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ACUTE INFLAMMATORY REACTION IN RATS AFTER INTRATRACHEAL INSTILLATION OF MATERIAL COLLECTED FROM A NYLON FLOCKING PLANT

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Several cases of interstitial lung disease have been diagnosed among workers at a nylon flock plant, but the etiologic agent for the disease outbreak was unknown. The results of a medical survey and industrial hygiene study indicated that the dust present in the plant may be responsible. Thus, airborne dust collected at the plant was examined for its inflammatory potential in rat lungs. The endpoints measured were: (1) breathing rates, (2) differential cell counts of bronchoalveolar lavage cells, (3) alveolar macrophage (AM) chemiluminescence, (4) albumin concentration and matrix metalloprotease activities in the acellular fluid from the initial bronchoalveolar lavage, and (5) pulmonary histopathology. In the first study, rats received a single dose of the airborne dust sample (10 mg/kg body weight) by intratracheal (IT) instillation. At 1 d post-IT, all inflammatory endpoints were significantly increased versus controls, but by 29 d post-IT they did not differ significantly from controls. Histopathology demonstrated mild to moderate, multifocal, suppurative pneumonia, usually centered around bronchioles, at 1 d post-IT. At 29 d post-IT, pulmonary inflammation was minimal to mild and characterized by alveolar histocytosis usually restricted to the immediate area of retained birefringent fibers. In subsequent experiments, airborne dust was extracted with water and the dust (washed airborne dust) and water extract (soluble fraction) were separated by centrifugation for further study. Nylon tow dust was prepared in the laboratory by milling uncut nylon strands (called tow) that had not been treated with the finish or dyes that are commonly used in the flock plants. Rats were administered a single dose of a dust sample (10 mg/kg body weight) or the soluble fraction (1.3 ml/kg body weight) by IT administration and the same endpoints were measured at 1 d post-IT. The dust samples caused significant increases in all of the inflammatory endpoints; however, the soluble fraction was much less active. Histological analysis of the lungs 1 d post-IT confirmed lung inflammation was occurring and tended to center around bronchioles. The results suggest that: (1) nylon flocking generates particles of respirable size that can interact with AM in the lung and can be detected in the lung 29 d after exposure, (2) the dust samples examined cause an inflammatory response, (3) water-extractable agent(s) from airborne dust contribute only minimally to the inflammatory response, and (4) the acute inflammatory response to these dusts is substantial when compared to other pathologic occupational dusts previously examined in our laboratory.

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Occupational interstitial lung disease (ILD) can result from exposure to various agents in the workplace, including crystalline silica, asbestos, coal dust, some metals (e.g., beryllium, cobalt), organic agents (certain bacteria, fungi, animal and plant proteins) and some chemicals (e.g., anhydrides, isocyanates) (Schwartz et al., 1994; Lopez & Salvaggio, 1994; Redlich, 1996; Rose, 1996). Several physician-diagnosed cases of occupational ILD have recently been identified among workers at a nylon flock plant (Kern et al., 1997, 1998). Nylon flock is finely cut nylon and is used for the manufacture of upholstery for furniture and automobiles, as well as many other products. However, the etiologic agent for the disease outbreak at the nylon flock plant was unknown.

Upon learning of ILD among workers from this plant, the National Institute for Occupational Safety and Health (NIOSH) conducted medical and industrial hygiene cross-sectional surveys to quantify respiratory morbidity and to identify work-related factors that may be causally related (NIOSH, 1998; Burkhart et al., 1999). The medical survey, which included a review of medical records of workers who were diagnosed with ILD, revealed a high prevalence of respiratory symptoms and respiratory diagnoses among workers from three departments. Knowledge of the processes in these departments, in conjunction with the medical survey and industrial hygiene results, indicated that the dust present in the plant may be responsible for this lung disease.

To investigate this hypothesis, a toxicological study was conducted. Airborne dust collected at the plant was examined for its inflammatory potential in rat lungs. In subsequent experiments, the airborne dust was extracted with water and the dust (washed airborne dust) and the water extract (soluble fraction) were separated by centrifugation for further study. The last material tested was dust prepared in the laboratory by milling uncut nylon tow. Although nylon tow can contain finish materials used in its manufacture, it did not contain any of the finish or dye components commonly used at the flock plant.

METHODS

Animals

Pathogen-free male Sprague-Dawley rats (200–300 g) were obtained from Hilltop Labs (Scottdale, PA) and were acclimated in an AAALAC-approved animal facility for at least 5 d before use. The rats were provided food and water ad libitum.

Collection of Airborne Dust

Dust samples from the plant were collected using a vertical elutriator operating at a flow rate of 7.4 L/min. Samples were collected onto polyvinylchloride filters, and after collection, dust was scraped from the filter surface into vials. Particle analysis of air samples collected at the flocking plant is reported in a companion article in this journal (Burkhart et al., 1999).

Preparation of Nylon Tow Dust

In order to obtain a sample of unprocessed nylon tow of suitable size for intratracheal instillation, strands of tow were milled using a Tekmar A 10 rotary knife mill. After milling, the bulk of the material was a tangled mass of nylon strands. However, examination of the inner surfaces of the mill revealed deposits of dust that were found to contain elongated particles similar to those seen in air samples from the plant. These particles were polydisperse, but average width and length, as measured by scanning electron microscopy, was approximately 2 μm and 14 μm, respectively.

Preparation of Washed Airborne Dust and Soluble Fraction

A 40-mg airborne dust sample was placed in a 17 × 100 mm polystyrene tube and was suspended in 5 ml of sterile, endotoxin-free water (BioWhittaker LAL Reagent Water, Walkersville, MD). The suspension was gently rocked at room temperature for 1 h, followed by centrifugation (1000 × g, 4°C, 10 min). The resulting supernate, called soluble fraction, was collected into a sterile 4-ml Nunc vial and stored at –80°C. The soluble fraction was analyzed for endotoxin using the Kinetic-QCL LAL Testing Made Easy 192 test kit (BioWhittaker, Walkersville, MD) following the routine assay methodology. The resulting pellet, called washed airborne dust, was dried under nitrogen at room temperature.

Dust Preparation for Intratracheal Instillation

The airborne, washed airborne, and nylon tow dusts were prepared as 10 mg/ml (w/v) suspensions in endotoxin-free Ca^{2+},Mg^{2+}-free phosphate-buffered saline, pH 7.4 (PBS; BioWhittaker, Walkersville, MD). The soluble fraction was made isotonic (141 m*M* NaCl) by the addition of 5 *M* NaCl prepared with endotoxin-free water (BioWhittaker LAL Reagent Water, Walkersville, MD).

Intratracheal Instillation

The rats were lightly anesthetized with an intraperitoneal injection of 0.7 ml 1% (w/v) sodium methohexital (Brevital, Eli Lilly and Company, Indianapolis, IN) and were intratracheally instilled using a 20-gauge 4-in ball-tipped animal feeding needle (Perfectum, New Hyde Park, NY). Rats received either a 10-mg/kg body weight dose of dust or an equivalent dose (1 ml/kg body weight) of PBS, which served as controls. For studies of the soluble fraction, rats received either a 1.3 ml/kg body weight dose of the soluble fraction or a 1.3-ml/kg body weight dose of PBS, which served as controls. This dose of the soluble fraction contained approximately the same amount of endotoxin as a 10-mg/kg dose of the airborne dust.

Breathing Rates

Breathing rates were determined using a flow plethysmograph that has been previously described in detail (Frazer et al., 1997). The plethysmograph chamber was constructed of an acrylic tube enclosed at both ends. One end of the chamber had a circular port that contained four 400-mesh stainless

steel screens. Pressure variations across the screens generated by flow into and out of the chamber were measured with a pressure transducer (Setra, Inc., Foxborough, MA). A digital oscilloscope (Tektronix, Inc., Wilsonville, OR) was used to record flow signals, which were transferred to a digital computer for analysis. To measure the rat breathing rates, a rat was placed in the flow plethysmograph, equilibrated with 10% CO_2, and its average breathing rate calculated based on the time between zero crossings of the flow signal at the beginning and end of an inhalation–exhalation cycle.

Bronchoalveolar Lavage and Lung Necropsy

Rats were euthanized with an intraperitoneal injection of 0.25 ml sodium pentobarbital (EUTHA-6, Western Medical Supply Inc., Arxadia, CA) and exsanguinated by cutting the right renal vein. Rats were euthanized 1 day after intratracheal (IT) instillations, and also 29 d after IT instillations in the airborne dust study. A tracheal cannula was inserted, and the left lung lobe was clamped off with a hemostat, ligated, and removed below the ligature for histopathology. Sections for electron microscopy were taken from the main stem bronchus in the upper, middle, and lower left lung. The sections were rinsed with Sorenson's phosphate buffer (pH 7.2) and postfixed with osmium tetroxide. The samples were cut longitudinally then dehydrated, dried, and mounted with double-stick tape on aluminum stubs and coated with gold/palladium. The right lung was lavaged through the cannula using ice-cold PBS, the first lavage being 3 ml and subsequent lavages using 4 ml until a total of 40 ml of lavage fluid was collected. The first bronchoalveolar lavage was kept separate from the rest of the lavage fluid. Bronchoalveolar lavage cells (BALC) were isolated by centrifugation (500 × g, 10 min, 4°C). The acellular supernate from the first lavage (BALF) was decanted, transferred to a 13 × 100 mm polystyrene tube, and frozen at –30°C for later analysis. The acellular supernate from the rest of the lavage was decanted and discarded. BALC were resuspended in HEPES buffer (10 m*M* *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 145 m*M* NaCl, 5.0 m*M* KCl, 1.0 m*M* $CaCl_2$, 5.5 m*M* D-glucose; pH 7.4), centrifuged (500 × g, 10 min, 4°C), and the supernate was decanted and discarded. The BALC pellet was then resuspended in 0.5 ml HEPES buffer and placed on ice. Cell counts and differentials were obtained using a Coulter Multisizer II and AccuComp software (Coulter Electronincs, Hialeah, FL). Lavage samples for electron microscopy were cultured on coverslips for 1 h, then fixed in glutaraldehyde and postfixed in osmium tetroxide. The cells were then dehydrated, dried, and mounted with double-stick carbon tape on aluminum stubs and coated with gold/palladium. Both the bronchi and the macrophages were imaged on a JEOL 6400 scanning electron microscope.

Alveolar Macrophage Chemiluminescence

Alveolar macrophage (AM) chemiluminescence (CL) assay was conducted in a total volume of 0.5 ml HEPES buffer. Resting AM CL was deter-

mined by incubating 0.5×10^6 AM at 37°C for 5 min with 0.04 mg% (w/v) luminol (Sigma Chemical Company, St. Louis, MO) followed by the measurement of CL for 15 min. To determine zymosan-stimulated AM CL, the assay was modified to include 1 mg unopsonized zymosan (Sigma Chemical Company, St. Louis, MO), which was added to the assay immediately prior to measurement of CL. Since polymorphonuclear leukocytes (PMN) do not respond to unopsonized zymosan, the CL measured is from AM (Castranova et al., 1990). Measurement of AM CL was done with an automated luminometer (Berthold Autolumat LB 953, Wallace, Inc., Gaithersburg, MD) at 390–620 nm for 15 min and the integral of counts per minute (cpm) versus time was calculated. Zymosan-stimulated CL was calculated as the cpm in the zymosan-stimulated assay minus the cpm in the resting assay.

BALF Albumin Concentrations

BALF albumin concentrations were determined using a Cobas Fara II analyzer (Roche Diagnostic Systems, Montclair, NJ). Albumin was determined colorimetrically at 628 nm based on albumin binding to bromcresol green (albumin BCG diagnostic kit, Sigma Chemical Company, St. Louis, MO).

BALF Matrix Metalloprotease Activity

Assay of matrix metalloproteases (MMP) was conducted to assess the potential inflammatory activation of these enzymes due to exposure to the three dust samples and soluble fraction. To determine BALF MMP activity, a fluorescent assay was used (Knight et al., 1992). For the assay, two 50-µl samples of BALF from each animal were prepared. One sample was used to detect MMP activity without activation (endogenous activity). The other was maximally activated by limited proteolysis with trypsin to remove the propeptide segment and expose the active site of the MMP (maximal activity). The endogenous sample (50 µl) was thawed and prepared immediately prior to assay by addition of 150 µl of 100 m*M* Tris (pH 7.6). For the maximal activity sample (50 µl), 1 µg trypsin (in 10 µl of 1 m*M* HCl) and 140 µl of 100 m*M* Tris (pH 7.6) with 5 m*M* $CaCl_2$ were added. The maximal activity sample was then incubated at 22°C for 24 h. After the 24-h incubation, 4 µg soybean trypsin inhibitor was added, and the sample was incubated at 22°C for an additional 30 min. For measurement, the entire contents of the sample was added to a 1-ml fluorescent cuvette in a water-jacketed bath and 800 µl of incubation medium added (100 m*M* Tris-HCl, pH 7.6, 0.2 *M* NaCl, 5 m*M* $CaCl_2$, 0.1 µ*M* $ZnCl_2$). One microgram of the fluorescent substrate was then added (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2) and the rate of change in fluorescence (328 excitation, 393 nm emission) was determined over 10 min. A standard curve of fluorescence versus nanomoles of substrate cleaved was constructed using a cleaved fluorescent peptide (Mca-Pro-Leu-Gly-Leu). MMP substrate digestion activity was expressed as nanomoles of fluorescent substrate cleaved per milliliter of BALF per minute.

Lung Histopathology

After the lung lavage was completed, the hemostats were removed and the lungs were inflated with 6 ml of 10% neutral buffered formalin. The left lung lobes, which were not lavaged, were routinely processed, embedded in paraffin, sectioned at 4 to 5 μm, and stained with hematoxylin and eosin and Gamori's trichrome. Slides were examined by a board-certified veterinary pathologist under bright field and polarized light.

Statistics

Data are expressed as means ± SE. Statistical differences were determined using one-way analysis of variance (ANOVA) with significance set at $p \leq .05$. When significant F values were obtained, individual means were compared to control using Dunnett's test with significance set at $\alpha = .05$.

RESULTS

Endotoxin

A water extract of airborne dust was made and the endotoxin content of this soluble fraction was determined (Table 1). Based on the amount of endotoxin per mg airborne dust, the amount of endotoxin received by IT administration of a 10-mg/kg body weight dose of the airborne dust was calculated and determined to be 112 ± 1 EU/kg. The dose of soluble fraction used for IT instillation, 1.3 ml/kg body weight, was chosen to deliver an endotoxin dose of approximately 112 EU/kg body weight. By using approximately equivalent dosages of endotoxin, the effect of endotoxin in the airborne dust inflammatory response could be evaluated.

Dust/Alveolar Macrophage Interaction

Figure 1 contains scanning electron microscope images of lavage material from control (A and C) and airborne dust-exposed (B and D) animals 1 d post-IT. Clear differences in cell geometry were evident. The control cells were predominantly spherical, while the exposed cells showed more diversity in shape. Control AM were also rather evenly distributed. In the exposed sample, the cells were often clustered around particles. Both exposed and control sample AM showed surface ruffling.

Figure 2 provides a closer view of AM interacting with elongated airborne dust particles. Analysis of these fibers suggests that they are shreds

TABLE 1. Endotoxin Content of Airborne Dust and Soluble Fraction

Quantity of airborne dust extracted (mg)	Extraction volume (ml)	Soluble fraction (EU/ml)[a]	Airborne dust (EU/mg)[a]
40	5	89.38	11.17

Note. One endotoxin unit (EU) = 0.1 ng endotoxin.

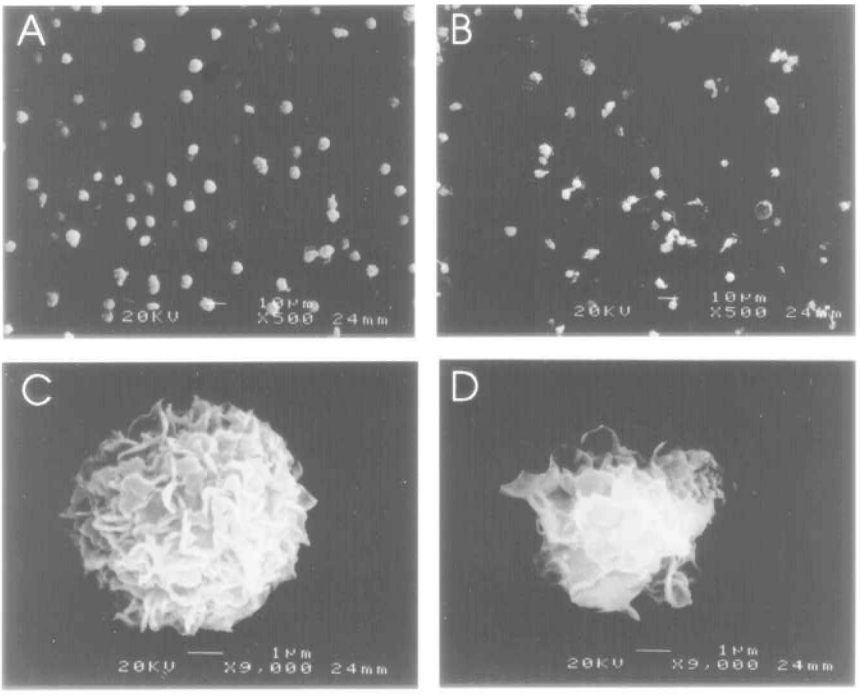

FIGURE 1. Scanning electron microscope images of lavage material from control (A and C) and airborne dust-exposed (B and D) animals at 1 d post-IT. Control cells tend to be evenly dispersed and spherical. Exposed cells are often clustered around particles and display more diverse geometry. Both show surface ruffing.

of nylon probably formed in the cutting/milling process at the plant. The association of these particles with AM indicates that the particles reached the lower region of the respiratory system after intratracheal instillation.

Figure 3 contains stereo pair scanning electron microscope images of lung sections from airborne dust-exposed animals at 1 d post-IT. Fibers with features consistent with the nylon shreds can be seen near the center of the photographs. Although stereo imaging is useful in resolving these particles from underlying material, note that shreds of cut lung tissue can be similar in appearance, making confirmation difficult.

Breathing Rates

To determine whether airborne dust caused a change in respiration, breathing rates were determined immediately prior to the IT administration of the airborne dust and immediately prior to sacrifice at 1 and 29 d after IT administration. Examination of the data demonstrates that only at 1 d post-IT was there a significant increase between the control rats and those that received airborne dust (Figure 4A). Subsequently, the effect of washed airborne and nylon tow dusts and the soluble fraction on breathing rates were determined and compared at 1 d post-IT. Airborne dust,

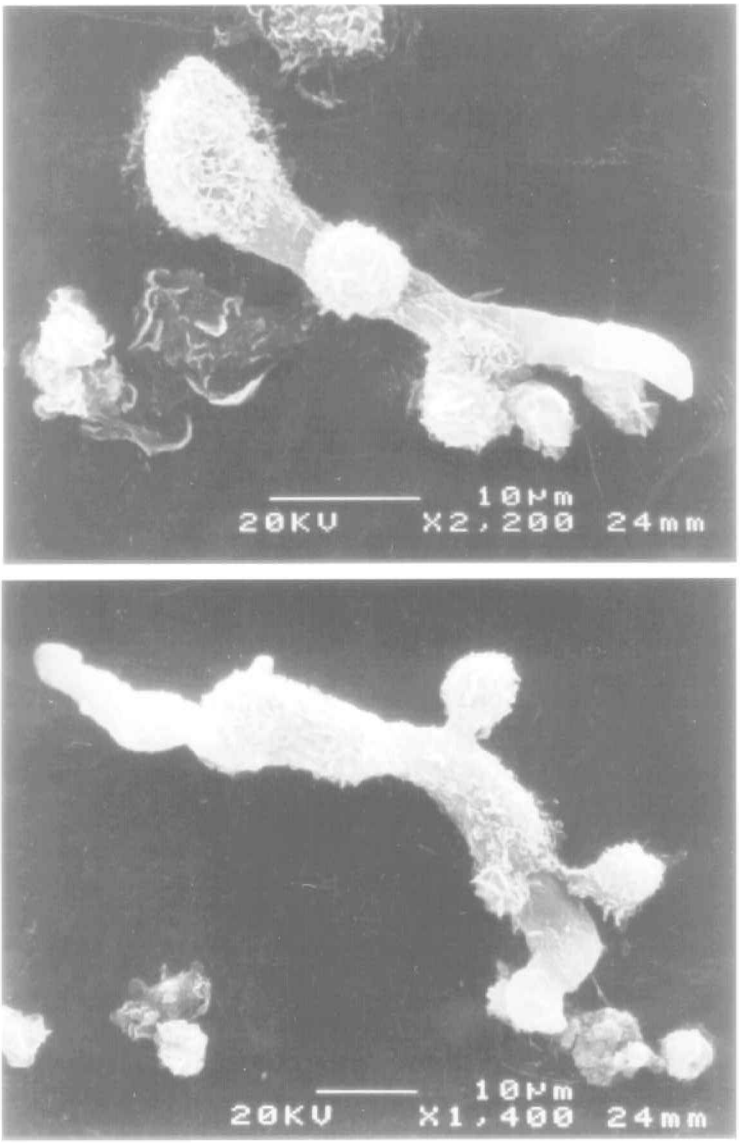

FIGURE 2. Scanning electron microscope images of lavage material of airborne dust-exposed animal showing AM interacting with elongated particles at 1 d post-IT. Analysis of these fibers suggests that they are shreds of nylon formed in the cutting/milling process at the flocking plant.

washed airborne dust, and nylon tow dust all caused a significant elevation in the breathing rates, but the soluble fraction did not significantly affect breathing rate (Figure 4B).

Cell Differentials

Initially, the effect of airborne dust on cellular differentials of the BALC was examined. The cellular differential data indicate that at 1 d

post-IT there was a significant increase in PMN harvested by bronchoalveolar lavage, but by 29 d post-IT the PMN levels had decreased to control levels (Figure 5A). There was no significant difference in AM cell yields at either 1 or 29 d post-IT. In later experiments, the effects of washed airborne and nylon tow dusts, and the soluble fraction on cellular differentials were determined at 1 d post-IT and compared to control. Airborne, washed airborne, and nylon tow dusts all caused a significant elevation in the PMN cell yields, but rats dosed with the soluble fraction did not have a significant increase in PMN cell yields (Figure 5B). AM cell yields were not significantly different from control for any of the treatments (Figure 5B).

AM Chemiluminescence

At 1 d post-IT, airborne dust caused a significant increase in AM CL versus control, but by 29 d post-IT the AM CL levels had decreased to control levels (Figure 6A). The effect of airborne, washed airborne, and

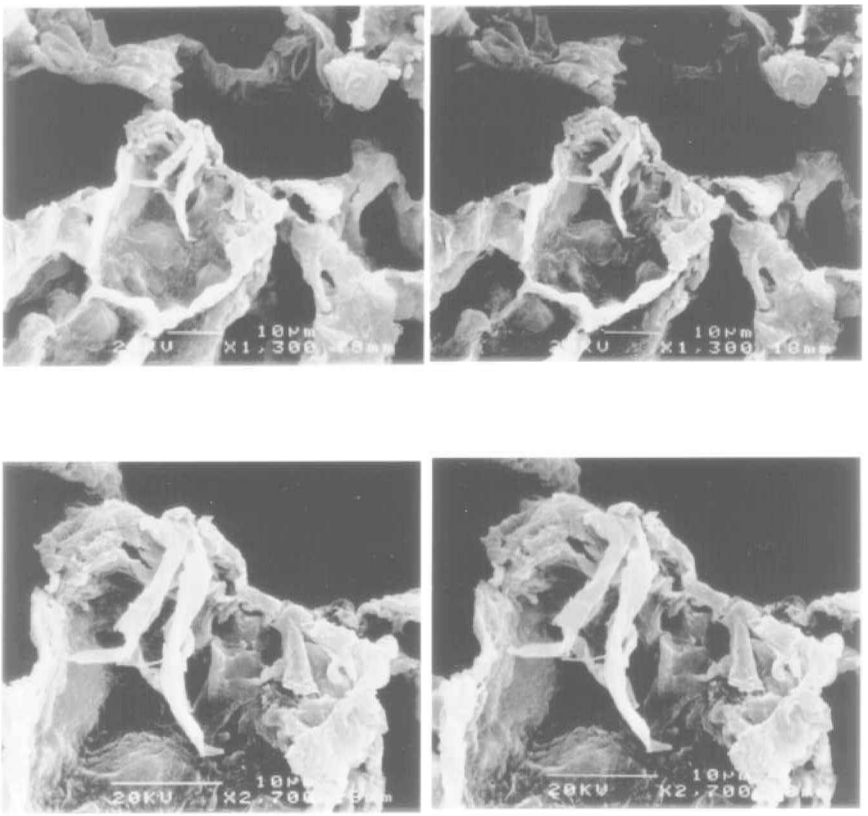

FIGURE 3. Stereo pair images at 2 magnifications of lung sections of airborne dust-exposed animals at 1 d post-IT showing fibers with features consistent with the nylon shreds observed in the plant environment (center of photograph). Although stereo imaging is useful in resolving particles from underlying material, shreds of cut tissue can have similar appearance, making confirmation difficult.

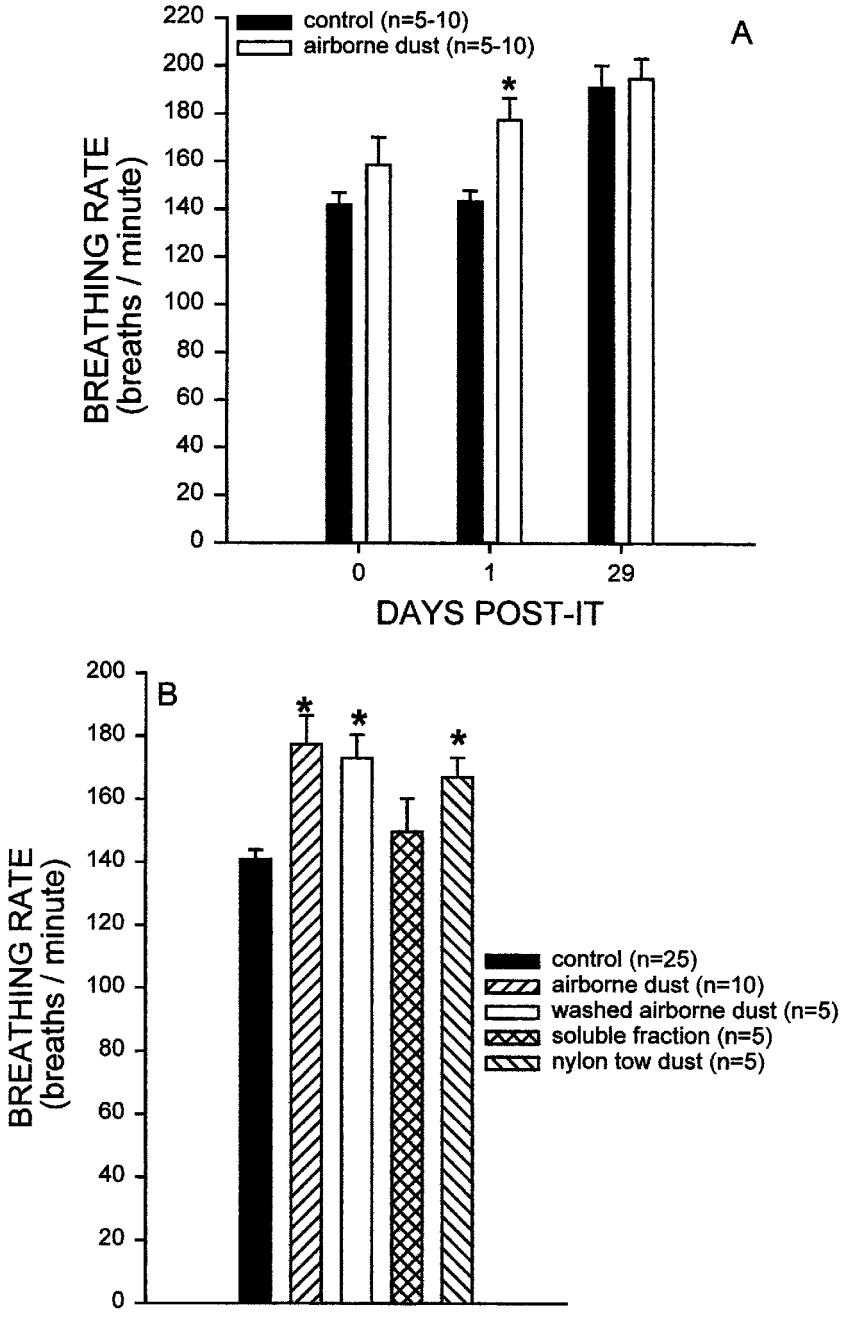

FIGURE 4. Rat breathing rates determined with flow plethysmograph. (A) Breathing rates of rats prior to (0 d) and at 1 and 29 d after administration of airborne dust compared to control. (B) Breathing rates of rats 1 d after administration of airborne dust, washed airborne dust, soluble fraction and nylon tow dust compared to control. An asterisk indicates a significant difference from control.

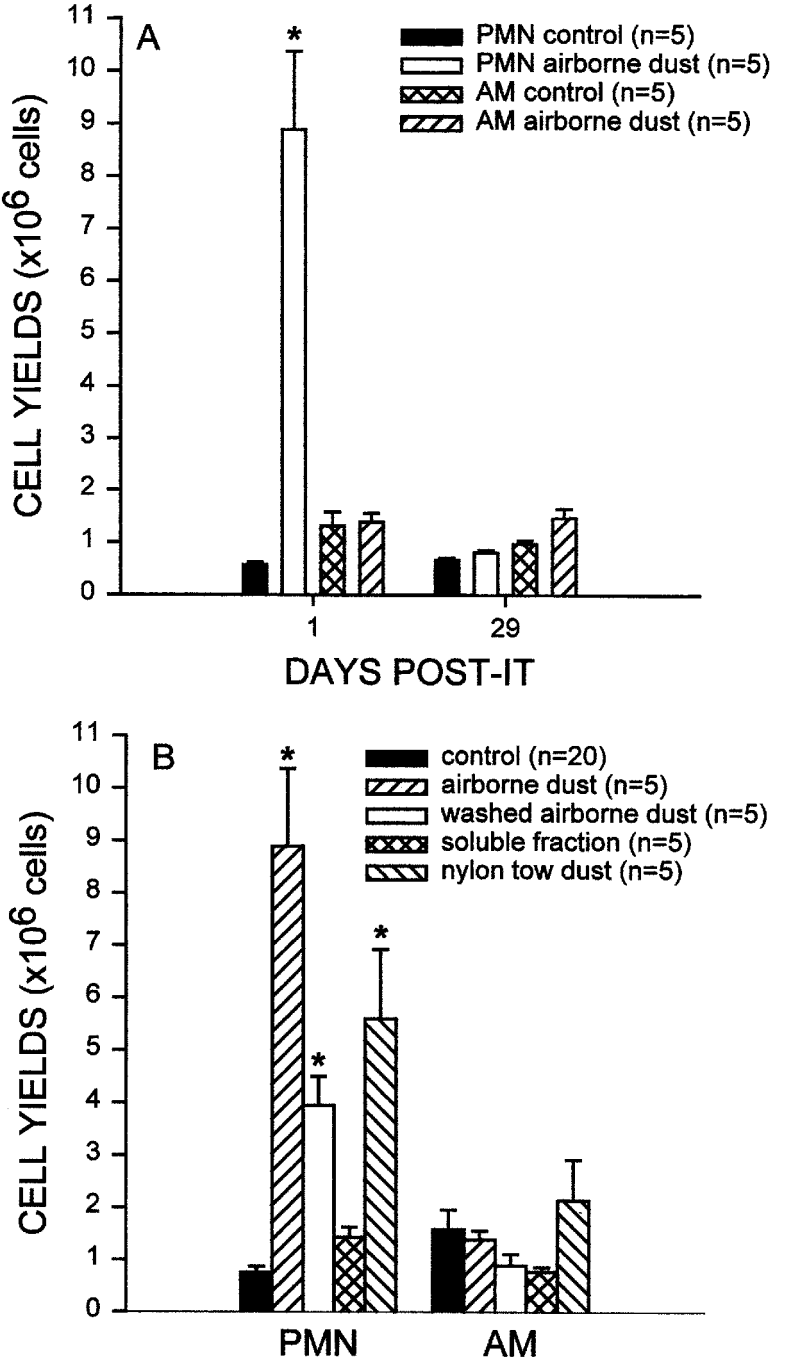

FIGURE 5. BALC differential cell counts. (A) Cell counts from rats at 1 and 29 d after administration of airborne dust compared to control. (B) Cell counts from rats 1 d after administration of airborne dust, washed airborne dust, soluble fraction, and nylon tow dust compared to control. An asterisk indicates a significant difference from control.

nylon tow dusts, as well as the soluble fraction, were next examined for their effects on AM CL at 1 d post-IT and compared to control levels. The data indicated that AM CL was significantly increased by all 3 dusts and the soluble fraction at 1 d post-IT (Figure 6B). However, AM activation in response to the soluble fraction was less dramatic than the other exposures.

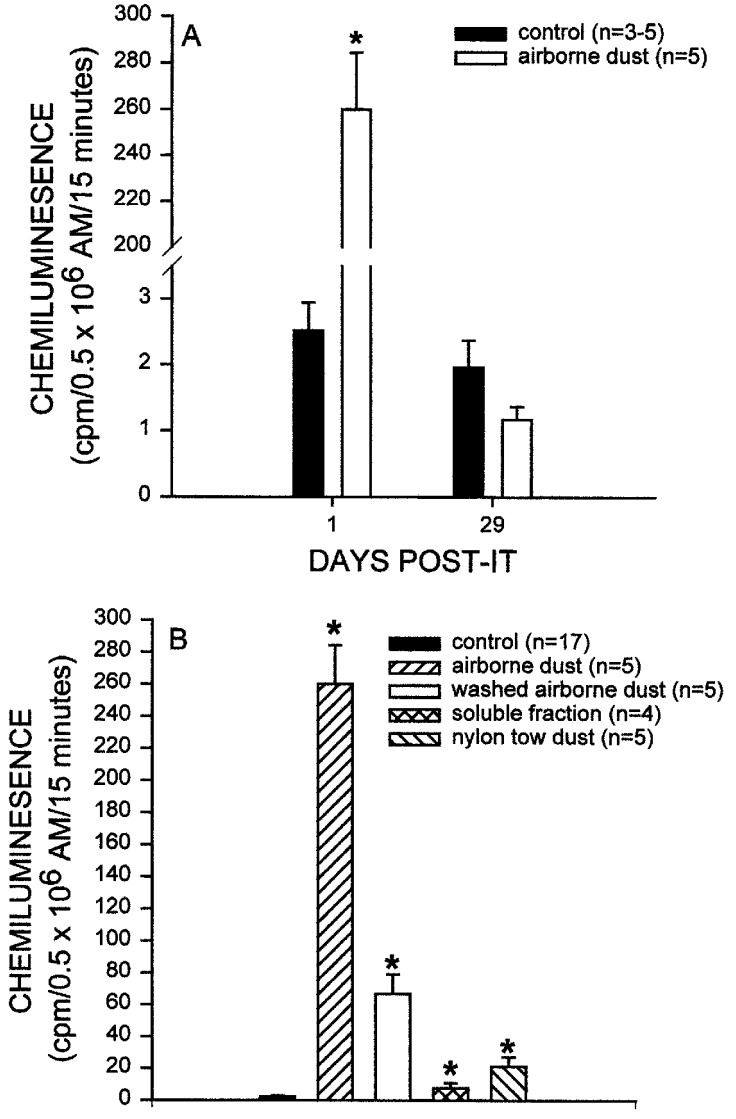

FIGURE 6. AM chemiluminescence. (A) Chemiluminescence from rats at 1 and 29 d after administration of airborne dust compared to control. (B) Chemiluminescence from rats 1 d after administration of airborne dust, washed airborne dust, soluble fraction, and nylon tow dust and compared to control. An asterisk indicates a significant difference from control.

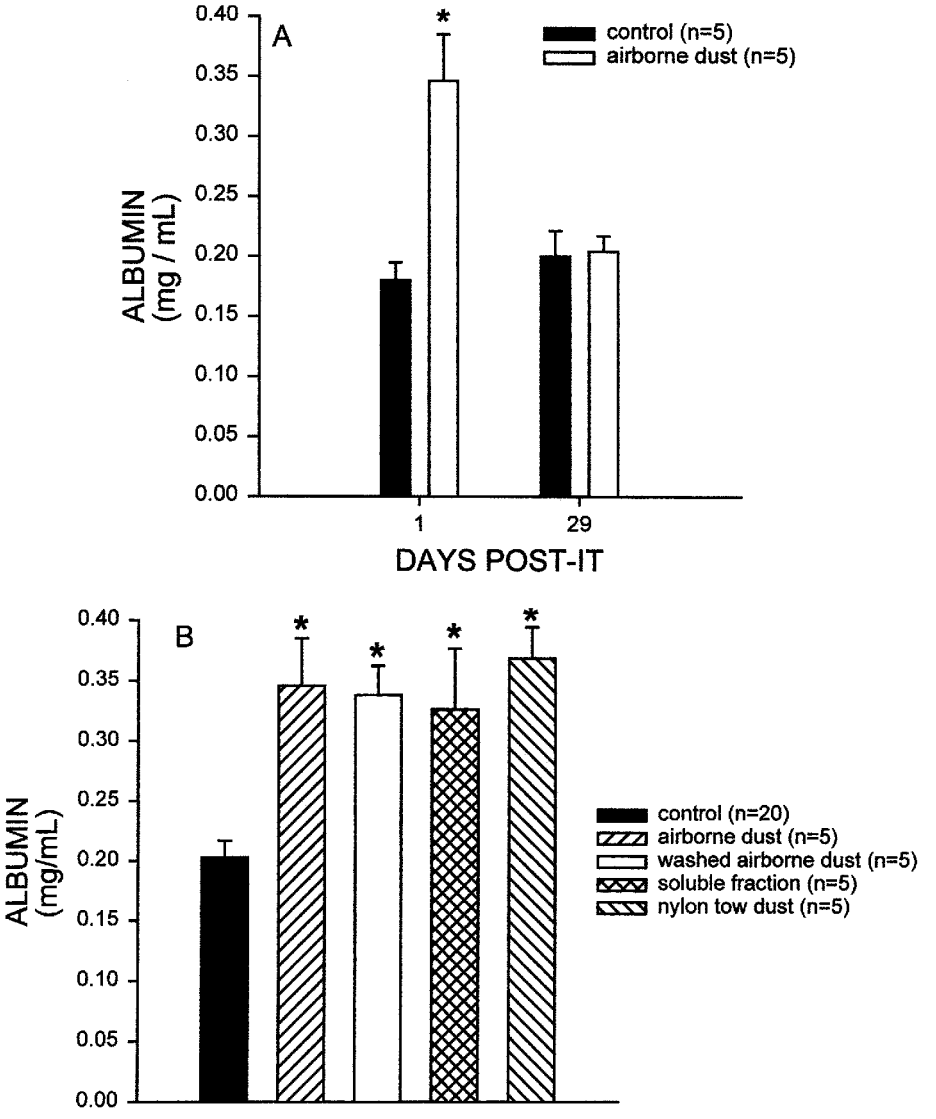

FIGURE 7. BALF albumin concentrations. (A) BALF albumin concentrations from rats at 1 and 29 d after administration of airborne dust compared to control. (B) BALF albumin concentrations 1 d after administration of airborne dust, washed airborne dust, soluble fraction, and nylon tow dust compared to control. An asterisk indicates a significant difference from control.

Albumin Concentration in BALF

Albumin that leaked into the lung from blood was measured in the BALF as a marker of a deterioration of the blood–gas barrier in the lung. In rats that were IT instilled with airborne dust, albumin concentrations in the BALF were significantly increased versus control at 1 d post-IT, but

declined to control levels by 29 d post-IT (Figure 7A). The effects of airborne, washed airborne, and nylon tow dusts, as well as of the soluble fraction, were also examined at 1 d post-IT. In comparison to control levels, all 3 dusts and soluble fraction caused a significant increase in albumin concentration 1 d post-IT (Figure 7B).

BALF Matrix Metalloprotease Activity

Results from analyses of MMP activity in acellular lavage fluid are given in Table 2. Exposure did not markedly affect endogenous levels of MMP. However, MMP activities of BALF samples maximally activated by limited proteolysis from animals given airborne, washed airborne, or nylon tow dust were significantly elevated above controls at 1 d post-IT. Maximal MMP activity returned to control levels by 29 d post-IT in BALF of rats exposed to airborne dust. The response to soluble fraction at 1 d post-IT was also significantly greater than control, although the magnitude of the reaction was somewhat lower than the response to the other particulate samples.

Lung Histopathology

Histopathologic assessment of rat lungs exposed to airborne dust at 1 d post-IT showed inflammation, consistent with BALC cell differentials. Mild to moderate, multifocal, suppurative pneumonia usually centered around bronchioles at 1 d post-IT was also observed. At 29 d post-IT, small foci of histiocytic alveolitis remained in all rats that had received airborne dust, and these foci were usually associated with birefringent fibers (Figure 8). Histiocytic inflammation was generally restricted to the alveoli near the fibers, with fiber length sometimes estimated in excess of 15 μm. No fibrosis was observed at either 1 or 29 d post-IT.

TABLE 2. MMP Activity in BALF

Group	Days post-IT	Endogenous MMP degradation of collagen (ng substrate/min/ml lavage)[a]	Maximally activated MMP degradation of collagen (ng substrate/ min/ml lavage)[b]
Control	1	0.036 ± 0.004	1.63 ± 0.08
Control	29	0.056 ± 0.010	1.64 ± 0.12
Airborne dust	1	0.058 ± 0.008	2.63 ± 0.26[c]
Airborne dust	29	0.049 ± 0.006	1.65 ± 0.07
Washed airborne dust	1	0.043 ± 0.010	2.87 ± 0.18[c]
Soluble fraction	1	0.048 ± 0.007	2.14 ± 0.17[c]
Nylon tow dust	1	0.038 ± 0.003	2.60 ± 0.22[c]

[a]Endogenous MMP levels from BALF without activation.

[b]Maximally activated MMP levels from BALF after limited proteolysis with trypsin to remove propeptide segment and expose the active site of the MMP.

[c]Significantly different ($p \leq .05$) from control group under Duncan's multiple comparison test ($\alpha > .05$).

FIGURE 8. Micrograph showing that at 29 d after IT administration of airborne dust the inflammation is histiocytic and restricted to the area around the retained fiber (indicated with arrow).

Similar histopathologic assessments were made at 1 d post-IT for washed airborne dust, soluble fraction, and nylon tow dust. The histological results were consistent with the BALC cell differentials, which indicated that airborne, washed airborne, and nylon tow dusts were the most inflammatory. Rats receiving soluble fraction were histologically indistinguishable from controls. Fibrosis was not associated with exposure to any of the three dusts or the soluble fraction.

DISCUSSION AND CONCLUSIONS

The primary objective of this investigation was to determine whether airborne dust, collected from an industrial plant, causes an inflammatory response in rat lungs. The experimental design of this investigation was to expose rats by IT instillation to a 10-mg/kg body weight dose of airborne dust and conduct toxicological studies and pathological examinations of lungs at 1 and 29 d post-IT. This dose of the airborne dust was chosen because other occupational dusts are known to cause inflammatory responses in rats at similar dosages. The two time points were selected to assess acute inflammation and its potential resolution.

Currently there is considerable debate in the field of respiratory toxicology concerning the appropriateness of IT instillation versus inhalation exposure. In the present study, the choice of exposure route was dictated by the limited quantities of thoracic respirable dust that could be practically collected by air sampling at the nylon flocking facility. Care was taken to sonicate the dust suspension before instillation to avoid aggregation of the nylon shreds. Histological evaluation indicates that bolus deposition was not a common phenomenon, since the deposited material appeared to be individual fiber-like shreds of nylon rather than aggregates of material. A recent study (Henderson et al., 1995) has demonstrated that carefully conducted IT instillation exposures can yield valuable mechanistic information that correlates well with results of inhalation exposure. We believe the present investigation has yielded important new information concerning the inflammatory potential of nylon fibers. However, an inhalation study would still provide interesting information concerning the effects of repeated exposures. For this reason, efforts have been made to identify an industrial process that would generate large quantities of nylon shreds for evaluation. Such a process has recently been identified and an inhalation study is being planned.

In terms of particle size, our approach was to collect and use elutriated dust for the instillations. Analysis of air samples at the flocking plant showed a wide variety of particle types and sizes (NIOSH, 1998; Burkhart et al., 1999). The largest particles observed in the air samples were the flock itself. These particles were nominally 1 mm long and 10–15 μm in diameter. Although these particles could deposit in the nose or mouth, because of their size they would not be expected to penetrate to the thoracic/

respirable regions of the lung. It was therefore deemed reasonable to attempt to exclude them from the samples used for intratracheal instillation. The vertical elutriator was originally designed for cotton dust sampling, and in a somewhat analogous way, the intent was to build a sampler that would exclude the larger cotton fibers and preferentially sample the smaller and more biologically relevant particles. Measurement of penetration characteristics of the elutriator has indicated an effective cut-off diameter of about 10 μm, which is similar to the defined curve that describes thoracic particulate mass. Thoracic sampling is intended for those materials that could be hazardous when deposited in the pulmonary conducting airways or gas exchange region. On the practical side, the elutriator provided a sufficiently high flow rate to obtain adequate mass for our experiments in a reasonable time, and was also less prone to clogging with flock, a problem experienced with some other samplers tried (NIOSH, 1998). Note that the separation of flock particles from the smaller components was not perfect, and one could occasionally detect flock fibers in the elutriated sample (Burkhart et al., 1999).

To measure the effect of airborne dust on pulmonary function, rat breathing rates were determined. Airborne dust caused a significant increase in breathing rate versus control at 1 d post-IT, but by 29 d post-IT there was no difference between control and airborne dust treatment groups. As a indicator of inflammation, PMN cell yield and AM CL were measured after IT administration of airborne dust. Both PMN cell yields and AM CL were significantly increased in rats that had received airborne dust versus control at 1 d post-IT, but no significant difference was observed for either parameter at 29 d post-IT. Albumin was measured in the BALF as a marker of a deterioration of the blood–gas barrier in the lung. Similar to the other toxicological measurements already described, at 1 d after IT administration of airborne dust the albumin concentration was significantly increased versus control, but by 29 d had decreased to control levels. Taken together, these data indicate that the airborne dust causes a significant acute inflammatory response in the lung accompanied by damage to the integrity of the lung blood–gas barrier.

Light microscopy indicated that following IT instillation, fibers present in the airborne dust can reach the gas exchange regions of the lung and interact with AM. In addition, fibers were still observed in the lungs at 29 d post-IT. Inflammation, assessed histologically at 29 d post-IT, no longer consisted of numerous disseminated foci. The inflammatory response consisted of a few small foci of predominantly histiocytic inflammation, usually restricted to the alveoli immediately around retained respirable fibers. The role of the persistent fibers, as well as the potential role of haptens and antigen presentation in challenge responses, remains to be investigated. However, this persistence may relate to the lymphocytic responses seen in exposed workers (Kern et al., 1998).

The nylon fibers, as well as chemical additives and biological conta-

minants, were suggested as possible causes of the inflammatory response induced by the IT administration of the airborne dust. To gain further insight into the role of additives and biological contaminants, a sample of airborne dust was extracted with water. The resulting dust sample, called washed airborne dust, was isolated by centrifugation and the water supernate, called the soluble fraction, was decanted and saved for further study. To investigate the toxicity of the nylon fibers themselves, nylon tow dust was prepared from the nylon material used at the beginning of the industrial process. The nylon tow dust had fiber properties similar to those observed in the airborne dust, but was not further treated or exposed to chemical or biological contaminants added during the flocking process and present in airborne dust samples collected at the plant. Thus, the nylon tow dust lacked the chemical additives and biological contaminants that the airborne dust contained as a result of industrial processing.

The experimental design of this component of our investigation was to expose rats by IT instillation to 10-mg/kg body weight doses of washed airborne dust or nylon tow dust and conduct toxicological studies and pathological examinations of the lungs. The dose of these two dusts, 10 mg/kg body weight, was equivalent to that used in the study of airborne dust and thus allowed the effects of these dusts to be compared. Toxicity of the soluble fraction was also examined. The dose of soluble fraction used, 1.3 ml/kg body weight, resulted in an endotoxin dose of approximately 112 EU/kg body weight, which was equivalent to the amount of endotoxin received from the 10 mg/kg body weight airborne dust exposure. These experiments differed from those conducted on airborne dust in that measurements were only made at 1 d post-IT. This was because the toxicological parameters being measured decreased to control values by 29 d post-IT in the airborne dust experiments, and limited amounts of the dust samples were available for study.

Similar to the observations for the airborne dust, rat breathing rates were significantly increased by washed airborne dust and nylon tow dust, but the soluble fraction did not significantly increase the rat breathing rate. PMN cell yield and AM CL were measured after IT administration of the dusts and soluble fraction as indicators of inflammation. PMN cell yields were significantly increased in rats that had received any of the three dusts, but the soluble fraction did not cause a significant increase in PMN cell yield versus control. AM cell yields from any of the treatment groups were not significantly different versus control. AM CL were significantly increased in rats that had received either dust or the soluble fraction versus control. Albumin was measured in the BALF to assess the deterioration of the blood–gas barrier in the lung caused by IT administration of the dusts and soluble fraction. All three dusts and the soluble fraction caused a significant increase in albumin in the BALF versus control.

These data indicate that the airborne dust, as well as the washed and nylon tow dusts, cause a significant inflammatory response in the lung and a reduction in the integrity of the lung blood–gas barrier. The observation that the washed airborne dust was inflammatory, but less than the airborne dust, does suggest that some additive(s) may contribute to the inflammatory response caused by the airborne (unwashed) dust. However, the soluble fraction does not always cause a significant inflammatory response, and thus the evidence for the role of the additives is not conclusively established by these studies.

In this study we measured the MMP activity of lung lavage because resident AM as well as inflammatory cells recruited into the lungs synthesize and secrete MMPs into the extracellular spaces (Horwitz & Crystal, 1976). MMPs are zinc-containing enzymes that degrade extracellular matrix proteins such as fibrillar and basement membrane collagens. Elevation of MMP activity has been shown to occur both in acute lung inflammation (Partridge et al., 1993) and in lung remodeling due to fibrosis (Denholm & Rollins, 1993). In this study we found that lung exposure to the various dust samples produced significant elevation of lung MMP levels at 1 d post-IT, and this is consistent with the significant elevations in other indicators of acute lung inflammation determined in this study. However, at 29 d post-IT, MMP activity of lung lavage fluid was not significantly different from controls.

In order to further evaluate the inflammatory response to the dusts and soluble fraction of the airborne dust, the results of this study were compared with those obtained in a previous study from our laboratory of other occupational dusts (Blackford et al., 1997). These data are presented in Table 3. The comparisons demonstrate that all three dusts examined in this study cause comparable or higher inflammatory responses, as indicated by PMN recruitment and AM CL, than those of other well-character-

TABLE 3. Comparison of Nylon Fiber Inflammatory Response to Other Known Inflammatory Dusts 1 d After Intratracheal Instillation

	Percent control	
Inflammatory agent	PMN cell yield	Zymosan-stimulated chemiluminescence
Airborne dust	1,182	11,441
Washed airborne dust	524	2,939
Nylon tow dust	743	925
Soluble fraction	190	340
Silica[a]	320	280
Coal[a]	60	120
TiO_2[a]	60	80
Carbonyl iron[a]	40	40

[a]Adapted from Blackford et al. (1997). Normalized to a 10-mg/kg dose assuming a linear dose response.

ized occupational dusts, such as coal mine dust and silica, which are known to cause interstitial lung disease.

The cases of chronic interstitial lung disease reported in workers at a nylon flocking plant were characterized pathologically as lymphocytic bronchiolitis (Kern et al., 1998). However, the pulmonary reaction of IT instilled rats was an acute inflammatory reaction characterized by PMN infiltration that resolved by 29 d post-IT. Considering the differences between the worker outcome and the rat response, what is the significance of the present study to the workplace health problem? First, the initial assumptions by plant officials was that airborne particulate nylon flock fibers were too thick to be respirable and that nylon was a biologically inert material. The present study proves that inflammatory nylon shreds are generated in the workplace. This information has been vital for recommending preventive measures. Second, although BAL parameters gave no indication of widespread inflammation in rats at 29 d post-IT, histological evaluation did indicate focal histiocytic alveolitis. It is possible that repeated exposures could result in a locus of deposition that would evolve into a site for chronic inflammation. Third, it is likely that repeated exposures would be required to induce a chronic lymphocytic reaction in the animal model. As previously stated, efforts have been made to identify an industrial process that would generate large quantities of nylon shreds for evaluation. Such a process has recently been identified and an inhalation study is being planned.

The most significant observations made in these studies are that: (1) nylon flock processing generates particles of respirable size that can interact with AM in the lung, (2) airborne dust collected at the plant is inflammatory and remains in the lung for at least 29 d after IT exposure, (3) nylon tow dust produced in the laboratory is also inflammatory, (4) water-extractable agent(s) contribute only minimally to the inflammatory response, and (5) the acute inflammatory response to the nylon tow dust is higher than that observed for other occupational dusts with well-documented deleterious effects on human health.

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