiled by Boggs (1992) and Lai (1992) shows that minute respiratory volumes (MRV) of a 300-g rat, a 35-g mouse and a 20-g mouse were 0.210, 0.040 and 0.025 L/min, respectively. The MRV of mice (average 25 g) in our study would be about 0.030 L/min. If it is assumed that dust deposition in the lung is roughly proportional to the MRV of the exposed animals and if Henderson et al. (1995) had included mice (ca. 25 g) in their study, the dust burden in the mouse lung would be about 0.11 mg quartz or 0.06 mg TiO_2. In the present mouse study, a single dose of 0.1 mg or 1 mg per mouse was chosen as low or high dose, respectively.

Bermudez et al. (2000), reported that the lung burden (determined at the end of the last exposure) in mice exposed to TiO_2 at 10, 50, or 250 mg/m^3 for 13 weeks (6 h/d, 5d/wk) was 5.2, 53.5 or 170.2 mg/g of dry lung tissue. The corresponding values for lung burden of TiO_2 per mouse calculated by these authors were approximately 0.2, 2, or 13 mg. It is noteworthy that the low dose (0.1 mg/mouse) of our study fell in between the values of the lung TiO_2 burden in mice exposed to 10 mg/m^3 for 1 week (extrapolated above using Henderson's data) and 13 weeks (Bermudez et al., 2000). The high dose (1 mg/mouse) was half the value of the body burden in mice exposed to 50 mg/m^3 for 13 weeks. This information is useful for qualitative comparisons of dust exposures by different pulmonary administrations as long as the bolus nature of the intratracheal instillation is recognized (Driscoll et al., 2000). It should also be noted that the residual lung burden determined at the end of the last inhalation exposure is the dynamic result from cumulative intermittent deliveries and continuous elimination.

When the mice were intratracheally instilled 0.1-mg TiO_2 particles, an increased number of macrophages, as compared with that in the lung of saline controls, was seen 7 days after the treatment. This increase was not visible 90 days after treatment and the lung tissue was apparently normal. When treated with the high TiO_2 dose (1 mg/mouse) and killed 90 days after the dust instillation, the mice showed that particles persisted in the lung, and numerous macrophages were present, presumably, continuous mounting defensive responses in the lungs. Tissue histopathological manifestations were minimal. These findings were consistent with the observations reported by Bermudez et al. (2000). In the lungs of the mice, which were exposed for 90 days to 50 mg/m^3 (TiO_2 lung burden of ca. 2 mg), Bermudez found increased numbers of macrophages and neutrophils and "elevated soluble indices of inflammation." These parameters remained elevated when examined at 26 weeks after the last exposure. The increases were not observed in mice exposed to10 mg/m^3 (lung burden ca. 0.2 mg).

In the present study, the dust suspensions for bolus instillation were freshly prepared and ultrasonicated before treatment. Despite these precautions, clumps of particles were still seen in the lungs, especially those that received the high doses. Exposures by inhalation would be expected to improve the distribution in the lung and minimize clump formation (Driscoll et al., 2000). For those animals that were instilled with 0.1 mg/mouse and killed 90 days after the treatment, MSS and SiO_2 particles, but not LSS and TiO_2, were still visible in the lungs. This observation indicated that TiO_2 and LSS were easier to clear from the lungs than MSS and SiO_2. However, when the animals were given 1 mg/mouse, all four dusts were visible in the lung regardless of whether the animals were

killed 7 or 90 days after the dust treatment. The high doses apparently overwhelmed the pulmonary clearance mechanisms.

Results of a particle-size distribution determination conducted after the animal treatment study showed that the respirable fraction of MSS isolated by Lovelace from the raw material had a bimodal distribution that included 70% fine dust with a mass median diameter (MMD) of 3 μm. To our surprise, it also contained 30% by volume of particles with diameters ranging from 30 to 400 μm with an MMD of 108 μm (Fig. 1B). Ultrasonication did not significantly alter the particle size distribution. The reasons for the presence of these large particles in the sample prepared as respirable dust are not known. The LSS, without ultrasonication, contained 5% large particles with an MMD of 81 μm; upon ultrasonication for 240 seconds, all these large particles disappeared (Fig. 1A). The sonicated LSS showed a bimodal distribution with MMDs in the respirable range. In general, the toxic insults to the lung from particles per unit weight decrease with an increase of particle size. The presence of large particles would quantitatively, but not qualitatively, influence the dose response. However, large particles are more persistent in the lung and therefore this fact may partially explain why MSS was more persistent than LSS in the lung.

LSS was more toxicologically reactive than TiO_2 in the mice; the difference was more evident at the high dose. Like TiO_2, LSS at the low dose produced very little tissue response. At the high dose, acute mild to moderate inflammation of the lung tissue, was observed among the LSS-treated mice. With the continuous irritation of the high-dose LSS dust in the lung, the acute inflammation, which was observed in the 7-d group, was transformed into chronic inflammation 90 days after dust treatment. However, the inflammatory manifestations were relatively mild with this dust.

MSS elicited a more severe reaction than the LSS. Inflammatory responses, which were not seen in animals of the LSS-LD groups, were detected in both the MSS-LD-7d and MSS-LD-90d groups. The inflammatory reactions, which were mild in the MSS-LD-7d group, were mild to moderate in the lung of the MS-HD-7d group. Fibrosis was observed in the MSS-HD-90d group.

Ozone pretreatment followed by MSS instillation was used to emulate the oxidative and reactive nature of actual Martial soil. As expected, mice receiving such treatment showed a greater pulmonary toxicity response than those receiving either compound alone; the toxic manifestations were especially discernible in ozone-pretreated animals instilled with the high-dose MSS. The O_3+MSS-HD-7d group revealed moderate pulmonary reactions with early focal moderate to severe fibrosis. The animals in the 90-d study had widespread intraalveolar debris, moderate alveolitis, and fibrosis. It is noteworthy that the ozone exposure (0.5 ppm for 3 hr) alone produced no overt lung injury when examined 7 days after the exposure, but focal mild alveolitis and peribronchiolar inflammation were noted 90 days after the exposure. The effects of ozone and MSS together appeared to be additive. Nevertheless, these effects were less severe than those seen in animal treated with high dose crystalline silica.

The pulmonary toxicity of the Hawaiian and San Francisco Mountain volcanic ashes have not been reported. However, fibrogenic activity or other pulmonary toxicity of volcanic ashes obtained from other locations (mostly from Mt. St. Helens) has been widely reported (Beck et al., 1981; Green et al., 1981; Schreider et al., 1985). Green et al. (1981) reported that victims died 10-16 days after the Mt. St. Helens eruption and autopsy revealed fibrosis in the lungs. The ash-induced fibrogenic responses were confirmed in rats intratracheally instilled with the ash (Green et al., 1981).

In the 1970s NASA conducted limited toxicity investigations on lunar soil samples. Intraperitoneal and subcutaneous injections of the lunar soil to mice resulted in acute inflammatory responses throughout the peritoneum or surrounding the subcutaneous injection site, respectively. Low-grade inflammatory reactions but no fibroblastic lesions were detected 20 months after the injection. Intratracheal injections of the lunar soil into guinea pigs were also carried out on three groups of 4 guinea pigs given 2 ml of saline containing 2% dust or no dust (3 dust-treated and only 1 saline control) (Holland and Simmonds, 1972). Two of the groups were killed on either 2 or 4 days after the injection. No information was given on exactly when the third group, which was held for long-term observation, was killed. Without referring to which group(s), the authors reported that a diffuse alveolar cell hypertrophy, septal edema, monuclear infiltration, and proliferation of alveolar macrophages were observed. The authors stated that "the tissue response seemed out of proportion to the amount of particle material present." Unable to discern the treatment effects because of a significant degree of spontaneous pulmonary pathology in both control and dust-treated guinea pigs, the authors concluded "the potentially harmful or innocuous nature of the dust remains to be investigated."

The Russians also conducted a series of studies to determine the toxicity of lunar soil they collected. A study was conducted in mice exposed to air (0.5 L/min) which was "passed through a layer" of preweighed sample of lunar surface material for 4 days (4 hr/d) (Kustov et al., 1974). According to the authors, pathomorphological examinations of internal organs showed no differences between the treated mice and controls exposed to clean air. Unfortunately, no information was presented on any weight loss after the air passed through the test material. Moreover, the authors also did not specify whether the lung was included in the examination.

Another Russian study (Kustov et al. 1989) was conducted in Wistar rats each injected intratracheally with a single dose of 50 mg (in 1% starch solution) lunar dust, or terrestrial analog (a basalt from Kamchatka, Russia). The compositions of the Russian lunar soil and terrestrial analog are comparable to U.S. counterparts. Six months after the dust treatment, the lung tissue revealed many fibrous cellular nodes that contained dust particles, macrophages, lymphocytes, plasmocytes, fibroblasts, and giant multinuclear cells. Terrestrial analog, like the extraterrestrial counterpart, also produced pneumoconiosis characterized by the development of fibrous cellular nodes, pronounced interstitial fibrosis and interlobular septa. These results are expected due to the massive dust dose used in these rats by the Russian investigators. Bermudez et al. (2000) reported that 13-week exposures (6 h/d, 5d/wk) of rats to 250 mg/m^3 TiO_2, a dust that at low concentrations produced little effects in rats, resulted in hypertrophy and hyperplasia of alveolar epithelium, and fibrotic changes; these effects were not seen in rats exposed to 50 mg/m^3 TiO_2 and in mice exposed to either concentration. The lung burden of dust in rats exposed to 250 or 50 mg/m^3 TiO_2 determined at the end of the exposures was 40 or 8 mg/rat, respectively. The dose of 50 mg/rat in the Russian study apparently seriously overloaded the lungs of the rats. It is well known that greatly overloading dust in rats impairs cellular responses to clear and sequester particles leading to a spectrum of histopathology that could include cancer (Hext, 1994; Morrow, 1992; Muhle et al., 1990; Trochimowicz, 1988).

(Kustov et al., 1989) also stated that the interaction of lunar soil and pulmonary tissue was accompanied by cellular autoimmune reactions. Unfortunately, experimental procedures to assess cellular autoimmunity, as well as the results to support the authors' interpretation were not presented. Therefore, a study is needed to determine whether lunar soil can induce cellular

autoimmune reaction. The need is further supported by findings implicating silica, a fibrogenic dust, in the induction of various autoimmune disorders (McHugh et al., 1994). In the present study, the ability of LSS and the other dusts to elicit production of antinuclear antibodies in mice was assessed. The results (Fig. 6) showed that silica and LSS dust were capable of increasing ANA; the increase was statistically significant only for the high-dosed silica and low-dosed LSS groups. This result suggests the possibility that LSS could induce cellular autoimmunity. However, the implications of this result are confounded by the apparent lack of a dose-response relationship. Moreover, in humans, ANA test is only an important adjunct for diagnosis of antoimmune diseases (Fritzler, 1986), other medical diagnostic tests are needed for disease prognosis because a considerable number of healthy persons also showed positive results on ANA (Tan et al., 1997; Kavanaugh, 1999).

The overall results of the present study showed that LSS and MSS are not simply nuisance dusts. It is reasonable to predict that inhalation exposures to LSS or MSS would produce more pulmonary responses than exposures to TiO_2, but less severe responses than crystalline SiO_2. Even though the chemical compositions of these dust simulants are similar to lunar or Martian soil, the results of the present study on the simulants would be no more than qualitative indicators of the hazards of the extraterrestrial soils. The results of the co-exposures to ozone and MSS suggest that Martian soil, a dust with a reactive surface and probably persistent in the lung like MSS if it is inhaled, could produce more severe injury than that caused by the simulant. These findings support NASA's intention to evaluate the oxidative and reactive Martian surface soil before sending humans to the hostile Martian environment (Sullivan, 2000, personal communication).

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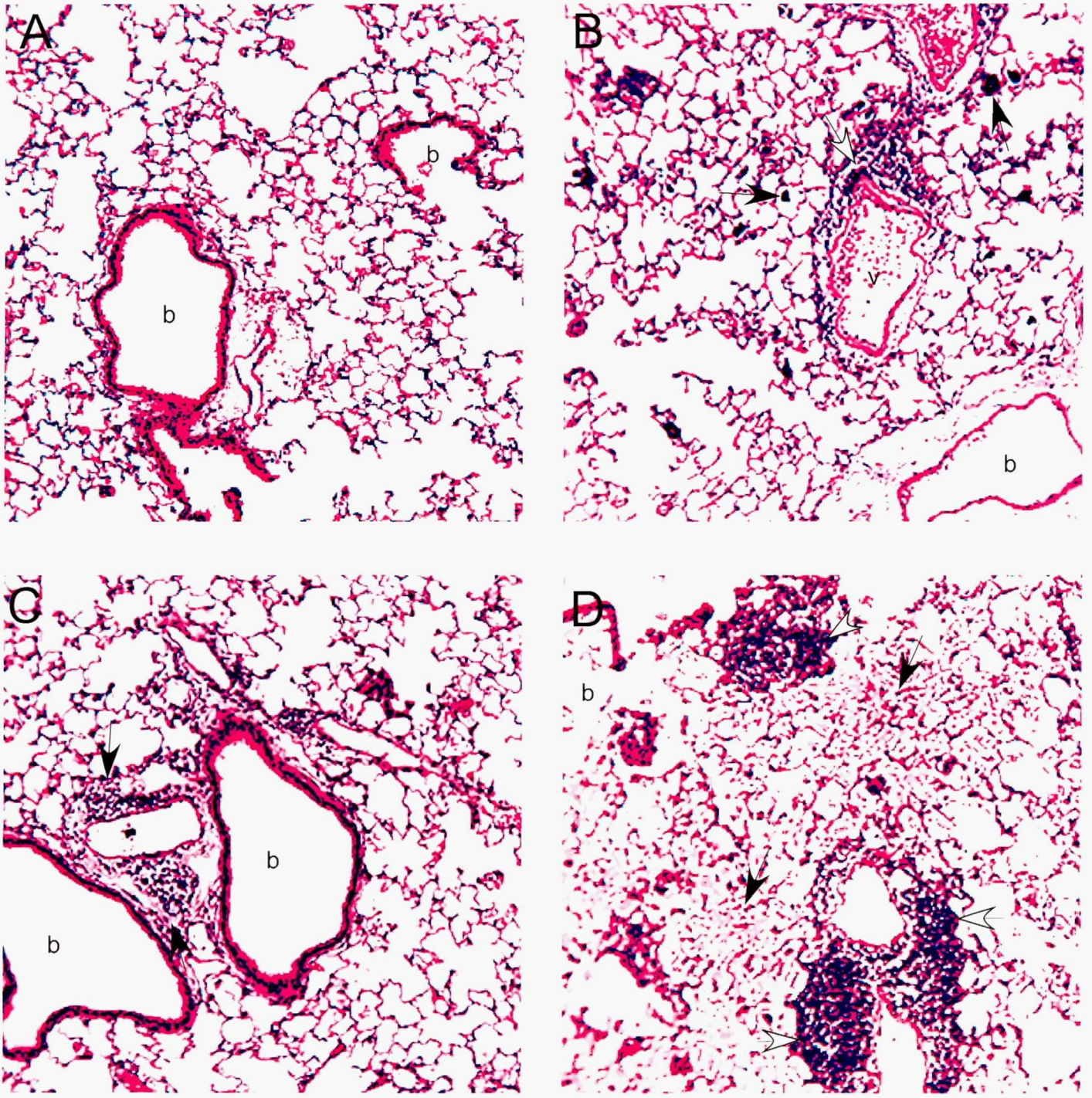

FIG. 2 Lung histopathology of mice intratracheally instilled with a single dose of 0.1 mg (LD) or 1 mg (HD) of TiO_2 (A, B) or quartz (C, D). The animals were killed 90 days after the dust treatment. The lung histology of mice in the TiO_2-LD-90d (A) was indistinguishable from that of saline controls, therefore, Fig. A is also used to illustrate normal lung histology. In the figures, ad = alveolar duct, b = bronchus, v = vessel; in (B): ⇒ = focal mild perivascular inflammation, → = clumps of particles; (C) shows mild focal fibrosis, → = mild perivascular and peribronchiolar inflammation; (D) shows moderate alveolitis, severe focal fibrosis, ⇒ = moderate to severe perivascular and peribronchiolar inflammation, → = intraalveolar debris and edema.

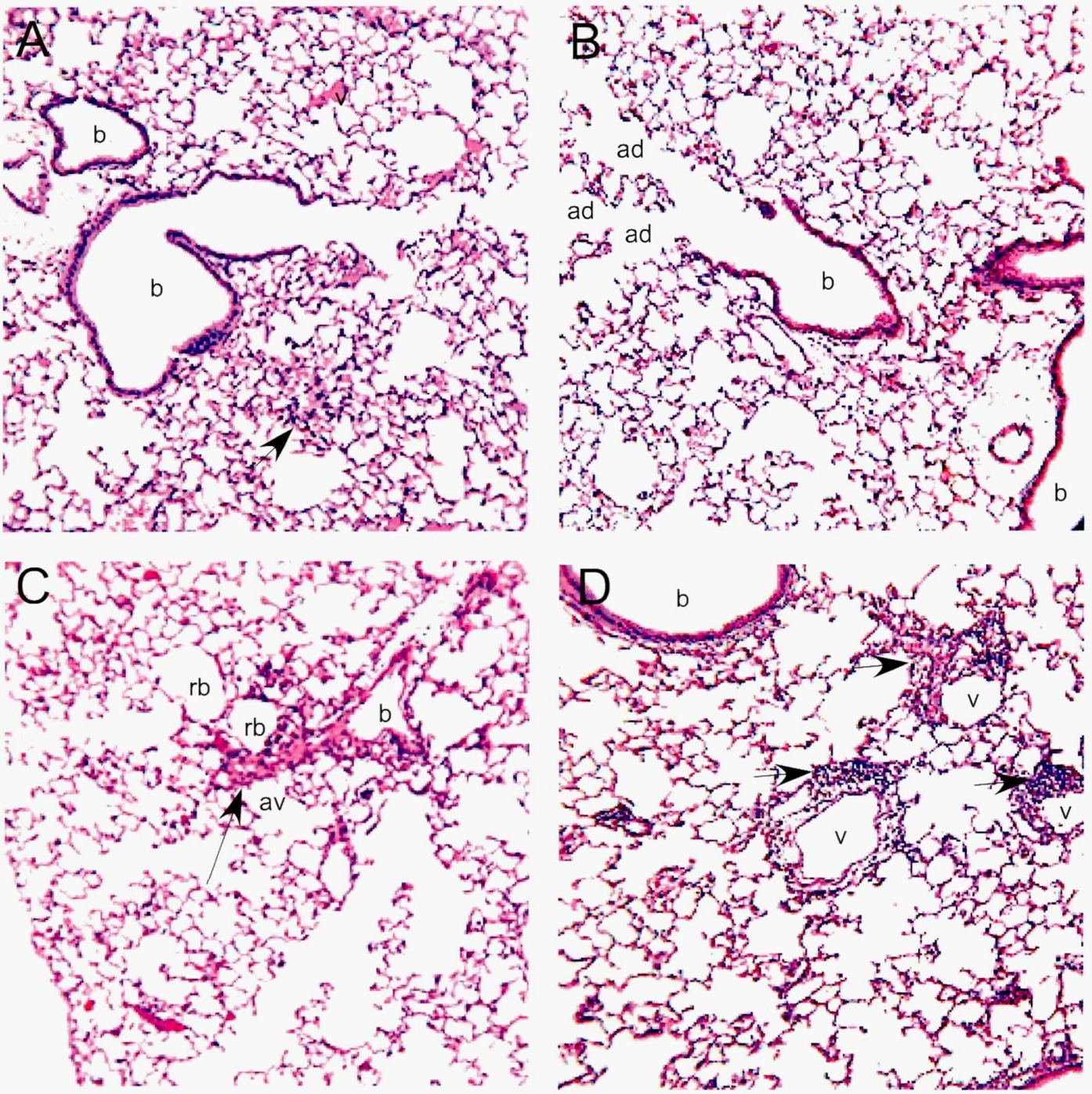

FIG. 3. Lung histopathology of mice intratracheally instilled with a single dose of 0.1 mg (A, B) or 1 mg (C, D) of lunar soil simulant and killed 7 days (A, C) or 90 days (B, D) after the treatment. In the figures, ad = alveolar duct, b = bronchus, v = vessel; in (A): ⟶ = particle-laden macro-phages; (B) shows no particles and normal tissue; (C) shows mild to moderate alveolitis with lymphocyte and neutrophile infiltration, ⟶ = alveolar damage, particle-laden macrophages, inflammatory cells; (D) shows mild alveolitis, ⟶ = septal thickening, blood vessel and bronchial inflammation.

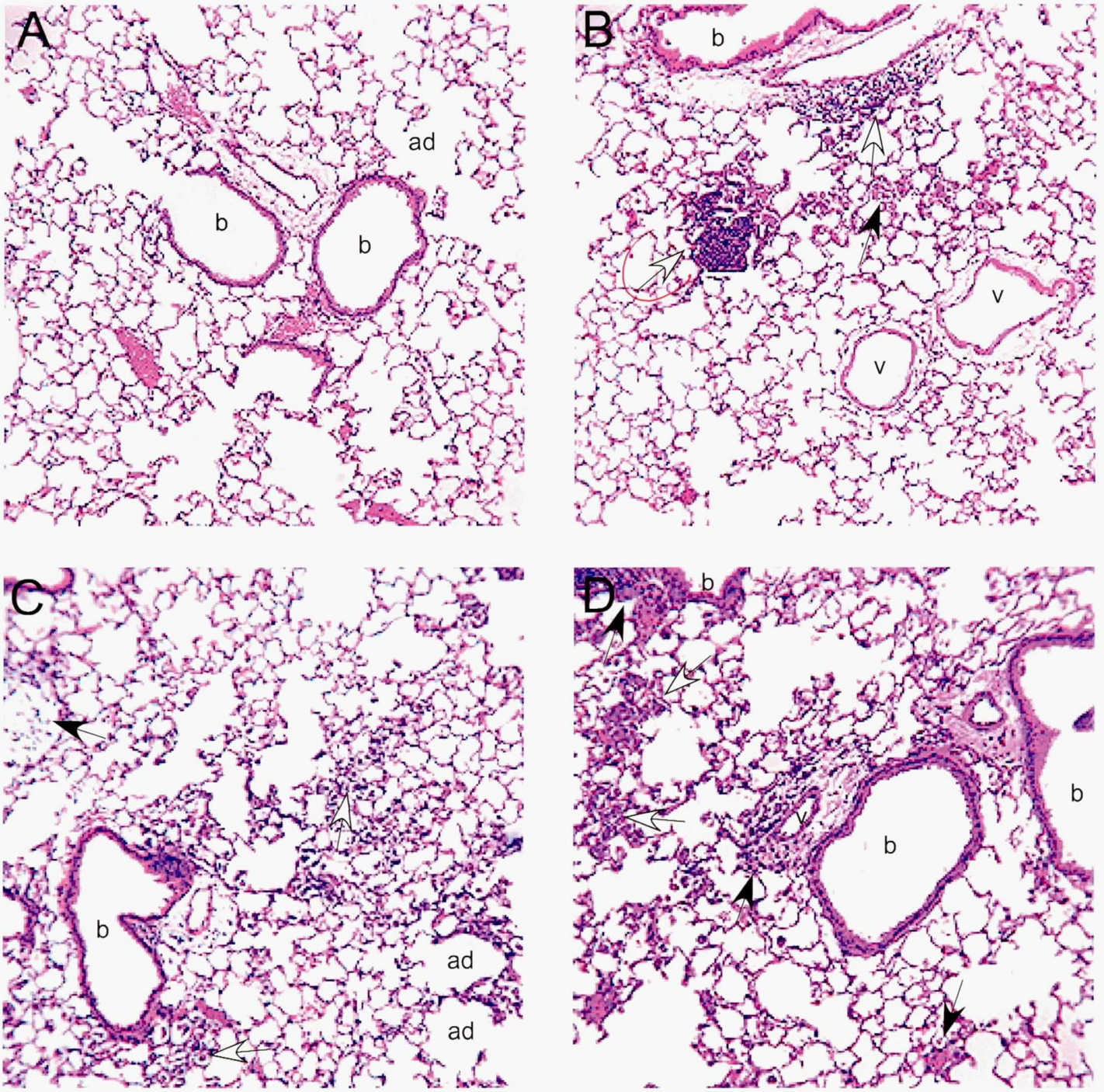

FIG. 4. Lung histopathology of mice intratracheally instilled with a single dose of 0.1 mg (A, B) or 1 mg (C, D) of Martian soil simulant and killed 7 days (A, C) or 90 days (B and D) after the dust treatment. In the figures, ad = alveolar duct, b = bronchus, v = vessel; (A) shows inflammation cells and scattered debris; in (B): ⟶ = particle-laden macrophages, ⇢ = fibrotic foci and inflammatory cells; in (C): ⇢ = particle-laden macrophages, ⟶ = inflammatory and intraalveolar debris; in (D): ⟶ = particle-laden macrophages, ⇢ = inflammation and fibrosis.

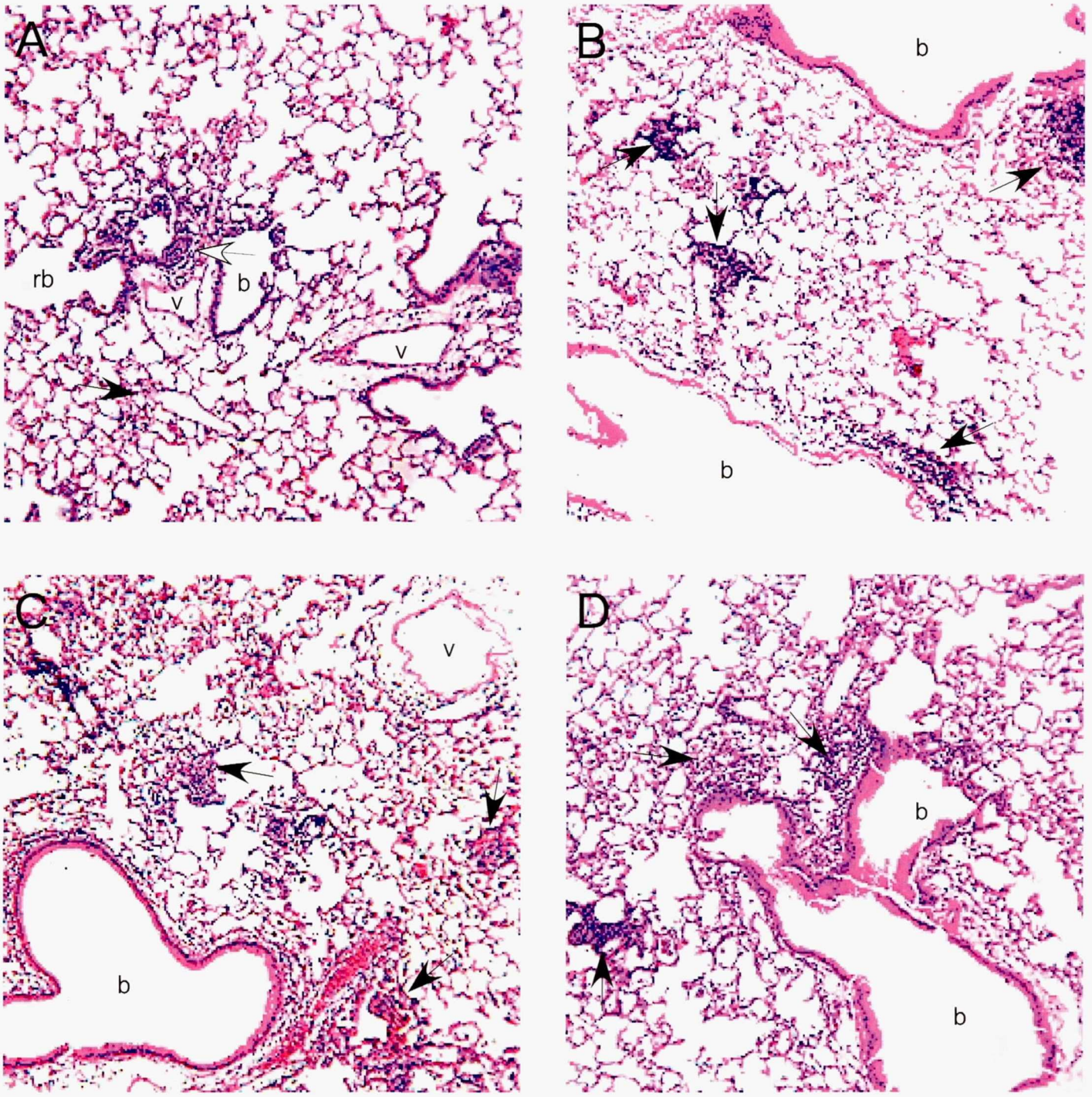

FIG. 5. Lung histopathology of ozone-pretreated mice each was intratracheally instilled with a single dose of 0.1 mg (A, B) or 1 mg (C, D) of Martian soil simulant and killed 7 days (A, C) or 90 days (B, D) after the dust treatment. The ozone exposure was 3 h at 0.5 ppm. In the figures, av = alveolar duct, b = bronchus, v = vessel; in (A): ⟹ = peribronchial inflammation, ➔ = focal inflammation; in (B): ➔ = focal inflammation and fibrosis; in (C): ➔ = focal inflammation, alveolitis, and intraalveolar debris; in (D): ➔ = septal thickening, fibrosis, wide-spread intraalveolar debris and alveolitis.

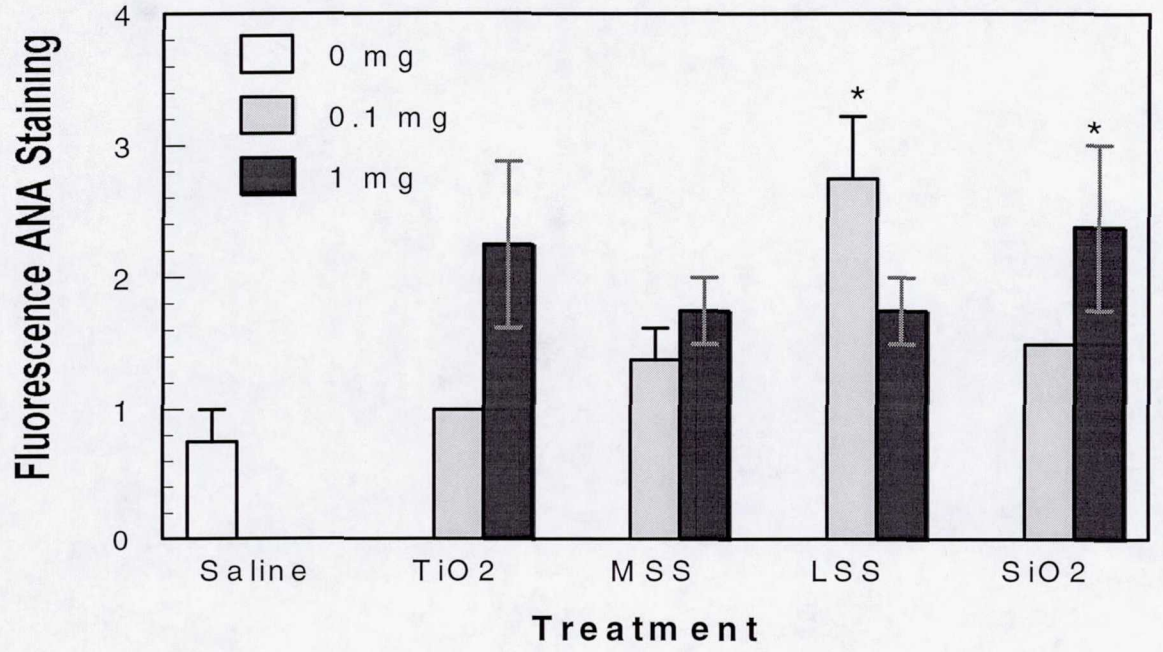

FIG. 6. Comparison of antinuclear antibody fluorescence intensity in serum of mice each was intratracheally instilled with 0 (saline only), 0.1, or 1 mg of Martian soil simulant, lunar soil simulant, TiO_2, or SiO_2. Serum samples were collected 90 days after the treatment. Each bar represents the mean (±SD) from 4 mice (* = statistical difference from that of saline, $p \leq 0.05$).

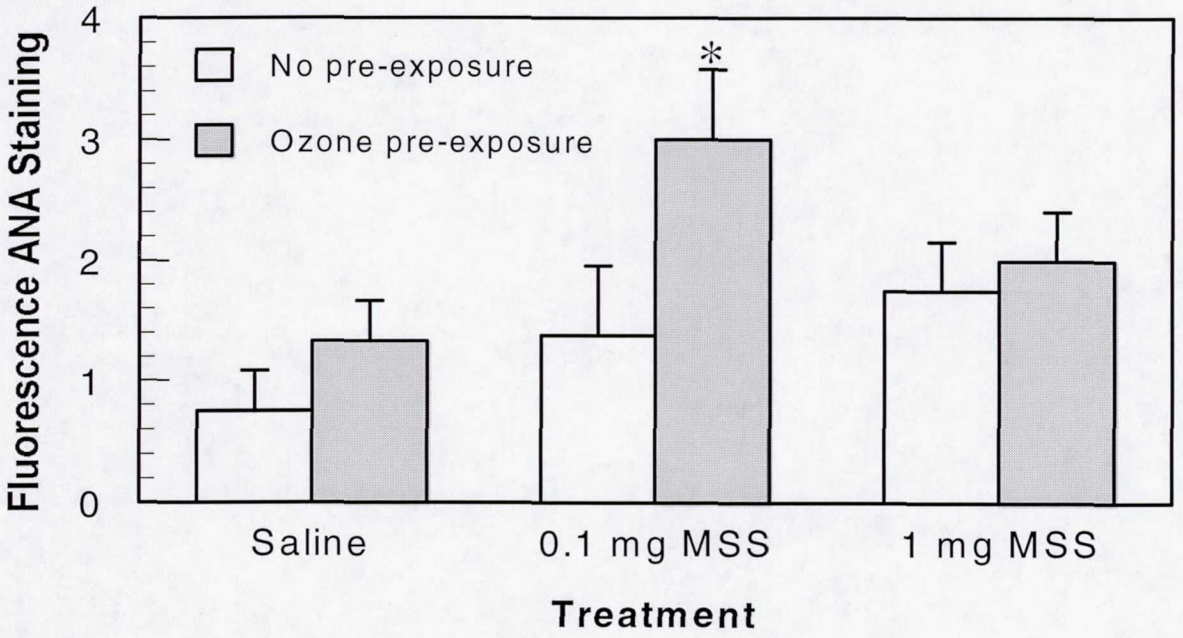

FIG. 7. Comparison of antinuclear antibody fluorescence intensity in serum of ozone-pretreated mice each was intratracheally 0 (saline only), 0.1, or 1 mg Martian soil simulant. Instillation was preceded by a 3-h exposure to 0.5 ppm ozone. Serum samples were collected 90 days after the treatment. Each bar represents the mean (±SD) from 4 mice (* = statistical difference from that of ozone-preexposed mice treated with saline, $p \leq 0.05$).

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