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ASSESSMENTS OF PULMONARY MACROPHAGE CLEARANCE RESPONSES TO INHALED PARTICULATES

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Abstract

An integrated bioassay program is being developed to evaluate the toxicity of inhaled particulate materials. The multi-disciplined approach combines studies on lung clearance mechanisms with pulmonary macrophage functional assessments based on cellular biology, biochemical and cytochemical evaluations on lung specimens from exposed animals. To validate this method, animals were exposed to asbestos, iron-treated asbestos, fiberglass, Mt. St. Helens ash or carbonyl iron particles. Deposition patterns, macrophage migration and phagocytosis were monitored in vivo at selected time periods after exposure. Our results showed that chemotactic factor generation by particles in vitro correlated with the corresponding macrophage recruitment responses in vivo. In addition, macrophage morphologic and functional characteristics were evaluated following exposures to aerosolized dusts. Our results suggest that scanning electron microscopy (SEM) techniques for investigating particle deposition and macrophage clearance provide an important component for evaluating the toxicity of inhaled particulate materials.

Key Words: Pulmonary Macrophage Phagocytosis, Macrophage Phagocytosis, Pulmonary Macrophages, Lung Macrophages, Lung Clearance, Inhaled Particles, Particle Clearance, Mononuclear Phagocytes, Macrophage Clearance

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Introduction

Occupational and environmental exposure to a variety of inhaled materials has been associated with the development of pulmonary disorders (12). One of the major goals for inhalation toxicologists is to prevent the occurrence of lung diseases by identifying materials whose exposure might be harmful to humans. In pursuing this goal, we are developing a biological screening program which is designed to assess the toxicity of inhaled particles using cellular biology, biochemical, physiological and morphological endpoints. One important component of this multidisciplined approach is the use of scanning electron microscopy to evaluate lung deposition patterns and macrophage clearance mechanisms (i.e., removal of inhaled particles from the distal lung by pulmonary macrophages) following exposure to aerosolized particulates.

Scanning electron microscopic (SEM) techniques are useful in evaluating pulmonary macrophage clearance functions, both in cell culture preparations as well as in the lungs of exposed animals. Since pulmonary macrophages are the cell-types which form the first line of defense in maintaining the sterility of the lung (1), studies of macrophage morphology, chemotaxis and phagocytosis are essential to an understanding of the clearance of inhaled particles. Accordingly, in this brief review, we will demonstrate how SEM can be utilized to assess particle deposition characteristics and macrophage clearance from the distal lung of exposed animals. This information, in combination with measurements of lung biomarkers such as enzyme and protein analyses, can be used to evaluate the lung responses to a number of inhaled materials.

Materials and Methods

Pulmonary Lavage

Male CD ® rats (strain Crl:CD ® BR, Charles River Breeding Labs, Wilmington, MA), 8-10 weeks old, were anesthetized by intraperitoneal injections of sodium pentobarbital (Nembutal ®). The trachea was isolated by blunt dissection and clamped. A small incision

was made two rings below the larynx, and a modified 19-gauge butterfly catheter was tied into place. The lungs were infused with Ca^{++}-Mg^{++} free, warmed (37° C) phosphate buffered saline solution from a reservoir located 10 cm above the animal's thorax (17). The fluid flowed freely into the lungs by gravity. When fluid flow was stopped naturally, the cannulus was dislodged from the reservoir and placed 10 cm below the thorax to collect fluid and lavaged cells from the animal. This broncho-alveolar lavage (BAL) procedure was carried out six times for each animal.

Fluid recovered by BAL was centrifuged (250 x g for 10 minutes) and the supernatant was removed. The cell pellet was resuspended in Eagles Minimal Essential Medium (Eagles MEM F-11, GIBCO, Grand Island, NY) supplemented with penicillin and streptomycin. Cell numbers and viability were counted using a hemocytometer and trypan blue solution. Cell differentials were carried out on Diff-Quik ® (American Scientific Products, Chicago, IL) stained cytocentrifuge preparations.

Preparation of Monolayers for Scanning Electron Microscopy

After an additional wash, a suspension of macrophages containing 2 x 10^5 cells was plated on Thermanox ® coverslips (Lux-Miles Scientific, Naperville, IL) placed inside Falcon culture dishes. The cells were incubated for 45 min in a CO_2 incubator (5%CO_2-95% humidified room air) at 37° C. Subsequently, the monolayers were rinsed vigorously in Eagles MEM to remove nonadherent cells and then either immersed in Karnovsky's fixative or processed for phagocytosis studies. Results of a pilot study showed that the plating efficiency was greater than 90%, as previously reported (18).

Following completion of fixation, coverslips were rinsed in a cacodylate-sucrose solution and dehydrated through a series of graded ethanol steps. Samples were critical-point-dried using carbon dioxide as the transitional fluid. Coverslips were mounted onto carbon stubs, gold-coated and studied in a JEOL JXA-35 scanning electron microscope.

SEM Analysis of Macrophage Morphology and Quantitation of Phagocytosis

The morphology and phagocytic capacities of pulmonary macrophages lavaged from the lungs of sham and dust-exposed rats were studied using scanning electron microscopy of randomly selected cultured cells. This analysis was implemented by selecting a field of view at low magnification to avoid observer bias. Then each cell in the field of view was analyzed at higher magnification. The characteristic surface morphology of each cell was recorded. Cells were characterized according to their ruffled membranes or unruffled, smooth surface characteristics. The surfaces of smooth macrophages were devoid of ruffled membranes, but frequently contained blebs or other membrane defects.

The phagocytic capacities of macrophages exposed to chrysotile asbestos or carbonyl iron particles were assessed using X-ray energy spectroscopy (see below) or backscattered imaging, respectively. To evaluate iron phagocytosis, the numbers of interiorized carbonyl iron beads were counted in individual macrophages using secondary and backscattered electron imaging modes (16). Virtually all of the phagocytic macrophages contained 1-5 beads, and the phagocytic rate was represented as percentages of phagocytic macrophages. As previously described, iron beads on the cell surface were easily delineated from internalized beads by their electron density and discrete shape.

Chemotaxis

In earlier reports we demonstrated that asbestos fibers interact with serum or lavaged proteins to generate complement-dependent chemotactic factors (19,20). Similarly, in the current study, pulmonary macrophage chemotaxis was used as a bioassay for serum complement activation as previously described (19,20). Briefly, the serum was separated from whole blood of rats. Pulmonary macrophages from unexposed rats were lavaged using phosphate buffered saline. Chrysotile asbestos fibers were obtained from the Jeffrey Mine in Quebec, Canada, and have been well characterized (14). Some chrysotile asbestos fibers were treated with ammonium ferrous sulfate according to the method of Flowers (7). The glass fibers (Code 100) were provided by Dr. James Leineweber, Johns Manville Co., Denver, CO (11). Ash from the Mt. St. Helens volcano eruption (May 1980) was obtained from the Environmental Protection Agency and is a heterogeneous mix of mineral particles. The major minerals in the ash were feldspar and orthopyroxene (8). The ash was utilized as a negative control since incubation of these particles with serum caused no significant complement activation (13,19,23). Normal heated sera (37°C for 45 minutes) and decomplemented sera (56° C for 30 minutes) incubated with chrysotile asbestos were used as additional negative controls.

Fresh serum was incubated with iron-treated chrysotile asbestos fibers, glass fibers, or Mt. St. Helens ash particles. Particles were incubated with sera at a concentration of 25 mg/ml. The sera-particle suspensions were incubated in 40 ml conical tubes for 45 minutes in a shaking water bath maintained at 37° C and subsequently decomplemented (56 °C for 30 min.) prior to removal of particles by centrifugation (250 x g for 10 minutes).

The chemotactic response of pulmonary macrophages was assessed using polycarbonate filters inserted into blind well chambers (15). The cells were plated in the upper portion of the chamber and incubated for 3.5 hours. Following termination of the assay, the filters (pore size = 5 μm) were fixed with ethanol and stained for light microscopy. To quantify macrophage migration, the numbers of macrophages which had migrated from the top to the opposite side of polycarbonate filters were counted in 20 predetermined high power fields.

Inhalation Studies

Animals were exposed to aerosolized chrysotile asbestos fibers, iron-treated chrysotile asbestos fibers, glass fibers, carbonyl iron particles, or Mt. St. Helens ash particles at concentrations ranging from 10 - 20 mg/m^3 total mass for 1, 3 or 5 hours in whole body inhalation chambers as previously described (17). Control rats were similarly sham-exposed to room air.

Immediately after cessation of exposure, particle deposition patterns for carbonyl iron, asbestos, iron-treated asbestos, fiberglass and volcanic ash exposed animals were investigated. Twenty-four or 48 hours after exposure to the dust aerosol, the animals were anesthetized with a lethal dose of pentobarbital and the lungs were infused with Karnovsky's fixative (i.e., 1% paraformaldehyde, 1% glutaraldehyde) through the trachea at a pressure of 15 cm of water. The fixed lung tissue was dissected and prepared for scanning electron microscopy as previously described (3,17). The numbers of macrophages which had migrated to sites of particle deposition (i.e., alveolar duct bifurcations) were recorded in both dust-exposed and sham-exposed rats. All bifurcation surfaces revealed by random dissection of critical-point-dried lung tissue were evaluated as previously described (17).

X-ray Energy Spectrometry of Cultured Macrophages

The percentages of chrysotile asbestos-containing macrophages in exposed, lavaged rats were determined by using scanning electron microscopy (at 20 kV) in conjunction with X-ray energy spectrometry (XES) (6,17). Individual cells were magnified to fill the viewing screen at x 6000. Each macrophage then was probed using the "spot" mode, i.e, a stationary beam of about 0.1 μm diameter. The beam was scanned across the cell surface in 5 to 6 sweeps while monitoring the number of specific X-ray counts generated per second for magnesium and silicon, the major elemental components of chrysotile asbestos (10). When a count rate higher than background was recognized, the beam was left stationary at this point on the cell, and the elemental peaks characteristic for Mg and Si were generated for 100 seconds on a cathode ray tube. Using X-ray energy microanalytical techniques, the numbers of characteristic X-rays collected in 100 sec were recorded by computer for the 2 elements. A ratio was then calculated by dividing the numbers of X-ray counts achieved for Mg by the counts for Si. Nonspecific X-ray counts (background) were automatically subtracted through computer programming. As a control, more than 150 cells from unexposed animals were analyzed with a range of 200 - 3540 counts. The range of counts for "naked" chrysotile asbestos fibers and macrophages containing asbestos were 6570 - 11400 and 5250 - 8760, respectively (mean = 7250 $\pm$ 2400). As a result, a minimum sum of 5000 counts, concomitant with peaks for Mg and Si were used as criteria for the identification of asbestos-containing pulmonary macrophages.

Results

Macrophage Morphology and Phagocytosis

Pulmonary macrophage morphology was evaluated by SEM according to surface characteristics. The majority of cells exhibited surface ruffles while the remaining macrophages exhibited smooth surfaces which generally were devoid of surface membrane projections. A representative macrophage monolayer is illustrated in Figure 1. Analysis of pulmonary macrophage phagocytosis of carbonyl iron spheres was carried out using both light microscopy (LM) and SEM. When the secondary and backscatter electron images were juxtaposed on the cathode ray tube of the SEM, the interiorized beads could easily be delineated from beads adhering to the macrophage surface (Figs. 2A and B, Table 1A). The data presented in Figure 3 demonstrates that analysis of *in vivo* macrophage phagocytosis by SEM was more accurate in evaluating particle phagocytosis when compared with light microscopy techniques. The two-dimensional LM technique tends to overestimate the numbers of phagocytic cells.

An X-ray microanalytic technique was utilized to quantify the percentages of lavage-recovered pulmonary macrophages containing chrysotile asbestos fibers at 0, 24, and 48 hrs after a 5 hr inhalation exposure to asbestos (Fig. 4, Table 1B). Immediately after the 5 hr exposure, 21% of the exposed population of recovered cells contained fibers and 38% of macrophages had phagocytized fibers 24 hrs after exposure. Analysis of macrophage phagocytosis by light microscopy resulted in an underestimate of the numbers of phagocytic cells (Table 1).

Chemotaxis

Addition of fiberglass, iron-treated chrysotile asbestos fibers, or carbonyl iron spheres to rat serum produced an enhanced pulmonary macrophage chemotactic response when compared with serum incubated with Mt. St. Helens ash particles (Table 2). Normal heated sera and sera decomplemented prior to incubation with chrysotile asbestos fibers provided additional controls. In another experiment, SEM was utilized to assess the *in vivo* phagocytic capacity of chemotactically active macrophages lavaged from the lungs of carbonyl iron exposed rats (data not presented). Several macrophages containing CI particles were observed in the secondary and backscatter electron image modes of the SEM (Fig. 5A and B).

Particle Deposition and Macrophage Accumulation In Vivo

SEM was utilized to assess particle deposition patterns and corresponding pulmonary macrophage accumulation at alveolar duct bifurcations following brief exposures to fiberglass, asbestos, iron-treated chrysotile asbestos, carbonyl iron and Mt. St. Helens ash. Our results demonstrated that these inhaled particulates, regardless of shape or chemical

TABLE 1A.
MORPHOLOGY AND IN VITRO PHAGOCYTIC CAPACITIES OF SHAM AND ASBESTOS EXPOSED PULMONARY MACROPHAGES

N	EXPOSURE	% RUFF MACS.	% PHAG MACS.
3	SHAM/48	78 ± 5	72 ± 4
3	ASB 1/48	66 ± 3	61 ± 8
3	ASB 5/48	64 ± 2	54 ± 5

% RUFF MACS. = % of ruffled macrophages.
% PHAG MACS. = % of phagocytic macrophages.
SHAM/48 = Sham exposure to room air and 48 h recovery period.
ASB 1/48 = 1 h exposure to asbestos and 48 h recovery period.
ASB 5/48 = 5 h exposure to asbestos and 48 h recovery period.

TABLE 1B.
PERCENTAGES OF ASBESTOS-CONTAINING PULMONARY MACROPHAGES

N	Technique	5-h/0-h Mean Percentage
2	LM*	15.2 ± 7
2	SEM-XES**	21.4 ± 2
		5-h/24-h
3	LM	29.5 ± 6
2	SEM-XES	37.8 ± 6
		5-h/48-h
3	LM	27.0 ± 8
2	SEM-XES	33.5 ± 3

5-h/0-h = 5 hrs. exposure and 0 recovery
5-h/24-h = 5 hr exposure and 24 hr recovery
5-h/48-h = 5 hr exposure and 48 hr recovery
* Light microscopy of several hundred lavaged cells
**SEM-X-ray energy spectrometry

composition, were deposited primarily at alveolar duct bifurcations, thus confirming the data of Brody and colleagues (4,5).

Following a 3 hr exposure, the majority of carbonyl iron spheres in the distal lung were located on alveolar duct bifurcations and adjacent duct surfaces in exposed rats (Figs. 6A and B). Within 24 hours, pulmonary macrophages had accumulated at sites of fiber/particle deposition in the lungs of fiberglass, iron-treated chrysotile and carbonyl iron exposed rats (Fig. 7, Table 3). In contrast, macrophages did not migrate to sites of particle deposition in animals exposed to aerosolized Mt. St. Helens ash, and this correlated with the inability of the volcanic ash to activate complement *in vitro* (Table 2). After phagocytizing iron particles on bifurcation surfaces (Fig. 8), pulmonary macrophages were observed on terminal bronchiolar surfaces, presumably exiting the distal lung via the bronchiolar mucociliary escalator (Figs. 9 and 10).

Discussion

Pulmonary macrophages play an important role in maintaining the sterility of the lung by phagocytizing and removing inhaled particles and bacteria. Phagocyte motility and endocytic capacities are prerequisites for efficient clearance of inhaled materials. The mechanism(s) through which macrophages identify inhaled particles on alveolar surfaces has not been well understood. Recently, we proposed that a variety of particles which activate complement *in vitro* and deposit preferentially at alveolar duct bifurcations will produce complement-derived chemoattractants *in vivo* after inhalation exposure. The generation of these factors could serve to recruit local macrophages to sites of particle deposition and thus, facilitate clearance of the inhaled particles or fibers. Here and elsewhere (21) we have tested a number of particulates including fiberglass, asbestos, iron-treated chrysotile asbestos, iron particles and Mt. St. Helens ash for generation of chemotactic factors. Using pulmonary macrophage chemotaxis as a bioassay for complement activation, we demonstrated that, with the exception of the volcanic ash, all of the particulates tested activated complement *in vitro*. In addition, we have exposed rats to aerosols of the particle types listed above and quantified macrophage accumulation using SEM. Without exception, all particles which activated complement *in vitro* produced pulmonary macrophage accumulation at sites of particle deposition *in vivo*.

Macrophage morphology and phagocytosis are important components of the lung's clearance reponse. Previously, we demonstrated that cellular morphological features correlated with functional potential of the cells, i.e., ruffled macrophages had a greater phagocytic and chemotactic activity in comparison to unruffled, smooth cells (18). Cell shape and surface morphology have been considered by few

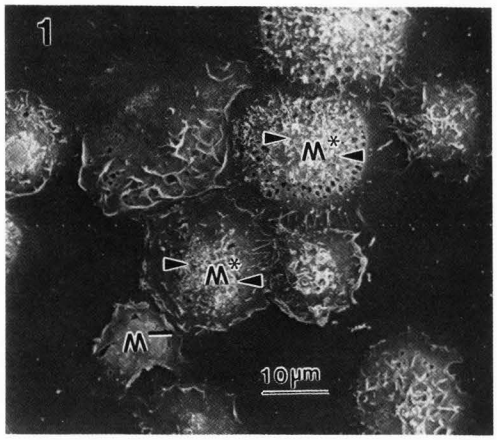
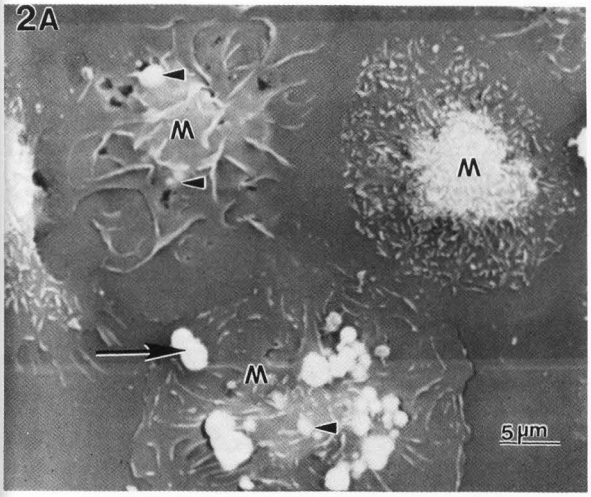
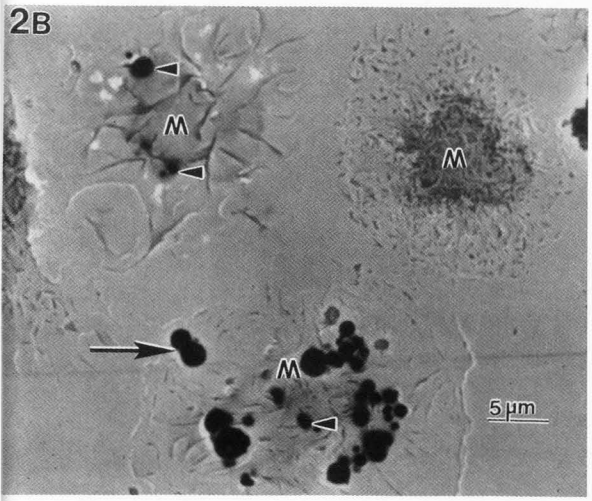


Figure 1. Scanning electron microscopy (SEM) of lavage recovered rat pulmonary macrophages (M) cultured on Thermanox cover slips for 45 minutes. Ruffled macrophages (M*) are characterized by ruffled membranes (arrowheads). Smooth macrophages (M-) can also be observed.





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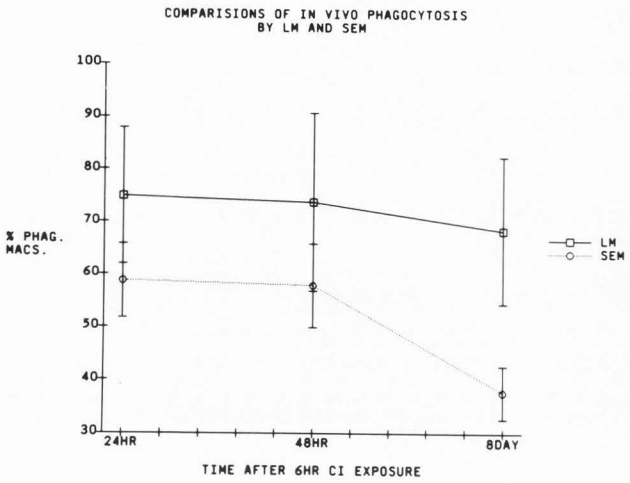


Figure 3. Assessments of macrophage phagocytosis of inhaled carbonyl iron particles by scanning and light microscopy (LM).
The LM technique tends to overestimate the numbers of phagocytic cells, since one is unable to discriminate between adsorbed and interiorized particles using this 2 dimensional method.

Figure 2A and B. SEM of pulmonary macrophage (M) phagocytosis of carbonyl iron particles in secondary (A) and backscatter electron images (B). The interiorized beads (arrowheads) can easily be distinguished from beads lying adjacent to the cell surface (arrow).

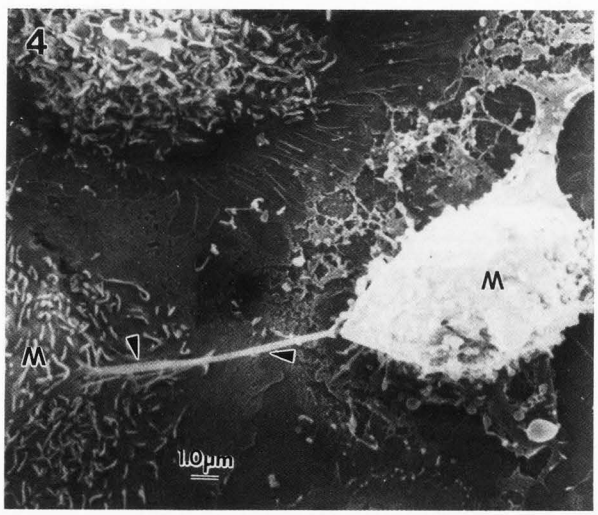
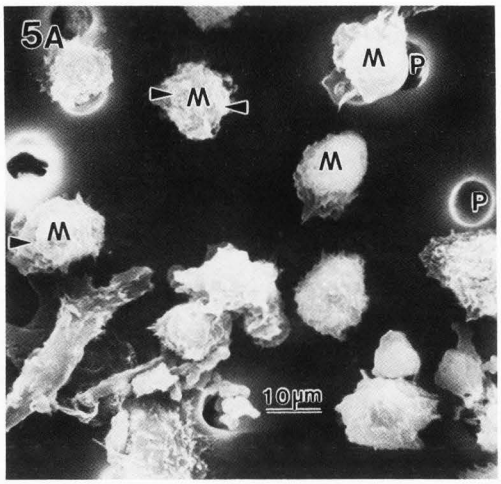


Figure 4. Scanning electron image of 2 pulmonary macrophages (M) sharing a chrysotile asbestos fiber (arrowheads). Cells were cultured for 45 min on Thermanox coverslips.



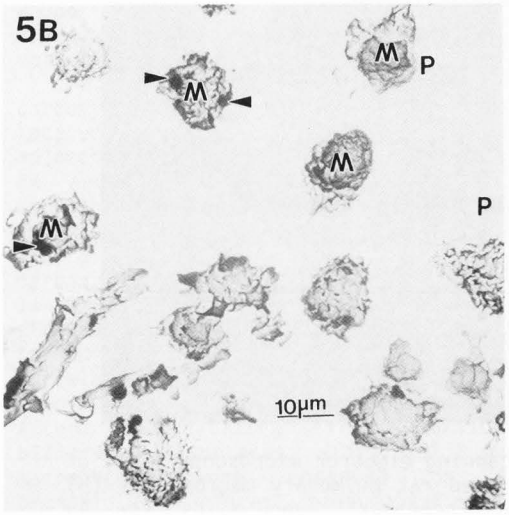
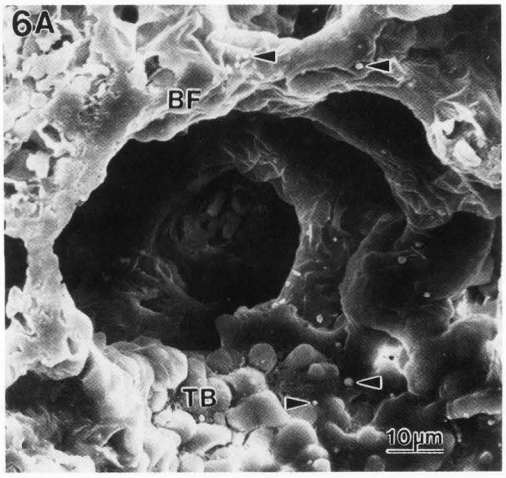


Figure 5A and B. Scanning electron micrographs of lavage recovered, carbonyl iron exposed macrophages (M) migrating across polycarbonate filters following introduction of a chemotactic stimulus. Cells are viewed in secondary (A) and backscatter (B) electron images of the SEM.



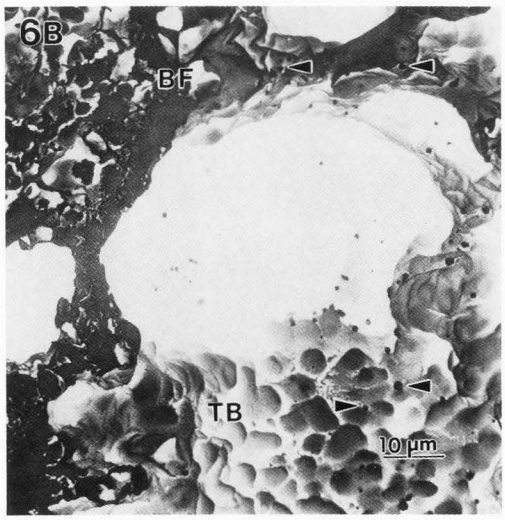


Figure 6A and B. Scanning electron micrograph (SEM) of critical point-dried lung tissue which reveals a terminal bronchiole (TB) and connecting alveolar ducts in a rat killed immediately after carbonyl iron exposure. The secondary (A) and backscatter (B) images reveal carbonyl iron particles (arrowheads) which have deposited on bronchiolar and bifurcation surfaces (BF). Note that several spheres not easily discerned by secondary imaging are readily observed in the backscatter mode.

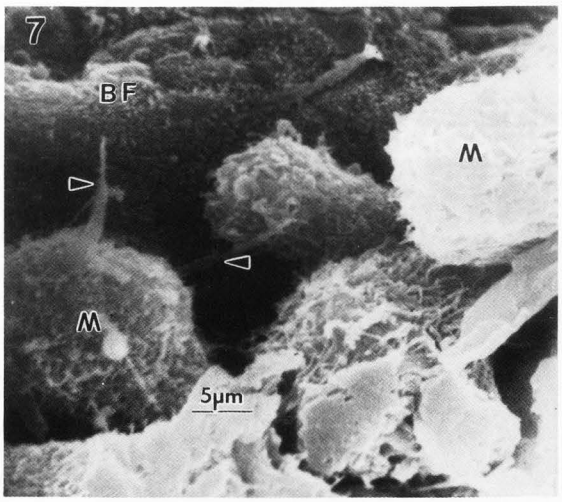


Figure 7. Pulmonary macrophages (M) have migrated to sites of asbestos fiber (arrowhead) deposition in a rat exposed to asbestos for 3 hrs.

TABLE 2.
MACROPHAGE CHEMOTACTIC RESPONSE TO FIBERGLASS AND FE-CHRYSOTILE ACTIVATED RAT SERA.

PARTICLE	N	1% ARS	5% ARS	10% ARS
FIBERGLASS	6	63.0 ± 7.8	155.3 ± 39.7*	277.3 ± 17.1*
FE-CHRYSOTILE ASBESTOS	6	40.7 ± 8.5	175.3 ± 43.5*	257.3 ± 10.1*
MT. ST. HELENS ASH	6	37.7 ± 12.3	55.0 ± 17.1	82.1 ± 29.1
NORMAL HEATED SERA	6	30.2 ± 15	62.5 ± 15.4	90.3 ± 13.6
DECOMPLEMENTED SERA + ASBESTOS	6	28.0 ± 17.1	39.3 ± 3.1	51.3 ± 17.5

*P<0.01 when compared with Mt. St. Helens activated, Normal heated, or Decomp. sera. (Student's t test).

TABLE 3.
PULMONARY MACROPHAGE ACCUMULATION IN DUST AND SHAM-EXPOSED RATS.

Exposure Group	N	Bifurcations Examined	Mean ± S.D.
SHAM 3/0	5	22	0.2 ± 0.4
SHAM 3/24	5	28	0.3 ± 0.6
SHAM 3/48	8	43	0.2 ± 0.3
FG 3/24	3	11	1.4 ± 0.8#
FG 3/48	3	6	2.2 ± 1.6#
C.I. 3/0	3	18	0.5 ± 0.7
C.I. 3/24	4	30	0.9 ± 0.6*
C.I. 3/48	4	25	1.5 ± 0.9#
MSH. 3/0	5	29	0.2 ± 0.4
MSH. 3/24	5	36	0.3 ± 0.4
MSH. 3/48	4	25	0.2 ± 0.5
CHRYS. ASB. 3/48	6	37	4.1 ± 2.6#
FE-CHRYS 1/48	3	10	2.8 ± 1.8#

*p<0.05 when compared with Mt. St. Helens or sham-exposed rats.
#p<0.01 when compared with Mt. St. Helens or sham-exposed rats.
CHRYS ASB. = CHRYSOTILE ASBESTOS FIBERS
FE-CHRYS. = IRON-TREATED CHRYSOTILE ASBESTOS FIBERS
C.I. = CARBONYL IRON PARTICLES
MSH. = MT. ST. HELENS ASH PARTICLES
3/0 = 3 h exposure to dust or air and no recovery period.
3/24 = 3 h exposure to dust or air and 24 hr recovery period.
3/48 = 3 h exposure to dust or air and 48 hr recovery period.

investigators. Waters and Gardner (22) have suggested that toxic metallic compounds produce alterations in the surface morphology of pulmonary macrophages that reflect varying degrees of cellular damage. Recently, we reported that the percentages of smooth cells could be increased experimentally by incubating macrophages with silica and this correlated with a depressed phagocytic capacity of the exposed cells (18). In addition, we showed that scanning electron microscopic techniques are important tools in assessing macrophage phagocytosis both in vitro as well as in vivo following dust exposures. Particles composed of iron or silica are ideal for visualization in the backscatter electron image of the SEM (2). The SEM technique for evaluating phagocytic capacities of cells is superior to LM techniques which do not permit the investigator to delineate between particles adsorbed from the cell surface from those which have been endocytosed.

In this brief review we have demonstrated the utility of scanning electron microscopic techniques in investigating deposition patterns and early cellular reactions in the lung following dust exposures. Using these methods, one can define the initial patterns of dust deposition and follow the time course of pulmonary macrophage participation in clearing inhaled dust particles. Once inside the phagocyte, the particle may remain static until events result in the migration of the cell toward an exit from the lung. Subsequently, the macrophage enters the bronchial surface to exit out of the lung via the tracheobronchial mucociliary escalator (9). Alternatively, particle clearance from alveolar regions to the airways may be retarded following deposition of more toxic materials such as silica or asbestos. The studies of lung clearance mechanisms described here can be combined with more applied investigations of biomarkers including differential cell counts and lung enzyme/protein levels to help assess the lung toxicity of inhaled materials.

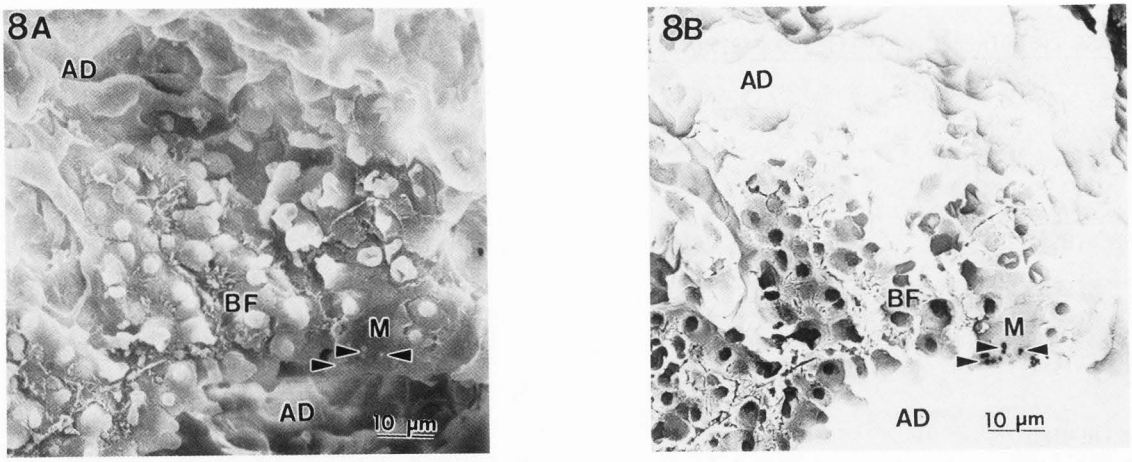


Figure 8A and B. A pulmonary macrophage (M) has migrated to an alveolar duct bifurcation (BF) in a rat exposed to carbonyl iron particles (arrowheads). The interiorized particles are viewed in secondary (A) and backscatter (B) images. AD: alveolar ducts.

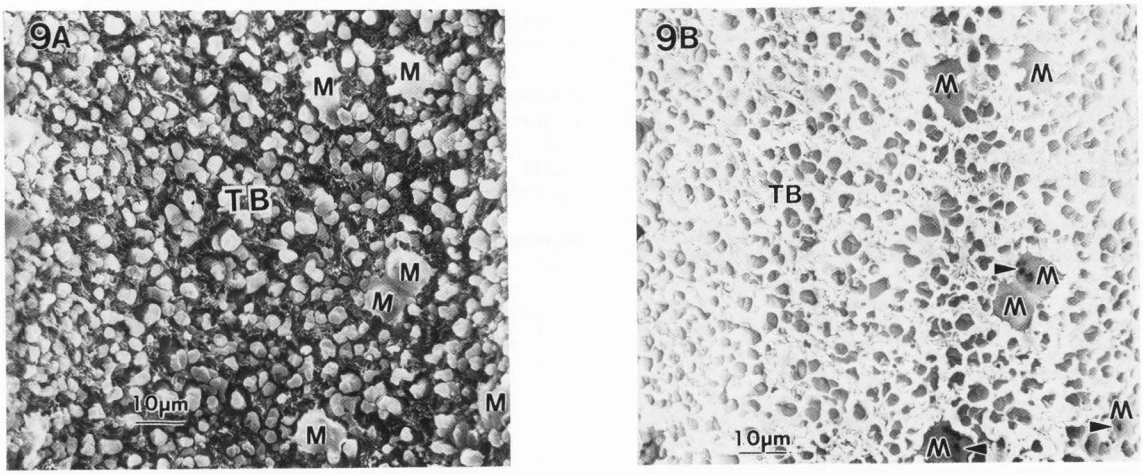
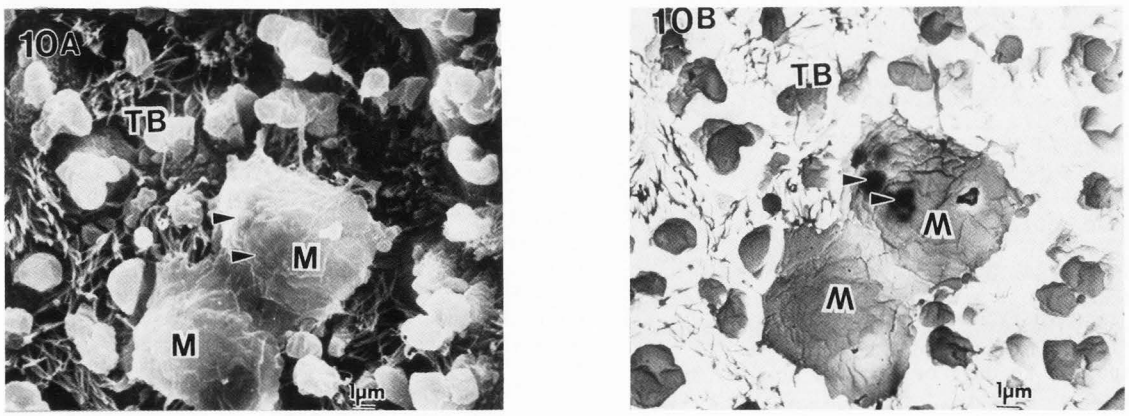


Figure 9A and B. SEM of pulmonary macrophages (M) which have travelled from alveolar regions to terminal bronchiolar (TB) surfaces. These phagocytes are exiting the lung via the bronchiolar mucociliary escalator. Many cells contain iron particles (arrowheads) and can be viewed in secondary (A) and backscatter (B) images.



Figs. 10A and B. A higher magnification SEM of 2 macrophages (M) seen in Fig 9. Note that the backscatter image is particularly useful for identifying phagocytized iron particles (arrowheads). TB: terminal bronchiole.

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Discussion with Reviewers

JL Abraham: What is the XES detection limit for chrysotile asbestos fibers? Can the authors state that a single unit fiber is detected? If not, what is the minimum mass needed for detection?

Authors: We have no information regarding the detection limit for measuring chrysotile asbestos fibers within exposed pulmonary macrophages. Although this method is superior to measurements made by light microscopy, we believe that the technique underestimates the percentages of phagocytic cells, primarily because we are unable to sample from every part of the cell using the spot mode.

VL Roggli: This study shows that there is a good correlation between the ability of a type of particulate to activate complement in vitro and its ability to induce macrophage migration to the point of particulate deposition in vivo. In this regard, it is of interest thaqt Mt. St.

Helen's volcanic ash has been reported to contain 1-6% crystalline silica. The data in Table 2 indicate that the macrophage chemotactic response to Mt. St. Helens ash exceeded the response for decomplemented sera and asbestos for each dilution of activated rat serum that was used, but the difference did not reach statistical signficance. These two bits of information together would seem to have implications for the sensitivity of the assays that were employed. Please comment.

Authors: The reviewer's point that the macrophage chemotactic response to volcanic ash exceeded the response for decomplemented sera is well taken. Mt. St. Helens ash-activated sera does not generate an increased chemotactic response when compared to normal heated sera (NHS), i.e., sera incubated at 37°C for 45 minutes; thus, the increase over decomplemented can be attributed to a spontaneous generation of chemotactic factor during the incubation period.

We have not tested crystalline silica for chemoattractant generation in serum or macrophage accumulation in vivo, but intend to carry out these experiments in the near future.

A Johansson: In your test system have you considered what would happen to the particles after they have been internalized by the macrophages? One clearance mechanism is the slow dissolution of relatively inert particles in the macrophage lysosomes.

Authors: Time course studies have been carried out on animals exposed for brief periods to chrysotile asbestos or carbonyl iron particles. Our results show that pulmonary macrophages migrate to alveolar duct bifurcations and phagocytize particulates within 24 hrs after exposure. Most of these cells are cleared from the bifurcations within 8 days postexposure and presumably have exited the lung via the tracheobronchial mucociliary escalator. Our data suggest that neither asbestos nor carbonyl iron particles are dissolved during this short time period. Conceivably, translocated particles may dissolve in the pulmonary interstitium.