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COMPARATIVE *IN VITRO* CYTOTOXICITY OF VOLCANIC ASHES FROM MOUNT ST. HELENS, EL CHICHON, AND GALUNGGUNG

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Dry sedimented volcanic ash samples from each of three widely separated volcanoes of the "Circum Pacific" region have been subjected to mineralogic analysis and in vitro *tests for cytotoxicity. The ash samples from the three different volcanoes varied in particle size, surface area, and concentration of silica. Total crystalline silica in the respirable fraction of ashes was 1.5% (Mount St. Helens, Moses Lake); 1.36% (Galunggung, Bandung-1); 1.95% (Gallunggung, Bandung-2); and 1.72% (El Chichon, Tuxtla). Hemolysis as an index of cytotoxicity was measured by* in vitro *tests on sheep blood erythrocytes and indicated wide differences in hemolytic activity among ash samples. Alveolar macrophage cytosolic (lactate dehydrogenase) and lysosomal (β-glucuronidase and β-*N*-acetyl glucosaminidase) enzymes were measured as an index of cellular integrity following dust exposure. Hemolysis and release of enzymes from alveolar macrophages were greater with volcanic ash from Galunggung (Bandung-1) and El Chichon (Tuxtla) than the other ashes. Although crystalline silica induced an effect similar to volcanic ash from Galunggung (Bandung-1) on the release of enzymes from alveolar macrophages, the hemolytic potency of silica was much greater. Light and electron microscopic observations of dust-exposed alveolar macrophages indicated that the ash particles were readily phagocytized. These results indicate that volcanic ash is moderately cytotoxic and that exposure may lead to overt reactions and the exacerbation of preexisitng chronic inflammatory processes.*

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INTRODUCTION

Volcanic eruptions in three widely separated volcanoes in the "Circum Pacific" region have exposed large numbers of people to respirable ashes and other potentially toxic agents. In addition to the immediate health hazards, delayed adverse effects pose a potential risk to populations respiring volcanic ashes intermittently or for prolonged periods (Baxter et al., 1981; Bernstein, 1983; Green et al., 1982; Merchant et al., 1982). The presence and persistence of respirable-size ash particles in ambient air is determined by the direction and force of explosion, wind, weather conditions, human activities, and size and density of the particles. The potential pathogenicity of the ash is dependent on the total respirable component, resuspended particulate levels, the relative cytotoxicities of the components of the ash, the amount inhaled, and a wide range of host susceptibility factors. It is likely that these volcanoes in the "Circum Pacific" will remain active for several years and thus remain a possible source of exposure to volcanic ashes in the future.

Most magmas before eruptions consist of a mixture of crystals and silicate melt. Following eruption into the atmosphere, the aerosolized magma immediately solidifies to particles of glass and crystals. Generally, two types of ashes are associated with separate explosions. A dark ash derived from the old volcanic cone, which usually represents the first eruption, consists of andesite with abundant plagioclase crystals (Hooper et al., 1980), and a pale ash, often found in subsequent eruptions, consists predominantly of dacite. The igneous rocks andesite and dacite contain several mineral phases. Furthermore, the mineral makeup and particle size distribution of volcanic ashes may differ considerably among samples, based on collection sites, distance, different eruptions, and volcanoes (Craighead et al., 1983; Frutchter et al., 1980; Hooper et al., 1980; Vallyathan et al., 1983a,b). Therefore, generalizations regarding cytotoxicity and pathogenicity of different volcanic ashes are difficult to make.

We therefore compared the cytotoxicities of the volcanic ashes from the recent eruptions of three volcanoes widely separated from each other by geographic and climatic conditions using short-term *in vitro* bioassays. Comparisons were made with known toxic (crystalline silica) and inert (barite) minerals with size fractions similar to the volcanic ashes. Although *in vitro* cytoxicity tests may not be predictive of *in vivo* pulmonary toxicity, these tests provide a rapid way of studying the effects of cell membrane integrity and relative cytotoxicity.

Hemolysis as an index of cytoxicity is commonly used in measuring mineral dust toxicity (Harington et al., 1971; Nash et al., 1966; Vallyathan et al., 1983a). Alveolar macrophages when exposed to dusts may release cytosolic and lysosomal enzymes, and this phenomenon is often used as an index of relative mineral toxicity (Beck et al., 1981; Chamberlain et al., 1979; Garrett et al., 1981; Schorlemmer et al., 1977). In this study we compared the hemolytic potential and release of cytosolic and lysosomal enzymes from alveolar macrophages exposed to respirable-size fractions of volcanic ash, crystalline silica, and barite.

MATERIALS AND METHODS

Sedimented volcanic ash samples from the Mount St. Helens volcanic eruptions on May 18, 1980, El Chichon eruptions on April 3, 1982, and from Galunggung eruptions of May 5 and June 3, 1982, were obtained and used in all the studies. Samples of Mount St. Helens ashes were collected from Moses Lake, 155 miles from the volcano, by industrial hygienists from the National Institute for Occupational Safety and Health (NIOSH). Ash samples from El Chichon were collected in Tuxtla, 45 miles from the eruption, by a U.S. Department of Agriculture research scientist. Two ash samples from Galunggung's eruptions, provided by the Director of the Indonesia Volcanology Survey, were collected from Bandung, approximately 160 and 165 miles away from the eruptions. The latter samples were collected without contamination during the ash fall from the volcanic eruptions of May 5 (Galunggung, Bandung-1) and June 3, 1982 (Galunggung, Bandung-2). The sedimented ash samples from Mount St. Helens (Moses Lake) and El Chichon (Tuxtla) were collected within 24 h of an eruption, and care was taken to minimize contamination with soil and other debris.

Ash samples were fractionated in a particle classifier to separate particles less than 10 μm maximum aerodynamic diameter, using an Accucut Particle Classifier (Donaldson Majal Division, St. Paul, Minn.). The respirable fraction (10 μm) by weight constituted 81% for Mount St. Helens (Moses Lake), 16.5% for Galunggung (Bandung-1), 8.8% for Galunggung (Bandung-2), and 6.2% for El Chichon (Tuxtla). Mineralogic analysis using X-ray powder diffraction was carried out in these fractions to determine the concentration of respirable silica polymorphs with a Philips automated diffractometer (Dollberg et al., 1983). In addition, the Talvite colorimetric method was used to confirm the concentration of total crystalline silica (Talvite, 1951). Scanning electron microscopy (SEM) combined with automated X-ray energy spectrometric (XES) analysis and image analysis were carried out on representative samples of the ashes to determine the microscopic morphology, elemental composition, area equivalent diameters, and concentration by number percentage. Each sample prepared on Nuclepore filters was analyzed at a magnification of X1000 for 31 elements using a LeMont DA-10 image analyzer (Stettler et al., 1982). Quantitative chemical analyses of 63 different elements were carried out either by proton-induced X-ray emission analysis (PIXE) (Bartsch et al., 1982) or by atomic absorption spectrometry. Surface area measurements were made by nitrogen adsorption technique (Bruneur et al., 1938).

In *in vitro* studies, the volcanic ash samples were compared with respirable-size crystalline silica (positive) and barite (negative) as mineral dust controls. Hemolytic activity was determined using a final concentration of 2% sheep erythrocyte preparation, according to the method of Harington et al. (1971), with modifications (sheep blood supplied by West Virginia University, Division of Animal and Veterinary Sciences, in heparinized vacutainer tubes). The test system consisted of 2 ml of 4% washed erythrocytes in phosphate-buffered saline (PBS) and 2 ml PBS with varying doses of respirable volcanic ash or mineral. The tubes were incubated for 1 h at 37°C with inverted mixings at 10-min intervals. After incubation the test tubes were spun at 500 X g for 5 min using a clinical centrifuge, and the optical density of the supernatant fluid was read at 540 nm against a buffer blank using a Gilford Spectrophotometer, Model 300N. Negative controls using blood and reagents were used to correct for any nonspecific background lysis. Percent lysis was calculated from the optical density readings of Triton X-100-lysed positive controls.

Rat alveolar macrophages obtained by bronchopulmonary lavage were used in assays measuring cytosolic enzyme lactate dehydrogenase (LDH) and selective release of lysosomal enzymes, β-glucuronidase (β-GLUC) and β-*N*-acetylglucosaminidase (β-NAG). Male Sprague-Dawley rats 12–14 wk old (250–300 g) were anesthetized with sodium pentobarbital (150 mg/kg body weight) and exanguinated by cutting the abdominal aorta. The lungs were then lavaged *in situ* 16–18 times with 4–5 ml per lavage of calcium- and magnesium-free Hanks' balanced salt solution of pH 7.4. Lavages were performed with warm (37°C) solutions, and a total volume 80 ml of lavage fluid was collected from each animal. Lavages were stored in ice and centrifuged at 500 X g for 10 min at 4°C. The sedimented pellets obtained from 3 or 4 rats were washed in cold HEPES medium containing 140 m*M* NaCl, 5 m*M* KCl, 10 m*M* HEPES (*N*-2-hydroxyethylpiperazone-*N*-2-ethane sulfonic acid), 5.5 m*M* glucose, pH 7.4, and pooled. In occasional cases when the lavages were contaminated with red cells, the pellets were lysed with 0.2% NaCl, vortexed, and immediately returned to isotonicity with an equal volume of 1.6% NaCl and diluted with HEPES medium. Macrophages, centrifuged and resuspended in a small volume of HEPES medium, were used in all the tests.

Cell counts were obtained from Trypan blue preparations using a grid hemocytometer. Periodically, air-dried cell smears were fixed in cold formalin and stained with hematoxylin-eosin and Geisma stains for differential counting of cell types. Cellular viability was evaluated in preparations using the Trypan blue exclusion test (Phillips, 1973). Approximately 95% of the cells obtained by lavage were alveolar macrophages. Alveolar macrophages (2×10^6 cells/ml) were incubated in the HEPES medium with and without dusts at 37°C for 2 h. After incubation they were centrifuged and resuspended in 0.4% Trypan blue and incubated for an additional 5 min. Cell viability was determined in aliquots by light-microscopic evaluations.

For the enzyme measurements, alveolar macrophages (2×10^6 cell/ml) were incubated with volcanic ashes or mineral dusts (1 mg/ml) in HEPES medium for 2 h at 37°C in a shaking water bath. Following incubation, the samples were centrifuged at 500 X g for 10 min at 4°C to sediment the cells and dusts. The supernatant fluid was then used in the measurements of enzymes released during the 2-h incubation with dusts. Total enzyme activities in the cells were determined in samples incubated without dust for 2 h. The effect of incubation was determined with cells incubated in buffer.

LDH activity was determined according to the method of Reeves and Fimignari (1963). β-GLUC activity was measured using *p*-nitrophenyl-β-*D*-glucuronide as the substrate, according to the method of Lockard and Kennedy (1976). β-NAG activity was assayed according to the method of Sellinger et al. (1960).

Hematoxylin-eosin- and Giemsa-stained microscopic preparations were examined by light microscopy for morphologic changes after exposure to dusts. Additionally, electron microscopic studies were made on macrophages exposed to dusts to evaluate the extent of cellular toxicity and phagocytosis. For these studies, the macrophage suspensions were fixed in buffered cold glutaraldehyde for 30 min, washed in Tyrode's buffer, and embedded in Epon. Sections were cut on a LKB OMU3 ultramicrotome and stained with saturated uranyl acetate and lead citrate. Stained sections were then examined in a Philips 201 transmission electron microscope and photomicrographed.

Optimal dust concentrations required to release detectable activities of enzymes were determined from a series of preliminary evaluations. Concentrations ranging from 100 μg to 2.5 mg/ml were used in these studies to evaluate the relative percent of enzymes released by silica and barite. Based on these tests, 1 mg/ml was selected as a satisfactory concentration for a wide range of minerals with differing toxicities.

Statistical Analysis

The data were subjected to statistical analysis using Student's two tailed *t*-test to compare the differences and their significances between dust-treated and controls. A Duncan's multiple-range test for variables was employed at an alpha value of 0.05 to rank the means and identify the dust that induced maximal hemolysis and release of enzymes.

RESULTS

The results of silica concentrations by particle number percentage, particle size, and surface area measurements by automated image analyses are presented in Table 1. The concentration of crystalline silica as estimated by XES analysis identification and quantitated by number as percentage of total inorganic particulate was higher than the quantitative measurements. Quantitative measurements of total crystalline silica by X-ray

TABLE 1. Particle Size, Surface Area, and Total Crystalline Silica Concentrations by X-Ray Diffractometry and Number Percentage by X-Ray Spectrometric Analysis in Volcanic Ashes, Silica, and Barite

Ash/mineral (source)	Number of particles analyzed	Median circular area equivalent diameter and range (μm)[a]	Surface area (m^2/g)[b]	Concentration of silica (%)	
				Number	XRD
Barite (Georgia Mines)	1573	0.87 (0.1-6.4)	5.8	3.1	NM[c]
Silica (Minu-Sil)[d]	1017	1.24 (0.08-6.6)	1.7	98.5	>99.0
Galunggung (Bandung-1)	1451	0.34 (0.1-11.9)	9.0	9.1	1.4
Galunggung (Bandung-2)	1020	1.47 (0.1-13.2)	3.8	28.9	1.9
El Chichon (Tuxtla)	1001	1.77 (0.09-10.7)	1.9	6.0	1.7
Mount St. Helens (Moses Lake)	1026	1.78 (0.09-10.2)	1.8	8.8	1.5

[a]Mean and range of circular area measurements and surface area analyses.
[b]Mean of 5 replicate tests.
[c]NM: not measured.
[d]Pennsylvania Glass and Sand Corporation.

diffractometry and by Talvite's colorimetric technique, on the other hand, showed remarkably lower levels in all ash samples (Table 1). The higher values in the concentration of crystalline silica by particle number percentage as identified by XES (Table 1) are probably due to the presence in the ash of large numbers of small silica particles, which on weight percentages by quantitative analyses are lower than then 2% level in all the ash samples. The results obtained by XES analyses further may lack a direct correlation with X-ray diffraction studies, due to the inability of XES to distinguish crystalline and noncrystalline particles, thereby classifying volcanic glass as silica.

Results of the quantitative elemental analyses of proton-induced X-ray emission (PIXE) and atomic absorption of 60 elements showed only a few major differences between the volcanic ashes. Ash from Galunggung (Bandung-1) showed higher concentrations of Mg, Si, S, K, Ca, and Fe than in the ash from Galunggung (Bandung-2). Mg, Si, S, Ca, and Fe concentrations were also higher in the Galunggung (Bandung-1) ash compared to all other ash samples.

Particle size measurements by automated image analyses on randomly selected fields of view indicate that 99% of the particles by count in all volcanic ash samples were within the respirable range of less than 10 μm in diameter (Fig. 1). Ash samples from Mount St. Helens had the greatest respirable fraction by weight and count. In contrast, the weight percentages of respirable fractions in the original ash samples from El Chichon and Galunggung were significantly less. These differences in the respirable fractions are probably due to the distances in the site of ash collected or due to wind velocities during the volcanic eruptions.

The results of the relative hemolytic activity of the volcanic ashes in comparison with positive (crystalline silica) and negative (barite) cytotoxic minerals are presented in Fig. 2. Volcanic ashes from Galunggung (Bandung-1) and El Chichon showed the greatest hemolytic activities on a weight basis when compared to the other ash samples (data not presented). Since the ash sample from Galunggung (Bandung-1) had the smallest median particle diameter (0.34 μm) and highest surface area (9 m^2/g) in comparison to the other ash samples, we conducted an experiment to evaluate the effect of similar surface area on hemolysis. Calculated weights providing equivalent surface area measurements (0.005 m^2/ml) were used for each ash and mineral sample and hemolysis was determined in 5 replicate samples. When equal surface area concentrations (calculated by weight to 0.005 m^2/ml) were added to the hemolysis test system, the ashes did not induce similar rates of hemolysis (Table 2). The volcanic ash from El Chichon showed the greatest hemolysis when similar surface area concentrations by weight were present in all the test systems.

To determine whether any substances are dissolved from the ash into the medium and thereby influence the hemolysis, two sets of experiments were performed. In one, ash samples were mixed in PBS and incubated

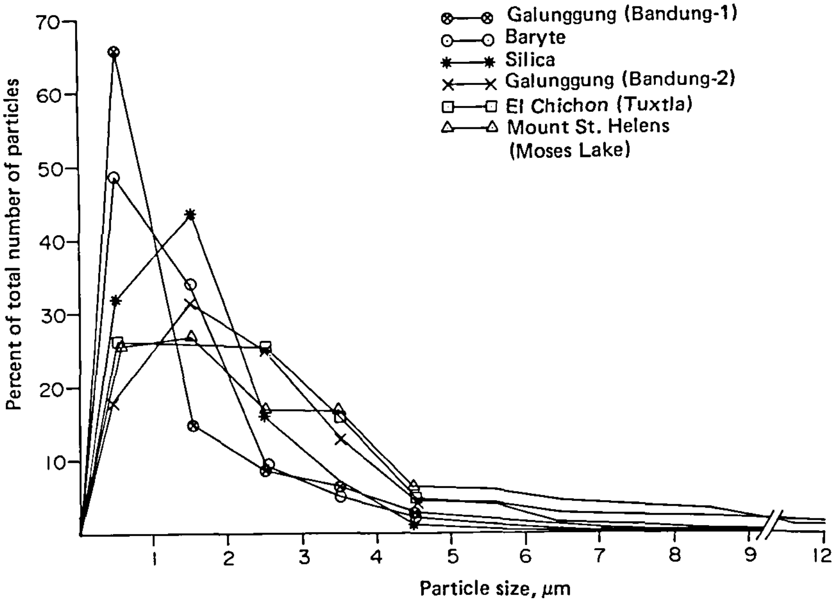

FIGURE 1. Distribution of particle by size in each volcanic ashes and minerals.

TABLE 2. Hemolytic Activity of Volcanic Ashes, Silica, and Barite with Similar Surface Areas of 0.005 m^2/ml

Mineral/ash (source)	Surface area (m^2/ml)	Dust concentrations (mg/ml)[a]	% Hemolysis[b]
Barite (Georgia Mines)	5.8	0.862	2.3 ± 0.25
Silica (Minu-Sil)[c]	1.7	2.941	90.0 ± 0.71
Galunggung (Bandung-1)	9.0	0.555	3.3 ± 0.13
Galunggung (Bandung-2)	3.8	1.315	4.4 ± 0.26
El Chichon (Tuxtla)	1.9	2.631	31.5 ± 2.10
Mount St. Helens (Moses Lake)	1.8	2.777	8.4 ± 0.10

[a]Concentrations of dust required to give surface areas in the first column.
[b]Mean and standard error of 4 replicate tests.
[c]Pennsylvania Glass and Sand Corporation.

with inverted mixings every 10 min for 1 h at 37°C. The supernatant obtained after centrifugation was then tested for osmolality using an automated osmometer (Precision Systems, Inc., Osmette-A). In the second experiment, the supernatant was tested for hemolysis. Following hemolysis, the osmolality was again determined. The leachates induced no hemolysis and there were no differences in the osmolality.

A linear realationship between hemolysis and increasing concentrations of dust was found for all the dusts (Fig. 2). This dose-dependent linear relationship was proportional to increasing concentrations of dusts up to the 50% hemolysis level, with correlation coefficients of 0.99–1.0 and slope intercepts close to 1 (Fig. 2). The 50% hemolytic concentrations (HC50) for volcanic ashes were: 7.41 mg/ml (Galunggung, Bandung-1); 7.81 mg/ml (El Chichon, Tuxtla); 13.81 mg/ml (Galunggung, Bandung-2); 15.63 mg/ml (Mount St. Helens, Moses Lake). In the case of crystalline silica and barite, the HC50 levels were 2.40 and 29.24 mg/ml, respectively.

The release of cytosolic and lysosomal enzymes from alveolar macrophages after exposure to ashes and other minerals is presented in Table 3. After exposure for 2 h, each of the ashes and minerals proved to be cytotoxic to macrophages. Although each ash/mineral tested produced elevations in the levels of LDH released from alveolar macrophages, silica and volcanic ash from Galunggung (Bandung-1) were remarkably more potent than the other dusts (Table 3). Silica caused an approximate threefold increase in the release of all three enzymes, while the volcanic ash from Galunggung (Bandung-1) induced an approximate fourfold increase in cytosolic LDH and threefold increase in the two lysosomal enzymes. This

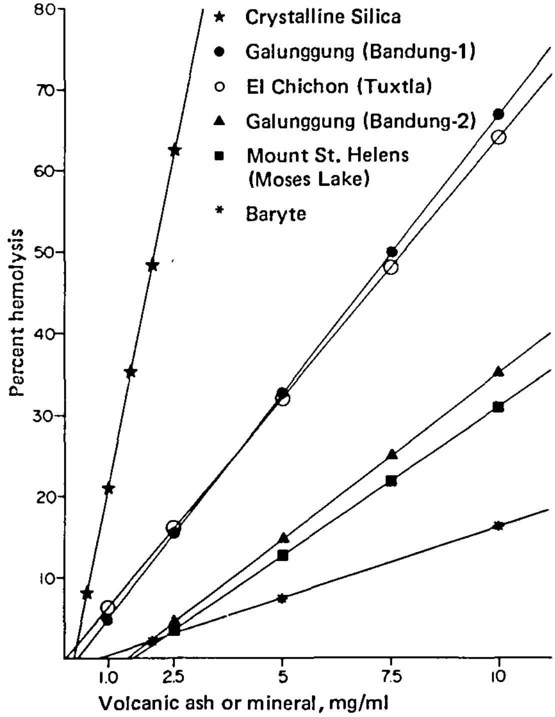

FIGURE 2. Dose response of hemolysis to volcanic ashes and minerals after 60-min incubation. The slopes and points indicate the linear regression analysis results for a minimum of 10 replicate experiments.

TABLE 3. Effect of Exposure of Alveolar Macrophages to Volcanic Ashes, Silica, and Barite

Ash/mineral (source)	Enzymes released (units/2 X 10^6 cells•2 h, 1 mg/ml)		
	LDH[a]	β-NAG[a]	β-GLUC[a]
Control	12.3 ± 1.0 (15)[b]	11.7 ± 1.8 (12)	7.3 ± 1.0 (13)
Barite (Georgia Mines)	34.1 ± 3.6 (4) $p = 0.001$[c]	19.5 ± 4.4 (4) p = NS	16.2 ± 3.8 (4) p = NS
Crystalline silica (Minu-Sil)	44.7 ± 4.3 (7) $p = 0.005$	31.8 ± 7.3 (5) $g = 0.05$	25.8 ± 6.7 (5) $p = 0.05$
Galunggung (Bandung-1)	50.8 ± 2.9 (9) $p = 0.001$	29.9 ± 4.5 (7) $p = 0.025$	23.2 ± 1.7 (8) $p = 0.001$
Galunggung (Bandung-2)	29.0 ± 2.7 (8) $p = 0.005$	15.5 ± 2.3 (6) p = NS	12.0 ± 1.0 (7) $p = 0.025$
El Chichon (Tuxtla)	32.5 ± 2.0 (8) $p = 0.001$	18.3 ± 2.6 (6) $p = 0.05$	13.0 ± 1.6 (7) $p = 0.025$
Mount St. Helens (Moses Lake)	30.7 ± 2.2 (9) $p = 0.001$	14.8 ± 1.9 (7) p = NS	11.5 ± 1.5 (8) $p = 0.05$

[a]LDH, lactate dehydrogenase; β-NAG, β-*N*-acetylglucosaminidase; β-GLUC, β-glucuronidase.
[b]Mean and standard error or replicate number of experiments indicated in parentheses.
[c]*p* Values based on control experiments performed on same number of tests. NS, not significant.

indicates all the ashes and minerals induced a defect in membrane integrity in the macrophages merely from contact. On the other hand, silica and volcanic ashes from Galunggung (Bandung-1) and El Chichon (Tuxtla) induced significant releases of lysosomal enzymes β-NAG and β-GLUC, thereby indicating phagocytosis and disruption of phagolysosomal membrane (Table 3). Barite, an "inert" dust, and volcanic ashes from Galunggung (Bandung-2) and Mount St. Helens (Moses Lake) failed to exert a marked effect on lysosomal enzyme release.

The results of experiments with Trypan blue exclusion showed limited, insignificant differences in the cell viability of alveolar macrophages exposed to volcanic ashes at 250 μg/ml. On the other hand, silica (16 ± 7%) and barite (18 ± 3%) induced membrane defects causing the dye to stain the macrophages. However, no consistent results were obtained from 4

FIGURE 3. Transmission electron micrograph of an alveolar macrophage exposed to volcanic ash. Many phagolysosomes and lysosomes are present in the cytoplasm. Phagocytized ash particles (electron-dense) are seen in the phagolysosomes (arrowheads). N, nucleus; PH, phagolosomes; L, lysosomes. X 12,250.

sets of experiments using 1 mg/ml dust, due to the aggregation of debris on cells. Cell viability experiments using 1 mg/ml dust were therefore considered not valuable and were discontinued.

Light and electron microscopic observations of dust-exposed macrophages indicated that the particles from all dusts were readily phagocytized. Cellular necrosis and vacuolization were evident in a few cells. Transmission electron microscopy revealed disrupted cell membranes and disintegrated nuclei in ash and silica exposed macrophages. Large numbers of cells showed internalized electron dense particles within the phagolysosomes (Fig. 3).

DISCUSSION

This investigation was undertaken to compare the cytoxicity of mineral dusts with that of volcanic ashes from recent volcanic eruptions in the "Pacific Ring of Fire." In previous studies, Mount St. Helens ash appeared moderately cytotoxic and fibrogenic in both *in vitro* and *in vivo* tests (Green et al., 1981, 1982; Vallyathan et al., 1983a,b). By comparing the cytotoxicity of volcanic ashes with highly cytotoxic and fibrogenic crystalline silica and "inert" barite, we obtained an estimate of the relative toxicity of ashes from different eruptions and volcanoes.

The relationship between *in vitro* cytotoxicity and *in vivo* fibrogenicity of several minerals is not well established. Although, many minerals exhibit a good correlation between hemolysis and fibrogenesis, several others show false negative or false positive results (Green et al., 1982; Harington et al., 1971; Jaurand et al., 1980; Light and Wei, 1977; Nash et al., 1966; Nolan et al., 1981; Summerton et al., 1977; Vallyathan et al., 1983a). Therefore, it is often difficult to relate *in vitro* studies and extrapolate data to human conditions. Extreme caution should be exercised in relating experimental data to actual human diseases. The disparity between *in vitro* results and *in vivo* response may result from the different physical and chemical properties of mineral phases within the same mineral used. Furthermore, together with the differences in the cytotoxicity of different mineral phases, the duration of exposure and dose are important factors involved in the expression of pathogenicity. Severalfold differences in the hemolytic potencies of quartz obtained from different geological localities have been reported (Nolan and Langer, 1983). Since these differences in quartz cytotoxicity are mainly attributed to variations in surface area, we prepared dust samples to contain equivalent surface areas and tested for hemolysis. The results indicate that ash from El Chichon (Tuxtla) is the most hemolytic, based on equal surface areas.

The release of LDH, a cytosolic enzyme, was measured as an index of cellular membrane integrity and viability. An increase in the LDH activity after incubation with dust would be an indication of macrophage plasma membrane damage resulting in the leakage of enzyme from the cell. Release of this cytosolic enzyme is often considered as a nonspecific reaction.

On the other hand, the presence of the lysosomal enzymes β-NAG and β-GLUC in the incubation medium would indicate phagocytosis and a defect in the phagolysosomal membrane.

Our *in vitro* biological tests for cytotoxicity with samples of volcanic ashes showed moderate erythrocyte membrane defects and leakage of cytosolic and lysosomal enzymes from the alveolar macrophages. The cytotoxic effect of volcanic ashes on erythrocyte cell membranes as measured by hemolysis was only moderate in comparison to crystalline silica. The volcanic ashes from Galunggung (Bandung-1) and El Chichon caused the highest hemolysis per unit weight. Although all the volcanic ashes induced the release of cytosolic and lysosomal enzymes from the alveolar macrophages, the extent of release was significantly higher for volcanic ash from Galunggung (Bandung-1). Comparable levels of enzymes were also released by crystalline silica. Barite, on the other hand, was not toxic to erythrocytes and induced the release of moderate levels of cytosolic enzyme LDH from alveolar macrophages.

From these *in vitro* studies it is reasonable to presume that the volcanic ashes are moderately cytotoxic to alveolar macrophages. Furthermore, it is evident that the ash from Galunggung (Bandung-1) is markedly cytotoxic to macrophages. Although *in vitro* cytotoxicity and the relative potential for fibrogenicity are difficult to correlate, our earlier *in vivo* animal studies have shown the volcanic ash from Mount St. Helens is moderately fibrogenic (Green et al., 1982; Vallyathan et al., 1983b).

Increasing evidence suggests that alveolar macrophages participate in the pathogenesis of lung disease (Brain, 1980). The release of macrophage cytosolic and lysosomal enzymes when exposed to volcanic ashes and silica suggests a loss of cellular integrity. Furthermore, this toxic effect is more marked with samples of volcanic ash from Galunggung (Bandung-1) and silica. The consequences of this toxic reaction on lung tissues are at present unknown, but it is possible selective release of these lysosomal enzymes into the extracellular medium may induce inflammation and proliferation of fibroblasts leading to the development of fibrosis. Recent evidence suggests that exposure to volcanic ash may also lead to the inhibition of superoxide anion release, and this may have implications with regard to the ability of macrophages to combat respiratory infections (Castranova et al., 1982). Whether such toxic effects will interact with preexisting endemic respiratory diseases to accelerate or exacerbate these diseases remains to be seen.

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