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Alveolar Type II Cell Response in Rats Exposed to Aerosols of Alpha-cristobalite

Robert B. Low,* Kevin O. Leslie,† David R. Hemenway,‡ Marlene Absher,§ Kenneth B. Adler,‖ Michael S. Giancola,* and Pamela M. Vacek¶

From the Department of Physiology and Biophysics, the Department of Pathology,† the Department of Civil and Mechanical Engineering,‡ the Department of Medicine,§ University of Vermont, Burlington, Vermont; the Department of Anatomy, North Carolina State University, Raleigh, North Carolina;‖ and the Department of Medical Biostatistics, University of Vermont, Burlington, Vermont¶*

Alpha-cristobalite causes pulmonary interstitial disease in humans and experimental animals. Aerosol exposure of rats to cristobalite for 8 days results in early and sustained alveolar type II cell hyperplasia in areas of inflammation characterized by the presence of macrophages and polymorphonuclear leukocytes. Irregular interstitial fibrosis and coalescence of alveoli are apparent by day 120. The inflammatory response is characterized by increased lavage cell recoveries, principally macrophages and neutrophils. Lavage recoveries of protein, nonpolar lipid, phospholipid, and saturated phosphatidylcholine also are increased. The recovery ratio for two important surfactant phospholipids, phosphatidylcholine and phosphatidylglycerol, is decreased at all points following exposure. Our morphologic analyses, together with results correlating lavage cell and lipid recoveries, point to the potential importance of macrophages and neutrophils in the epithelial cell response to cristobalite exposure. (Am J Pathol 1990, 136: 923–931)

The silicon dioxide polymorph alpha-cristobalite has been implicated in human pulmonary interstitial disease.[1,2] Our group recently has shown that 8-day aerosol exposure of rats to alpha-cristobalite results in rapid and severe inflammation that persists long after exposure has ceased.[3–5] Interstitial fibrosis becomes apparent histologically by 2 months, when collagen deposition also is increased.

Altered alveolar epithelial cell function is a hallmark of lung injury that leads to interstitial disease.[6] Pulmonary injury induced by exposure to mineral dusts leads to changes in epithelial cell structure, alveolocapillary permeability, and surfactant recovery.[7–14] Significant alterations in bronchoalveolar lavage surfactant lipid recovery and/or composition also occur in Adult Respiratory Distress Syndrome[15] and in animal models of acute lung injury involving N-nitroso-N-methylurethane,[16] oxygen,[17,18] bleomycin,[19,20] and intratracheal administration of silica, as reported most recently.[21] Similar results have been reported for human idiopathic pulmonary fibrosis.[22,23] It appears in the latter case that changes in the recoveries of the surfactant phospholipids, phosphatidylcholine (PC) and phosphatidylglycerol (PG), may be related to prognosis.

The purpose of the present studies was to determine the effects of alpha-cristobalite exposure on bronchoalveolar lavage surfactant lipid recovery, and to relate changes to alterations in epithelial cell morphology. A particular focus was on the relative recoveries of PC and PG, which we demonstrate to be altered. We also explored the relationship between lavage surfactant lipid and cell recoveries. A preliminary report of these findings has appeared elsewhere.[24]

Methods

Animal Model and Aerosol Exposure

Details regarding aerosol exposure have been provided elsewhere.[4,5] Male 175-g to 200-g Caesarian-derived, barrier-maintained Fischer 344 rats (Taconic Farms, Germantown, NY) were exposed for 6 hours a day for 8 days to alpha-cristobalite in horizontal flow chambers[25] at a concentration of 58 mg/cu m. This resulted in the deposition

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Address reprint requests to Robert B. Low, PhD, University of Vermont, Department of Physiology and Biophysics, Given Building, Room E-211, Burlington, VT 05405.

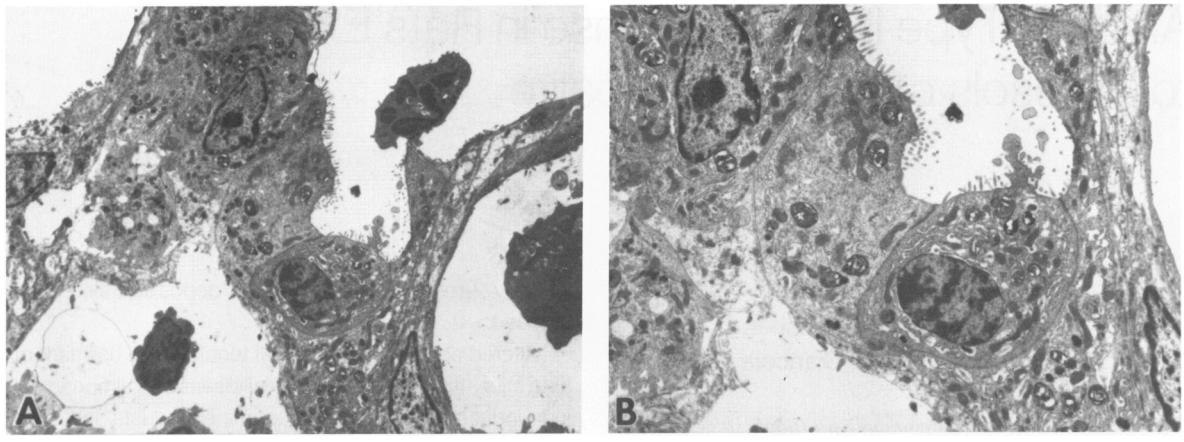

Figure 1. *Electron micrographs of lung tissue at day 12 after exposure at 58 mg/m³. Cuboidal type II cells characterized by prominent microvilli and many cytoplasmic lamellar bodies line the walls of involved alveoli.* **A:** *×1500;* **B:** *×2000; actual magnifications.*

of 380 ± 80 μg/lung (1360 ± 440μg/g tissue; mean ± SD, n = 4), as measured on ashed lung tissue by the molybdenum blue technique.[26] An additional study involved exposure of animals to levels of 7.5 and 27.5 mg/cu m. The alpha-cristobalite (C&E Mineral Corp., King of Prussia, PA) was 98% pure cristobalite, with 2% alpha quartz as determined by powder x-ray diffraction and had 2.4μg/g aluminum, but no other significant metal contamination as measured colormetrically. More than 95% of the particles were of respirable size (≤5 μm aerodynamic diameter) as measured by impactor (Sierra Instr., Carmel, CA) during the exposure. Animals were killed on days 0, 5, 12, 60, and 120, day 0 being when the exposure was terminated.

Histology

For morphologic studies, lungs were perfused vascularly with saline before immersion fixation with glutaraldehyde via the trachea at physiologic pressure.[4,11] The results of light microscopic evaluation have been reported previously.[4] For electron microscopic studies, random 1-mm portions of lung lobes were taken from a mid-cross section, postfixed in osmium tetroxide, embedded in Spurr's low-viscosity resin, sectioned at 30 to 50 nm on an ultramicrotome, and counterstained with uranyl acetate-lead citrate before examination with a Zeiss EM-10 electron microscope (Oberkochen, West Germany).

Lavage Analyses

Bronchoalveolar lavage techniques were as previously described.[20,27] Briefly, animals were anesthetized with intraperitoneal sodium pentobarbital (0.2 ml/100 g), after which the pulmonary vasculature was perfused *in situ* with 30 ml phosphate-buffered saline (PBS). The lungs then were removed prior to lavage ten times with calcium- and magnesium-free PBS, at 0.033 ml/g body weight per instillation. The resultant lavage fluids were centrifuged immediately for 10 minutes at 500*g* and the cell-free supernates frozen at −30°C prior to biochemical analyses. Lavage cell enumeration was by hemocytometry and differential counting on slides prepared by cytocentrifugation, methanol fixation, and May-Grunwald-Giemsa staining.[4,5] Lavage cell recoveries were calculated from lavage cell concentration, differential, and lavage volume recovered.

Lavage biochemical analyses generally were as described previously.[11,20,27] Protein concentration was determined as described by Lowry et al.[28] Total lipids were separated in conventional fashion for further fractionation by silicic acid chromatography.[27] Lipid phosphorus was determined by phosphate analysis and converted to phospholipid by multiplying by 25.[29] Phospholipids were analyzed by one-dimensional thin-layer chromatography according to Touchstone et al,[30] except that quantification of individual lipid spots was by phosphate analysis.[27] Percent phospholipid that was saturated was measured using the osmium method of Mason et al.[31] Nonpolar lipids were quantitated from malachite green-stained, one-dimensional chromatograms in silica gel, as described previously.[20] Controls were performed as before[20] to test the accuracy of the lipid analytic techniques. In all cases, recovery was calculated by multiplying the concentration of each biochemical constituent by the total volume of lavage fluid recovered.

Electrophoresis of unconcentrated lavage proteins was performed in sodium dodecylsulfate (SDS) 8.5% polyacrylamide slab gels as described by Laemmli[32] that subsequently were silver stained.[33] Lavage samples were mixed 9:1 with 10× SDS sample buffer and boiled briefly

Table 1. *Lavage Cell and Protein Recovery*

	Total cells	Macrophages		Polymorphonuclear leukocytes		Lymphocytes		Protein
	($\times 10^{-6}$)	Total ($\times 10^{-6}$)	Percentage	Total ($\times 10^{-6}$)	Percentage	Total ($\times 10^{-6}$)	Percentage	(mg)
Day 0								
Control (n = 3)	10.0 ± 3.0	10.0 ± 3.0	99.6 ± 3.0	0.03 ± 0.04	0.3 ± 0.3	0.04 ± 0.04	0.3 ± 0.3	1.8 ± 0.5
CRS (n = 3)	36.7 ± 2.8*	18.1 ± 2.6	49.7 ± 10.3*	18.7 ± 4.9*	50.3 ± 10.3*	0.00 ± 0.00	0.0 ± 0.0	9.0 ± 2.4*
Day 5								
Control (n = 3)	16.9 ± 3.0	16.6 ± 2.9	98.0 ± 1.0	0.18 ± 0.20	1.0 ± 1.0	0.17 ± 0.03	1.0 ± 0.0	5.3 ± 0.4
CRS (n = 3)	32.5 ± 3.8	9.8 ± 3.0	29.7 ± 6.1*	22.9 ± 1.6*	70.7 ± 5.7*	0.00 ± 0.00	0.0 ± 0.0*	6.2 ± 1.5
Day 12								
Control (n = 2)	7.9 ± 1.9	7.6 ± 1.9	96.5 ± 0.7	0.16 ± 0.04	2.0 ± 0.0	0.11 ± 0.03	1.5 ± 0.7	3.1 ± 0.7
CRS (n = 3)	78.7 ± 14.0*	37.6 ± 8.9*	48.3 ± 11.2*	38.7 ± 12.4*	48.7 ± 11.2*	2.84 ± 0.34*	3.7 ± 0.6*	8.5 ± 1.5*
Day 60								
Control (n = 2)	10.9 ± 2.3	10.7 ± 2.3	99.0 ± 0.0	0.11 ± 0.02	1.0 ± 0.0	0.00 ± 0.00	0.0 ± 0.0	2.9 ± 0.4
CRS (n = 3)	101.7 ± 11.5*	50.3 ± 3.3*	49.7 ± 3.1*	49.3 ± 7.8*	48.3 ± 2.9*	0.50 ± 0.29*	0.5 ± 0.2	11.0 ± 1.5*
Day 120								
Control (n = 3)	11.2 ± 1.2	11.1 ± 1.2	98.7 ± 0.6	0.07 ± 0.02	0.7 ± 0.3	0.07 ± 0.02	0.7 ± 0.3	5.1 ± 2.9
CRS (n = 3)	66.5 ± 10.0*	34.0 ± 8.0*	50.7 ± 4.9*	30.7 ± 1.9*	46.7 ± 4.6*	1.80 ± 0.04*	2.7 ± 0.6	12.2 ± 1.2*

Data expressed as mean ± SD. CRS = alpha-cristobalite. Exposure level: 58 mg/m^3.
* $P < 0.05$ *versus* control.

just prior to electrophoresis. Approximately 2.5 μg of protein was layered in each sample well.

Data were analyzed by two-way analysis of variance comparing groups by day. The significance of differences between control and exposed animals on each day of death was adjusted for multiple comparisons by the Bonferoni method.[34] One-way analysis of variance was used to examine differences between days and to assess the linearity of significant differences. Finally, the relationships between lavage cell and biochemical parameters were assessed by computing Pearson's correlations.

Results

Morphology

A description at the light microscopic level of the morphologic changes observed following cristobalite exposure, including examples of alveolar type II cell hyperplasia, has appeared elsewhere.[4] Briefly, the lungs of rats in the period immediately following 8-day exposure are characterized by widespread inflammation, and mild general thickening of alveolar walls. The inflammation is characterized by the presence of macrophages and polymorphonuclear leukocytes (PMN) both within the interstitium of the alveolar wall and adherent to the alveolar epithelium. Involved foci also are characterized by a more compact and cuboidal layer of alveolar lining cells, particularly in the region of the alveolar ducts where the inflammatory changes begin to concentrate by day 5. Type II epithelial cell hyperplasia is indicated by electron microscopic evaluation of alveolar regions (Figure 1). Lesions that characterize the response to cristobalite for up to a year[4] are prominent by day 120. The periodicity of the focal lesions as they relate to terminal airway distribution is apparent, with uniform spacing throughout the peripheral lung. The architecture of alveoli within fibrotic, inflammatory foci predominantly is one of irregular interstitial thickening and occasionally coalesced walls of alveoli of grossly abnormal size and shape. Involved alveoli often are filled with granular or foamy macrophage aggregates as well as PMN, and invariably are lined by cuboidal, hyperplastic type II cells. The degree of histologic abnormality is directly related to exposure dose.[35]

Lavage Analyses

Lavage cell data are shown in Table 1. Total cell recovery was increased, particularly at the later times. This was due early on to increases in the recovery of PMN and at later times to increased recoveries of both macrophages and PMN. Lymphocyte recovery was increased significantly

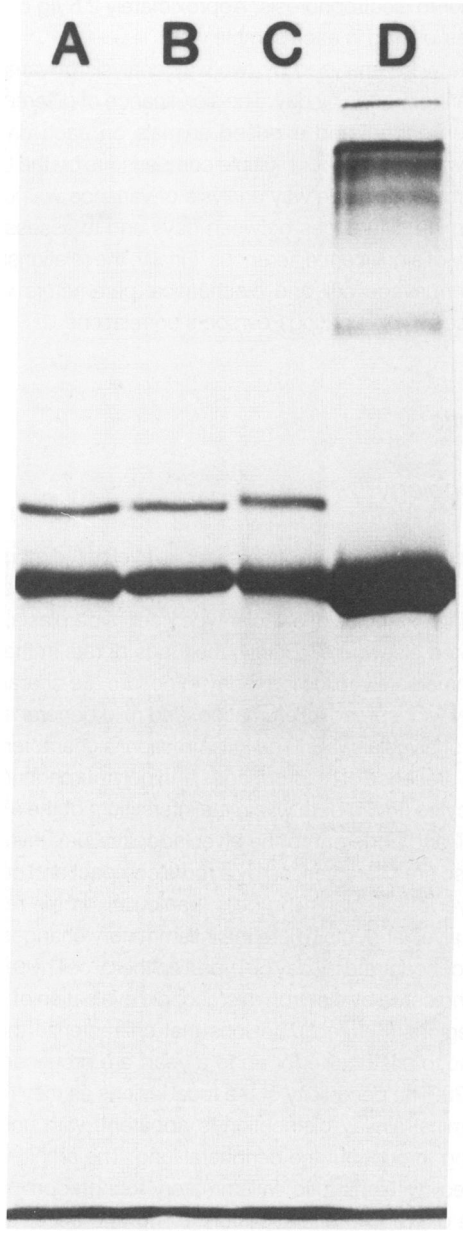

Figure 2. *Sodium dodecylsulfate polyacrylamide gel electrophoresis of lavage fluids from animals exposed at 58 mg/m³. Lavage samples were electrophoresed without prior concentration and the resultant electrophoretograms were silver stained.* **A:** *control;* **B:** *day 12;* **C:** *day 120;* **D:** *albumin standard.*

at days 12 and 120, although even then contributed only slightly to total cell recovery. These changes were reflected in the percentages of each cell type. Again, lavage cell abnormality was directly related to exposure dose.[35]

Lavage Protein

Lavage total protein recovery was increased significantly immediately after exposure and again by day 12 postexposure, with highest values occurring at days 60 and 120 (Table 1). The spectrum and proportions of the prodominant proteins recovered was similar for all exposure groups to that of controls, consisting primarily of serum albumin, based on analyses of sodium dodecylsulfate polyacrylamide gel electrophoretograms (Figure 2).

Phospholipid

Phospholipid recovery from the lungs of exposed animals was elevated significantly by day 0, reaching highest values at days 60 and 120 (Table 2). Recovery of saturated phospholipid was elevated significantly at all times, remaining stable at days 0 and 5, before becoming increasingly elevated. Most of this was phosphatidylcholine (PC), a high percentage of which was saturated. Phosphatidylglycerol (PG) recovery was increased only at day 12. Noteworthy was the significant fall in the ratio of these two phospholipids (Table 2) to a constant, abnormal value at all time points, due predominantly to increases in the recovery of PC. Lower exposure levels indicated the degree of abnormality in phospholipid values was dose related (Table 3).

Nonpolar Lipid

The major nonpolar lipids recovered were cholesterol, cholesterol ester, and triglyceride, the former two constituting most of the recovered nonpolar lipid (Table 4). Nonpolar lipid recovery was elevated at day 12, while cholesterol recovery was increased at days 12 and 120. Only one control value was available for day 60, but comparison with pooled control data indicated cholesterol recovery at day 60 for exposed animals was increased at this time as well ($P < 0.05$).

Correlations Between Lavage Cell and Biochemical Parameters

There were significant positive correlations of total cell recovery with the recoveries of total and saturated phospholipid, PC, and PG for exposed but not for control animals (Table 5). There were no correlations between total cell recovery and nonpolar lipids (not shown).

There were significant positive correlations between both total macrophage and PMN recoveries and the same phospholipid variables for the exposed animals, with the exception of PMN *vs.* PG. There were no significant correlations between macrophage parameters and the PG/PC ratio for either control or exposed animals. At the same time, there was a strong negative correlation between the

Table 2. *Lavage Phospholipid Recovery*

Day/Group	Total phospholipid (mg)	PC		PG recovery (mg)	PG/PC
		Recovery (mg)	Percent saturation		
Day 0					
Control (n = 3)	1.9 ± 0.3	1.6 ± 0.2	56 ± 2	0.23 ± 0.03	0.15 ± 0.01
CRS (n = 3)	4.5 ± 0.4*	4.2 ± 0.1*	64 ± 2	0.20 ± 0.00	0.05 ± 0.00*
Day 5					
Control (n = 3)	1.8 ± 0.5	1.8 ± 0.0	55 ± 4	0.23 ± 0.06	0.13 ± 0.04
CRS (n = 3)	4.4 ± 0.8*	3.8 ± 0.7	64 ± 6	0.20 ± 0.03	0.05 ± 0.00*
Day 12					
Control (n = 2)	0.6 ± 0.1	0.6 ± 0.1	63 ± 6	0.05 ± 0.01	0.09 ± 0.00
CRS (n = 3)	8.4 ± 1.2*	7.3 ± 1.1*	62 ± 11	0.43 ± 0.05*	0.06 ± 0.01*
Day 60					
Control (n = 2)	2.2 ± 0.0	1.8 ± 0.0	50 ± 1	0.26 ± 0.01	0.15 ± 0.01
CRS (n = 3)	10.0 ± 1.5*	9.0 ± 1.4*	65 ± 1*	0.37 ± 0.02	0.04 ± 0.00*
Day 120					
Control (n = 3)	3.3 ± 1.3	2.7 ± 1.2	49 ± 2	0.39 ± 0.11	0.15 ± 0.02
CRS (n = 3)	11.1 ± 1.3*	10.0 ± 1.7*	62 ± 1*	0.53 ± 0.09	0.05 ± 0.00*

Data expressed as mean ± SD. CRS, alpha-cristobalite; PC, phosphatidylcholine; PG, phosphatidyglycerol. Exposure level, 58 mg/m^3.
* $P < 0.05$ *versus* control.

PG/PC ratio and both PMN recovery and percent PMN for the control group. Significant negative correlations between percent lymphocyte and phospholipid recoveries were noted only within the control group, but primarily were due to one day 12 value, which had a high percent lymphocyte value and low phospholipid recovery. The PG/PC ratio was negatively correlated with percent lymphocytes in the control group, but was positively correlated with both percent and total lymphocytes recovered in the exposed animals. The latter correlations, again, were due to the day 12 values, which had the highest lymphocyte recoveries and somewhat elevated PG/PC ratios, as compared with the other exposed animals.

Discussion

Alpha-cristobalite exposure leads to an increase in lavage protein, an increase in recoverable surfactant lipids, and a decrease in the ratio of two specific surfactant lipids, phosphatidylcholine and phosphatidylglycerol. These changes occur during a period of lung injury and alveolar type II cell hyperplasia, particularly in regions of the lung characterized by increased numbers of macrophages and PMN. There are corresponding changes in the recoveries of lavage cells, principally macrophages and PMN, in several cases correlating with altered lipid profiles.

The pattern of alveolar type II cell hyperplasia and lavage protein and lipid recoveries may reflect a subtle form of silicotic alveolar proteinosis[8,36,37] in which alveoli become filled with amorphous proteinaceous material composed of serum proteins and surfactant phospholipid. We did not observe any typical morphologic changes characteristic of this condition in the current study, although this may be merely a reflection of the dosage of alpha-cristobalite and the degree of acute alveolar injury in this model.

The rise in lavage total protein is characteristic of acute lung injury. This increase could be due either to gross damage to the alveolocapillary membrane or to altered flux of serum proteins[38,39] as Dethloff et al[13,40] have reported from studies of lung injury due to intratracheal instillation of silica. Serum proteins also could accumulate at the alveolar surface as a result of tissue injury at more proximal airway sites. Our preliminary analyses indicate albumin remains the predominant protein. It will be important to examine this further, particularly in terms of in-

Table 3. *Lavage Biochemical Analyses Exposure to Lower Concentrations of Cristobalite*

Exposure level	Protein recovery (mg)	Total phospholipid recovery (mg)	PC		PG recovery	PG/PG
			Recovery (mg)	Percent saturation		
0	2.3 ± 0.3	2.8 ± 0.2	2.4 ± 0.2	48 ± 5	0.34 ± 0.03	0.15 ± 0.01
7.5	5.1 ± 0.4*	5.5 ± 1.2*	4.9 ± 1.1*	50 ± 3	0.36 ± 0.10	0.08 ± 0.02*
27.5	4.0 ± 0.7*	5.9 ± 1.8*	5.2 ± 1.7*	49 ± 6	0.49 ± 0.22	0.08 ± 0.01*

Data expressed as mean ± SD (n = 3). Results are from animals 120 days after exposure to alpha cristobalite. PC, phosphatidylcholine; PG, phosphatidylglycerol. Exposure level as indicated.
* $P < 0.05$ *versus* control.

Table 4. *Nonpolar Lipid Recovery*

Day/Group	Total nonpolar lipid (μg)	Cholesterol		Triglyceride		Cholesterol ester	
		Recovery (μg)	Percent total NPL	Recovery (μg)	Percent total NPL	Recovery (μg)	Percent total NPL
Day 0							
Control (n = 3)	347 ± 91	84 ± 19	26 ± 9	10 ± 18	2 ± 4	235 ± 84	66 ± 7
CRS (n = 3)	401 ± 67	174 ± 127	41 ± 29	19 ± 17	5 ± 5	177 ± 112	46 ± 32
Day 5							
Control (n = 3)	514 ± 220	70 ± 97	11 ± 16	59 ± 102	9 ± 16	370 ± 97	78 ± 20
CRS (n = 3)	671 ± 682	231 ± 244	31 ± 16	94 ± 101	12 ± 11	266 ± 319	44 ± 41
Day 12							
Control (n = 2)	165 ± 31	85 ± 19	54 ± 22	3 ± 4	1 ± 2	77 ± 47	45 ± 20
CRS (n = 3)	1132 ± 299*	770 ± 342*	66 ± 13	12 ± 17	1 ± 2	341 ± 27	32 ± 11
Day 60							
Control (n = 1)	320	90	28	0	0	230	72
CRS (n = 3)	745 ± 290	381 ± 207	50 ± 10	14 ± 24	3 ± 5	350 ± 140	47 ± 12
Day 120							
Control (n = 3)	405 ± 147	101 ± 48	24 ± 3	50 ± 17	12 ± 2	212 ± 65	54 ± 9
CRS (n = 3)	1083 ± 269	490 ± 164*	45 ± 10	150 ± 6	14 ± 3	394 ± 231	35 ± 14

Data expressed as mean ± SD. CRS, alpha-cristobalite; NPL, nonpolar lipid. Exposure level: 58 mg/m^3.
* $P < 0.05$ *versus* control.

creased recoveries of high molecular weight proteins, which might provide evidence for altered permeability[13,39]; or for proteins that are present as minor constituents quantitatively but that nonetheless are important markers of inflammation or altered immune function.[4,13,40]

Dethloff et al[40] report no change in PG or PC as percentage of total phospholipid following intratracheal exposure of rats to Min-U-Sil (U.S. Silica Products, Pittsburgh, PA), whereas Begin et al[14] report an increase in their sheep model, also involving intratracheal administration. We found a fall in percent PG, as Kawada et al[21] recently have reported to result in rats following intratracheal exposure. Potentially important variables that might explain differing results include dose, the specific silica polymorph used, the method of exposure, time, and animal species. In any case, our results demonstrating a fall in the PG/PC ratio are similar in this regard to several other models of lung injury.[15,16,18–20,41,42] Recent studies reporting decreases in percent PG and highlighting the potential usefulness of measuring lavage PG in staging and prognosis in human idiopathic pulmonary fibrosis[22,23] indicate the potential importance of this change. At the same time, results from ours and others' studies using animal models suggest the value of using PG measurements to stage disease may be limited, since the changes occur in an early and sustained manner.

Also of interest in the context of our present findings, we have found modest changes in surfactant lipid recovery and PG/PC ratio with little tissue histopathology only immediately after exposure of animals to similar doses of amorphous silica, after which the lungs return to normal (see Hemenway et al[5]; data not shown). Similarly, there is an immediate, transient change in lavage cells and biochemical composition following exposure to alpha quartz (Hemenway et al[5]; not shown), although a substantial change in surfactant lipid recovery occurs at much later points (approximately 1 year) when fibrosis develops.[12]

The alterations we observed in lavage lipid recovery are not likely due to increases in the contribution of tissue or plasma lipids, for reasons discussed previously.[19,20] Rather, we believe the changes are due to altered surfactant synthesis, degradation, and/or clearance.[43–46] The association of the observed pattern of lipid recovery with type II cell hyperplasia suggests that increased synthesis is at least partly responsible. This possibility is reinforced by our finding of increased recovery of cholesterol, much of which may be related to surfactant production.[47] In related studies, Dethloff et al[40] report disproportionately larger increases in intracellular *versus* extracellular phospholipid following intratracheal silica instillation, while others have found increased surfactant lipid synthesis after a similar insult.[10,48,49] A recent preliminary report indicates increased surfactant lipid synthesis following intratracheal exposure to silica.[50] Finally, the careful experiments of Kawada et al[35] support a role for increased synthesis, although additional factors also appear to be involved. Studies of whole lung surfactant lipid content and metabolism in our model might further resolve this question. The changes in PG and PG/PC ratio may reflect a return of type II cell lipid metabolism to a pattern seen during fetal and postnatal lung development.[20,51] In any case, whether or not these effects are the result of direct interactions between alveolar epithelial cells and the offending agent remains to be shown.

Changes in lavage phospholipids similar to what we report here have been associated with altered lung compliance[19,20] and surfactant activity.[41] Disproportionate changes in PG could lead to abnormal surfactant func-

Table 5. *Cell-biochemical Correlations Protein and Phospholipid*

	Total cells	Macrophages		Polymorphonuclear leukocytes		Lymphocytes	
		Total	Percentage	Total	Percentage	Total	Percentage
Protein							
Control	0.37	0.38	0.06	0.36	0.23	0.27	0.08
CRS	0.49	0.49	0.36	0.44	−0.40	−0.03	−0.02
Phospholipid							
Control	0.10	0.11	0.33	−0.17	−0.35	−0.46	−0.65*
CRS	0.73*	0.74*	0.45	0.61*	−0.53*	0.30	0.31
Saturated phospholipid							
Control	0.14	0.15	0.27	−0.15	−0.35	−0.38	−0.59*
CRS	0.78*	0.75*	0.37	0.71*	−0.46	0.29	0.27
PC							
Control	0.15	0.16	0.32	−0.12	−0.32	−0.40	−0.61*
CRS	0.72*	0.73*	0.46	0.61*	0.53	0.27	0.28
PG							
Control	0.12	0.14	0.41	−0.33	−0.53	−0.52	−0.71*
CRS	0.58*	0.57*	0.39	0.49	−0.48	0.49	0.52
PG/PC							
Control	0.14	0.16	0.44	−0.68*	−0.90*	−0.52	−0.71*
CRS	−0.32	−0.38	−0.25	−0.23	0.22	0.55*	0.55*

Given are the r values for Pearson's correlations. CRS, alpha cristobalite. Exposure level, 58 mg/m^3.
* $P < 0.05$.

tion,[51] although compensatory changes in the proportions of other anionic phospholipids might well compensate.[19,51–53] Altered proportions of neutral lipids, such as cholesterol, also may modulate surface activity.[54–56] This remains an important area for follow-up study.

Chronically increased recovery of lavage phospholipid in animals exposed to alpha-cristobalite also is of interest, as lung surface active material appears to have immunosuppressive activity.[57–59] Altered amounts and proportions of lavage phospholipids might contribute to a failure of normal regulation of lymphocyte function.[4,12] It will be important to examine this possibility further in terms of defining lymphocyte subclasses and assessing their *in vivo* and *in vitro* function in comparison with pulmonary surfactant levels and eventual parenchymal disease.

Beck et al[60] have concluded that the prolonged occurrence of macrophages and PMNs at the alveolar surface might be an important marker and determinant of mineral toxicity. Measurements of surfactant lipids additionally appear useful for following altered epithelial cell function. The correlations of total cell, macrophage, and PMN recoveries with surfactant lipid parameters complement our morphologic evaluation and are consistent with the possibility that these cell types play a role in causing altered epithelial cell function, as they are thought also to be involved in the overall pathogenesis of silicosis.[12,61–63] This possibility is consistent with the conclusion of Absher et al[4] that there may be a correlation between fluctuations in alveolar cell populations and overall tissue pathology in this model.

Of additional note is the strong negative correlation observed between percent and total PMN and the PG/PC ratio within the control but not the experimental group. The ratio of these two surfactant phospholipids may be very sensitive to increases in PMN, with the maximal response reached after only small increases in these cells. Further significant increases in PMN then would have no additional effect, leading to a loss of the negative correlation. Alternatively, small rises in PMN may trigger a decrease in the PG/PC ratio to a "baseline" level, below which no further drop can occur despite further increases in these cells. Finally, it could be that a normal control mechanism is disrupted by exposure to cristobalite.

It must be borne in mind that the relationships between lavage cells and phospholipids are based at this time largely on correlation studies. Experiments that manipulate the macrophage, PMN, and surfactant lipid response will be required to demonstrate the importance and causal nature of these connections.

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