Hypertrophy and Hyperplasia of Alveolar Type II Cells in Response to Silica and Other Pulmonary Toxicants

by Bruce E. Miller* and Gary E. R. Hook*

Alveolar Type II cells serve two major functions in the lung, both of which are essential for the preservation of normal lung function. First, Type II cells synthesize and secrete pulmonary surfactant, and second, they function as progenitor cells for maintaining the alveolar epithelium. The Type II cell population of the lung is quite sensitive to the deposition of toxicants in the distal lung, responding in two principal ways. Damage to the Type I epithelium stimulates Type II cells to proliferate and subsequently differentiate to replace the injured Type I cells. Second, a portion of the Type II cell population may become hypertrophic. Both of these events are frequent findings in the diseased or damaged lung. The Type II cell changes are often associated with increases in surfactant pools. In those cases where ultrastructural characteristics of hypertrophic Type II cells were examined, the appearance of these cells was consistent with that of an activated cell type. Alterations in the lamellar body compartment are a common finding in hypertrophic Type II cells, with increases in both lamellar body size and number. It is likely that the hypertrophic, or activated, Type II cells account for the increased levels of surfactant found in the lungs after exposure to a variety of toxic agents. We examined, in detail, Type II cell hyperplasia and hypertrophy induced by silica deposition. Both Type II cell hyperplasia and hypertrophy were prominent responses. The proliferative response led to an approximate doubling of the number of Type II cells in the lung. The hypertrophic response of the Type II cell population was a result of activation of biosynthetic pathways for surfactant-associated phospholipids and protein. The significance of this activation and the underlying mechanisms responsible for it are unknown, but in view of the relatively generalized nature of the hypertrophic response, the activation could be an important component in the lung's response to toxic insult.

Introduction

Two major functions of alveolar Type II cells have been defined. First, alveolar Type II cells synthesize and secrete pulmonary surfactant, a function that is well documented (*1*). Few would doubt the fundamental importance of the Type II cell in maintaining the patency of the alveoli and distal airways. Second, Type II cells serve as stem cells for the replacement of Type II cells that are damaged as a consequence of pulmonary disease or chemical-induced injury (*2*). In addition, autoradiographic evidence indicates that the Type II cell is the progenitor cell for the Type I epithelium in the developing lung (*3*).

Although Type II cell function is of fundamental importance to the well-being of the organism, very little is known about the pathobiology of the Type II cell. Very few studies have examined Type II cell function under conditions of naturally occurring or chemically induced lung disease. Perturbations of the surfactant system accompany a number of human respiratory conditions, including the neonatal and adult respiratory distress syndromes (*4,5*), idiopathic pulmonary fibrosis (*6*), and alveolar proteinosis (*7,8*), but the causes of the surfactant abnormalities and their relationship to the progression of disease have not been delineated. In addition, in experimental animals, alterations in surfactant levels have been reported following exposure of the lung to a variety of chemical and physical agents, suggesting that Type II cells and the surfactant system may be important components in the response of the lung to injury.

Our laboratory is interested in the mechanisms through which toxic agents influence the pulmonary surfactant system. Of the many agents reported to affect the surfactant system, perhaps the most potent is silica. The increases in surfactant that occur in response to silica deposition in the lung have been well documented (*9–12*), and recently we have extended those studies to the cellular level in an attempt to understand the underlying mechanisms through which silica is acting.

*Biochemical Pathology Group, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Address reprint requests to: G. E. R. Hook, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

In this review we will discuss our recent findings concerning the effects of pulmonary toxicants on the Type II cell population. Our major focus will be the changes in Type II cells caused by silica and how those changes may account for the increased levels of surfactant phospholipids found in the lungs of silica-exposed rats. However, we emphasize that, while the response of the surfactant system to silica has been the most well-studied example of surfactant changes in chemical-induced lung injury, it is probable that many other compounds could act through similar mechanisms.

The Type II Cell

The alveolar epithelium of the lung (Fig. 1) is composed primarily of two cell types, Type I and Type II cells. Type I cells account for approximately 97% of the surface area of the alveolar epithelium (*13*). These squamous cells provide a large surface area, allowing for the efficient exchange of gases between the alveolar space and the vascular compartment. Type II cells are cuboidal cells, and although they occupy only about 3% of the alveolar surface in the rat lung, they constitute approximately 63% of the total alveolar epithelial cells (*13,14*). Type II cells are typically found in corners or niches of alveolar septa and are characterized by surface microvilli and abundant mitochondria and rough endoplasmic reticulum. The most distinctive ultrastructural feature of the Type II cell is the cytoplasmic lamellar inclusion body, the intracellular storage site of pulmonary surfactant (Fig. 1).

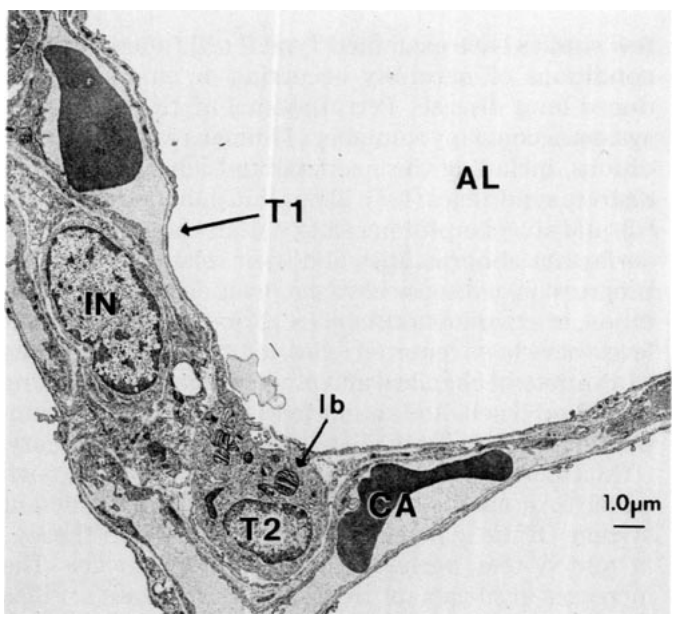

FIGURE 1. Alveolar septa in a normal rat lung. AL, Alveolar lumen; CA, Capillary; IN, interstitial cell; T1, Type I cell; T2, Type II cell; lb, lamellar body adapted from Miller et al. (*41*).

Type II cells, in addition to producing surfactant and serving as a stem cell population for the maintenance of the alveolar epithelium, have several other functions—all of which are essential for lung homeostasis. These include metabolism of foreign compounds and transepithelial water movement. These Type II cell functions have been discussed in detail in a recent review (*15*) and will not be discussed here.

Type II Cell Hyperplasia

Damage to the alveolar region of the lung results in a characteristic cellular response, regardless of the injurious agent. Type I cells, perhaps because of their relatively large surface area [approximately 4500 μm^2, (*13*)] and attenuated cytoplasm, are quite sensitive to toxic agents. Among the first cellular changes to be observed following exposure to a pulmonary toxicant is damage to Type I cells. [Type I cells appear to be incapable of dividing (*16*).] Subsequent to the proliferative burst, a sufficient number of Type II cells differentiate to replace the damaged Type I cells and restore the integrity of the alveolar epithelium (*17,18*). Differentiation of Type II cells into Type I cells takes approximately 2 days (*19*). This response is now generally recognized as a common reparative tissue reaction to alveolar injury (*2*) and appears to be essential for the normal tissue architecture being restored (*20,21*).

Evaluation of Type II cell hyperplasia typically has been based on qualitative histological examinations by light microscopy. For quantitative determination of the Type II cell proliferative response, electron microscopic, morphometric analysis has been the usual approach (*22*). This methodology has been used to examine the cellular changes occurring in the lung parenchyma in response to oxygen (*14,23*) and asbestos (*24,25*). However, electron microscopic morphometric techniques are lengthy, tedious, and expensive; thus they are not suitable for routine use.

Light microscopy has been used to study Type II cell population kinetics. The usual approach has been to label proliferating cells with ^{3}H-thymidine, followed by determination of the labeling index, i.e., the proportion of the total cells incorporating ^{3}H-thymidine. For example, Adamson and Bowden (*26*) used the labeling index to determine that Type II cell proliferation was maximal 2 to 3 days after intratrachael instillation of silica into the lungs of mice. These same authors (*27*) showed that exposure to short crocidolite asbestos fibers (mean length of 24 μm), on the other hand, produced only a slight degree of Type II cell hyperplasia (*28*). The labeling index method has also been used to study Type II cell proliferation in lung injury induced by oxygen (*29*), cadmium (*30*), butylated hydroxytoluene (*31*), and nitrogen dioxide (*32*). In all of these studies, it was found that the proliferative burst of the Type II cell population occurred within several days of the beginning of the exposure.

Although the labeling index approach has been useful, it suffers from two major disadvantages. First, the pro-

cedure merely detects cells that have incorporated ^{3}H-thymidine. Whether or not that incorporation actually reflects the degree of cell proliferation may be difficult to ascertain. Second, it does not provide a measure of the actual increase in cell number. Such information is useful when one desires to examine how a toxic agent can affect the cellular makeup of the lung parenchyma (*14,23,24*). This information could be important in understanding the cellular mechanisms responsible for the chronic effects of toxic agents, e.g., pulmonary fibrosis, alveolar proteinosis, etc.

We have recently developed a simple light microscopy-based morphometric procedure for Type II cell quantitation (*33*). This procedure takes advantage of the observation that, in the alveolar region of the lung, Type II cells are the only cell type containing alkaline phosphatase activity (*34,35*). The alkaline phosphatase activity is limited to the luminal membrane of the Type II cell and provides a convenient marker for the Type II cell at the light microscopic level. This staining pattern was confirmed by electron microscopic histochemistry (*33*). Figures 2 and 3 show the alveolar regions of a control and a silica-treated rat lung stained for alkaline phosphatase activity. With this technique, Type II cells are easily discernible from other cell types, and the Type II cell hyperplasia induced by silica is readily apparent.

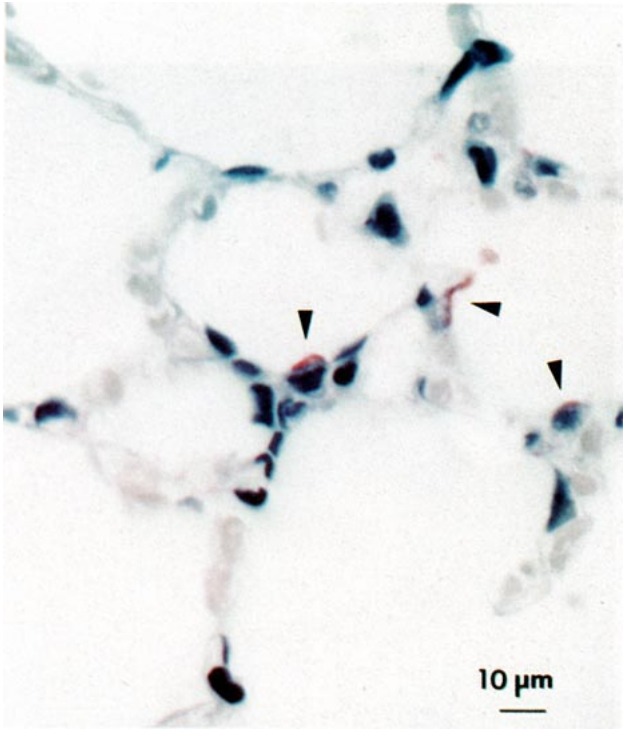

FIGURE 2. Distribution of alkaline phosphatase staining in the alveolar region of a normal rat lung. Alkaline phosphatase reaction product appears as a bright red precipitate. Sections were *counterstained with Harris' hematoxylin. Arrows indicate luminal* staining of Type II cells.

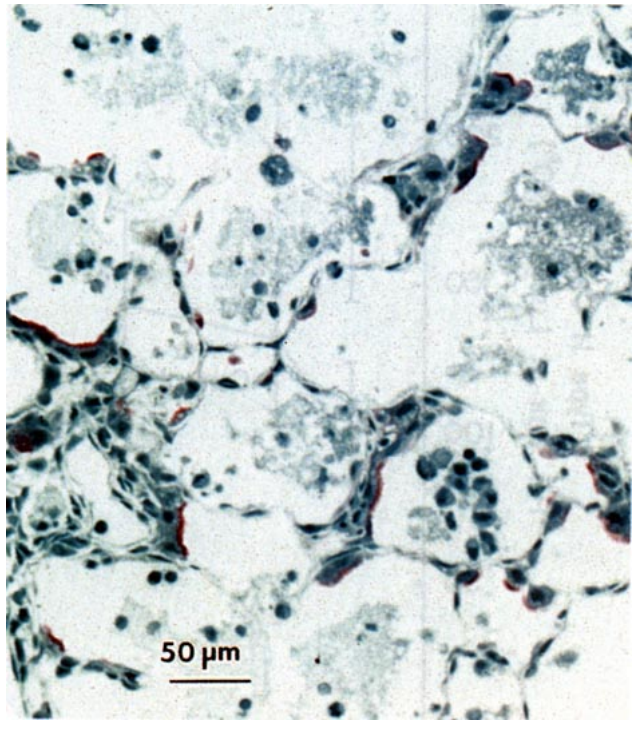

FIGURE 3. Alkaline phosphatase staining of the alveolar region of a *rat lung 14 days after exposure to 10 mg of silica. The hyperplastic* response of the Type II cell population is easily discernible.

Type II cell hyperplasia can be quickly and easily quantitated using the morphometric procedure described by Miller et al. (*33*). The morphometric procedure requires that only alkaline phosphatase-positive cells with nuclear profiles be counted. Although polymorphonuclear leukocytes contain alkaline phosphatase activity, these cells are easily distinguished from Type II cells by their characteristic, segmented nuclear morphology. In addition, the alkaline phosphatase activity of polymorphonuclear leukocytes is distributed throughout the cytoplasm of the cell, whereas staining of Type II cells is confined to their luminal surfaces. Type II cells in the lungs of rats, humans, and guinea pigs stain positively for alkaline phosphatase, but those cells in the lungs of mice and hamsters do not stain by our procedure (Miller and Hook, unpublished data).

We have used the alkaline phosphatase procedure to quantitate changes induced by silica in the Type II cell population of the lungs of rats. Following a single intratracheal injection of 10 mg silica, the Type II cell population approximately doubled over a 4-week time period (Fig. 4) (*33*).

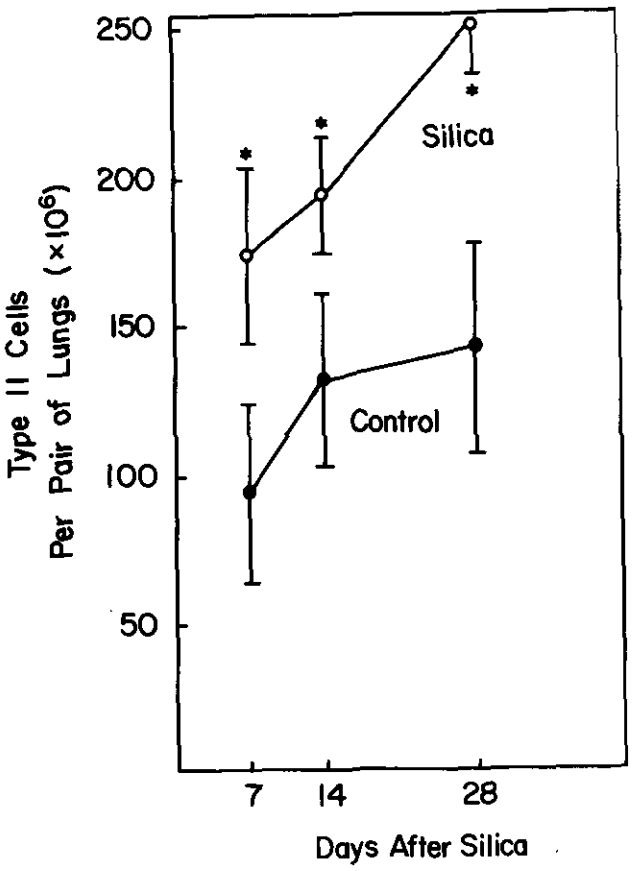

FIGURE 4. Changes in Type II cell population as a function of time after dosing with 10 mg silica. Each point represents the mean ± SD from three or four animals. Asterisk (*) indicates significantly different from corresponding control value ($p < 0.05$). From Miller et al. (*33*).

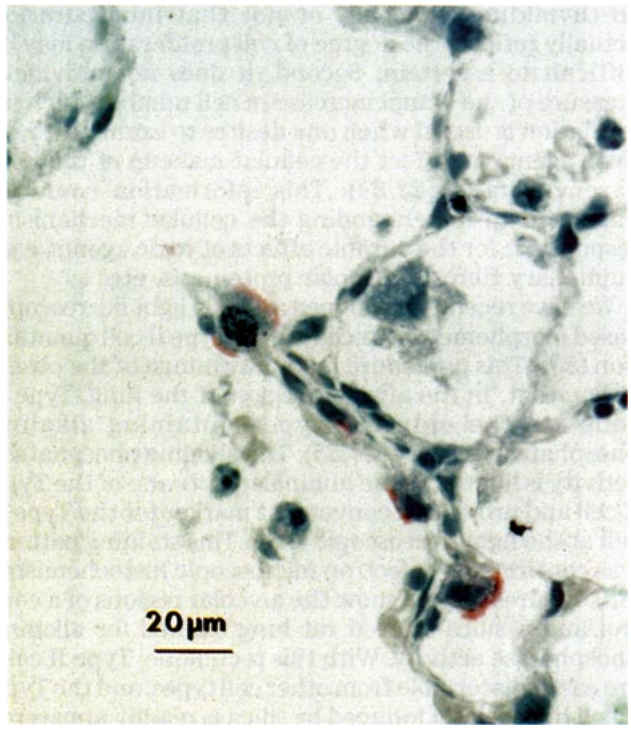

FIGURE 5. Alkaline phosphatase staining and autoradiography of a Type II cell *in situ*. ^{3}H-thymidine (2 µCi/g body weight) was given 90 min before sacrifice.

An additional advantage of the alkaline phosphate identification of Type II cells is that the technique can be coupled with ^{3}H-thymidine and autoradiography to identify Type II cells that may be actively engaged in synthesizing DNA (Fig. 5). Autoradiography does not interfere with the staining of the Type II cells. In addition, the procedure is also applicable to the study of Type II cells under conditions of *in vitro* cultivation (Fig. 6). When combined with ^{3}H-thymidine labeling and autoradiography, this technique should be extremely useful for examining Type II cell population kinetics, especially under conditions involving lung damage and disease.

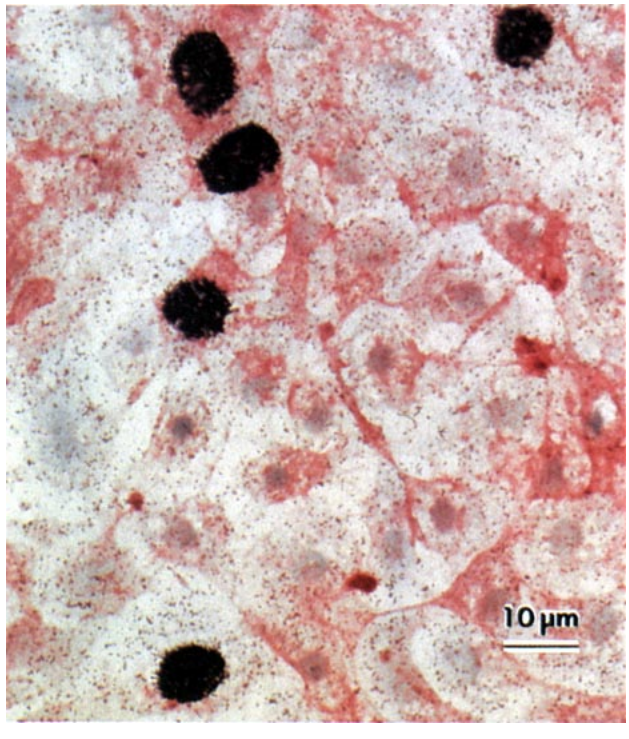

FIGURE 6. Alkaline phosphatase staining and autoradiography of Type II cells in primary culture. The cells were incubated with ^{3}H-thymidine for 24 hr.

Type II Cell Hypertrophy

Type II cell hypertrophy is another frequently reported response of the Type II cell population to lung injury. Numerous investigators have described the presence of

hypertrophic Type II cells following exposure to a pulmonary toxicant. The significance of the hypertrophic response of Type II cells following lung injury is not known, and the nature of the hypertrophic response has received very little attention, at least partly because of the difficulty in examining the response in a quantitative manner.

In many instances where Type II cell hypertrophy has been observed, alterations in lamellar bodies have also been reported. The most frequently reported lamellar body alterations in hypertrophic Type II cells are increases in lamellar body number and/or an increase in lamellar body size. For example, Aso et al. (*36*) reported that hypertrophic Type II cells in the lungs of rats given bleomycin contained increased numbers of lamellar bodies. A similar finding was reported by Kumar et al. (*37*) in mice. Recently, Balis et al. (*38*) reported that hypertrophic Type II cells in the lungs of rats exposed to 3 ppm of ozone contained increased numbers of lamellar bodies. Hypertrophic Type II cells in the lungs of asbestos-exposed rats were characterized by more numerous and larger lamellar bodies than seen in normal Type II cells (*24,25*). The intravenous administration of Freund's adjuvant to rabbits resulted in Type II cells that contained larger and more numerous lamellar bodies (*39*). Likewise, the lungs of rats exposed to cigarette smoke for 25 days contained hypertrophic Type II cells with increased numbers of lamellar bodies (*40*).

In the silica-exposed lung, the ultrastructural changes that occurred in the lamellar body compartment of hypertrophic Type II cells were evaluated morphometrically, and it was found that these cells contained lamellar bodies that were, on the average, twice the volume of those found in normal Type II cells (Fig. 7, Table 1)(*41*). In addition, hypertrophic Type II cells contained approximately 40% more lamellar bodies per cell. In the case of silica, the hypertrophic response appears to be progressive in nature with the number of hypertrophic Type II cells increasing over time (*42*).

In many of the studies cited previously, Type II cell hypertrophy occurred concomitant to alveolar injury. Diffuse alveolar injury, however, is apparently not a prerequisite for the induction of Type II cell hypertrophy. Gottschall et al. (*43*) reported that the antibiotic oxytetracycline resulted in hypertrophic Type II cells containing larger and more numerous lamellar bodies. This occurred in the absence of any evidence of alveolar epithelial injury. The authors suggested that the Type II cell changes were indicative of increased production of pulmonary surfactant. Similarly, Fringes et al. (*44*) recently reported that the hypolipidemic, peroxisome proliferating agent clofibrate produced, in the absence of Type II cell proliferation, hypertrophic Type II cells containing increased numbers of lamellar bodies.

In several instances (*36,38*), Type II cell hypertrophy appeared to precede Type II cell hyperplasia. In the study with bleomycin by Aso et al. (*36*), Type II cell hypertrophy was observed prior to any morphological signs

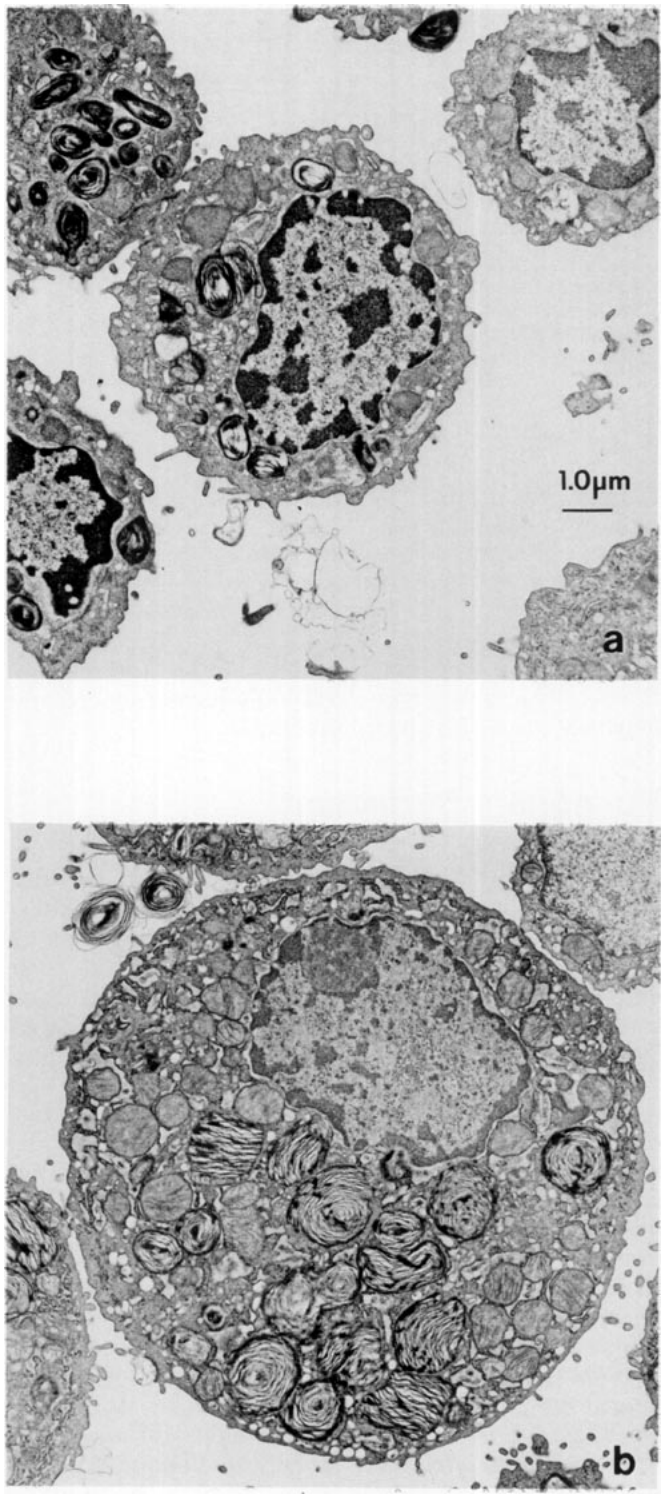

FIGURE 7. Electron micrographs of isolated Type II cells. (*a*) Type II cell isolated from control rat lungs; (*b*) hypertrophic Type II cell isolated from a rat lung 14 days after treatment with 10 mg silica. The cell profiles shown were chosen because they most closely approximated the mean caliper diameter of the cell type which they represent. From Miller and Hook (*70*).

Table 1. Morphometric analysis of control and hypertrophic Type II cells.[a]

	Type II cell population	
	Control[b]	Hypertrophic[b]
Cell volume, μm	354 ± 18[c] (311)[d]	523 ± 29[c] (261)[d]
Lamellar body volume, μ^3	0.32 ± 0.07 (381)	0.66 ± 0.10[c] (409)
Lamellar bodies per Type II cell	96 ± 43 (41)	131 ± 84[c] (32)

[a]Adapted from Miller and Hook (*41*).
[b]Values are mean of N determinations ± SD.
[c]Significantly different from control ($p < 0.05$).
[d]Number of individual profiles measures.

Table 2. Phospholipid composition of pulmonary surfactant isolated from normal rats by bronchoalveolar lavage.

Phospholipid	Composition, % of total phospholipid
Lysophosphatidylcholine	0.5
Sphingomyelin	1.1
Phosphatidylcholine	
Unsaturated	30.4
Disaturated	54.5
Phosphatidylinositol	3.7
Phosphatidylserine	1.1
Phosphatidylethanolamine	3.9
Phosphatidylglycerol	4.9

[a]From Dethloff et al. (*10*).

of Type I cell injury. Thus, Type II cell hypertrophy may be a response separate from that which induces Type II cell proliferation. Consistent with this reasoning is the observation that in several cases Type II cell hypertrophy has been shown to persist even in the absence of continuing Type I cell damage (*24,37,41,42*). The nature of the alterations in lamellar bodies in hypertrophic Type II cells suggest that these cells may have an increased capacity for surfactant production.

Pulmonary Surfactant

Pulmonary surfactant is absolutely essential for normal lung function. At end-expiration, when alveolar volume is minimal, surface tension forces would cause collapse of alveoli if surfactant were not present. An important biophysical property of surfactant is that upon compression, as occurs at end-expiration, its surface tension decreases to near zero. Thus, the presence of surfactant maintains alveolar stability at low lung volumes (*45*).

In the lung, surfactant is located in two distinct, but interrelated, compartments. Extracellular surfactant refers to surfactant that is present in the lumen of the alveoli and distal airways. This surfactant compartment may be removed quantitatively from the lungs by using bronchoalveolar lavage (*46*). With proper use of this technique, up to 95% of the extracellular surfactant can be recovered (*47*). Intracellular surfactant refers to the lamellar body compartment within Type II cells. This compartment can be recovered by any of several density gradient centrifugation procedures [for example (*10,48*)]. The development of reliable techniques for the isolation of surfactant has allowed changes in the surfactant compartments in response to pulmonary toxicants to be evaluated.

Isolated surfactant consists of about 90% lipid and 10% protein by weight (*49*). Phospholipids account for greater than 80% of the lipid component of surfactant. The phospholipid composition of pulmonary surfactant from the rat lung is shown in Table 2. The high percentage of phosphatidylcholine, and in particular, disaturated phosphatidylchloline accounts for the surface tension lowering ability of surfactant. In addition to its unique phospholipid composition, surfactant also contains several surfactant-specific proteins that are important in surfactant function (*50*).

A variety of compounds have been shown to affect surfactant levels in the lung. Increases in surfactant levels are a frequent finding in the lungs of toxicant-exposed animals. In the bleomycin-exposed lung, Type II hypertrophy was associated with increases in extracellular surfactant phospholipids (*36*). Exposure to oxidant gases also results in increases in surfactant-associated phospholipids. Increases in both the extracellular and intracellular pools of surfactant have been found following exposure to oxygen (*51,52*), ozone (*38*), and nitrogen dioxide (*53*). Inhalation of cadmium aerosol (*54*) and diesel exhaust (*32*) also resulted in increased extracellular surfactant.

Mineral dusts appear to be especially potent in promoting the accumulation of surfactant phospholipids. Increases in extracellular surfactant in response to asbestos deposition have been reported in several studies (*55–57*). Crystalline silica (alpha-quartz) is perhaps the most powerful inducer of surfactant phospholipids. The effects of silica on surfactant phospholipids have been particularly well studied. Fallon (*58*) was the first to report that silica causes an increase in the phospholipid content of the lung, an observation that was incorrectly attributed to macrophage influx and disintegration. Heppleston and his colleagues (*59–61*) showed that the majority of the increases in phospholipids in silica-exposed lungs resulted from large increases in phosphatidylcholine and disaturated phosphatidylcholine, which they suspected arose from pulmonary surfactant. These workers believed that the silica-induced pulmonary phospholipidosis was similar to alveolar proteinosis, a disease of the human lung characterized by the accumulation of large amount of lipid (*7*). Gabor et al. (*9*) demonstrated that silica deposition in the lungs resulted in up to a 12-fold increase in extracellular surfactant phospholipids. Dethloff et al. (*10–12*) examined the effects of silica on surfactant-associated phospholipids in detail. These authors found that, along with increases in extracellular surfactant, silica had an even more dramatic effect on the intracellular pool of surfac-

Table 3. Intra- and extracellular surfactant-associated phospholipid composition in the lungs of control and silica-exposed rats.

	Composition, % of total phospholipid			
	Intracellular		Extracellular	
Phospholipid	Control	Silica	Control	Silica
Lysophosphatidylcholine	1.5	0.5	0.5	1.0
Sphingomyelin	3.8	1.3	1.1	1.7
Phosphatidylcholine				
Unsaturated	29.5	32.8	30.4	35.2
Disaturated	51.8	49.4	54.5	49.2
Phosphatidylinositol	2.4	6.2	3.7	5.3
Phosphatidylserine	1.6	1.1	1.1	1.2
Phosphatidylethanolamine	5.0	4.2	3.9	3.1
Phosphatidylglycerol	4.5	4.5	4.9	3.4

[a]Intracellular surfactant is the lamellar body compartment within Type II cells. Extracellular surfactant refers to surfactant recovered by bronchoalveolar lavage. From Dethloff et al. (*10*).

tant. Greater than 120-fold increases in the intracellular surfactant pool could be induced (*10*). Despite these enormous increases, the phospholipid composition of the surfactant was similar to that in control lungs (Table 3). Approximately 85% of the increase in phospholipids in the lung caused by silica was associated with the surfactant system, indicating that the surfactant system is a specific target of silica (*10*).

An interesting finding in several of the studies mentioned above is that extracellular surfactant levels can remain elevated, even many weeks after exposure to mineral dusts. For example, Gabor et al. (*9*) found that extracellular surfactant levels remained elevated up to 26 weeks following exposure. Similarly, Richards and Curtis (*62*) found that the increases in extracellular surfactant produced by silica remained approximately five times higher than in control animals for as long as 48 weeks after exposure. Increases in surfactant following inhalation of chrysotile asbestos remained approximately 2.5-fold above control levels up to 50 weeks after exposure (*57*). Asbestos and silica are well known as etiologic agents in the development of pulmonary fibrosis. It is, therefore, conceivable that the increases in surfactant induced by these fibrogenic dusts are somehow related to the progression of the fibrotic process (*62*). In this regard, it is noteworthy that nonfibrogenic dusts do not elicit a surfactant response (*9,62*).

It is apparent from the preceding discussion that an increase in surfactant levels is a common response to lung insult. It should also be noted, however, that increased surfactant levels are not a generalized response of the lungs to all toxic chemicals. Some compounds appear to cause a decrease in extracellular surfactant levels. For example, the volatile, organic compounds trichloroethylene, carbon tetrachloride, and gasoline all appear to cause decreases in extracellular surfactant (*63,64*).

Surfactant Biosynthesis in the Injured Lung

Although surfactant metabolism in the toxicant-exposed lung has not been well studied, a number of investigators (*65–67*) have proposed that stimulation of biosynthesis is the underlying cause accounting for the accumulation of surfactant. Young et al. (*51*) found that, in the oxygen-exposed lung, production of surfactant-associated disaturated phosphatidylcholine was increased at least 4-fold. Richards and Lewis (*67*) found that tissue slices from silica-exposed lungs incorporated more [methyl^{14}C]choline into phosphatidylcholine than tissue slices from the control lungs. Miller and Hook (*66*) extended this finding and demonstrated that tissue slices from silica-exposed lungs had increased rates of incorporation of [methyl^{14}C]choline and ^{3}H-palmitate into lamellar body phosphatidylcholine and disaturated phosphatidylcholine; this was associated with an increase in the activity of cholinephosphate cytidylyltransferase, a key enzyme of the CDP-choline pathway for the *de novo* biosynthesis of phosphatidylcholine.

To better understand how exposure to toxicants may affect surfactant production, several investigators studied phospholipid biosynthesis in Type II cells, that were isolated from the lungs of exposed animals. Kikkawa et al. (*68*) found that Type II cells isolated from the lungs of rabbits exposed to bleomycin had a 1.6-fold higher level of phosphatidylcholine biosynthesis. Type II cells isolated from the lungs of rabbits exposed to oxygen incorporated radiolabeled choline into phosphatidylcholine and disaturated phosphatidylcholine at a greater rate than Type II cells from unexposed rabbits (*52*).

Wright et al. (*69*) reported that the activities of several enzymes involved in phospholipid biosynthesis were elevated in Type II cells isolated from the lungs of rats exposed to nitrogen dioxide. Type II cells isolated from the lungs of silica-treated rats also have an increased capacity for the production of surfactant-associated phospholipids (*70*).

Miller et al. (*41*) also showed that hypertrophic Type II cells could be isolated from the lungs of rats exposed to silica by intratracheal instillation. Not all Type II cells in the lungs of silica-exposed rats became hypertrophic, and the process of Type II cell hypertrophy was progressive (*42*). The method developed by Miller et al. (*41*) could separate hypertrophic from nonhypertrophic Type II cells. Compared with nonhypertrophic Type II cells, the hypertrophic Type II cells incorporated radiolabeled choline and palmitate into phosphatidylcholine and disaturated phosphatidylcholine at a greater rate (*70*).

In the silica-treated lung, activation of the CDP-choline pathway for the biosynthesis of phosphatidylcholine appears to be the mechanism accounting for the increased production of phosphatidylcholine and disaturated phosphatidylcholine by hypertrophic Type II cells. Increased activities of the two terminal enzymes of the CDP-choline pathway, cholinephosphate cytidylyltrans-

ferase (EC 2.7.7.15) and cholinephosphotransferase (EC 2.7.8.2), were found in the hypertrophic Type II cells (Miller and Hook, unpublished observations). Further, the increase in the activity of cholinephosphate cytidylyltransferase was shown to result from an increase in the activity of the membrane-bound and not the soluble form of the enzyme. The physiologically active form of the enzyme appears to be the membrane-bound form (*71*).

In addition to increased biosynthesis of surfactant-associated phospholipids, the biosynthesis is the major surfactant-associated protein [M_r 26,000–36,000, SP–A (*72*)] was increased approximately 7-fold in the hypertrophic Type II cells (Miller and Hook, unpublished observations). Thus, hypertrophic Type II cells represent a population of Type II cells in which the activation of biosynthesis of both the phospholipid and protein components of surfactant has occurred.

The Significance of Type II Cell Hyperplasia and Hypertrophy

In most of the examples of increased surfactant levels discussed above, Type II cell hyperplasia alone cannot seem to account for the increase. In the case of silica, during a period when Type II cell numbers in the lungs increased only 2-fold, intracellular pools of surfactant phospholipids increased 20-fold (*33,66*). There seems little doubt that the increased levels of surfactant seen in the lungs of silica-exposed rats is due to the presence of hypertrophic Type II cells. Within the hypertrophic Type II cells, increased levels of surfactant phospholipids appear to be brought about through activation of biosynthetic pathways. In fact, the hypertrophic state of the cells may arise from enhanced biosynthesis of surfactant phospholipids.

An important question that must still be addressed is what does the Type II cell activation and the accumulation of surfactant mean in terms of pulmonary function? Do increased levels of surfactant serve to somehow protect the lungs, as suggested by the results of several studies (*62,73*)? The activation of Type II cells and the significance of the surfactant increases is an area which has received very little attention, but in view of the apparent generalized nature of the hypertrophic response, an important physiological function of the surfactant system may yet await discovery.

We thank Gary J. Baker for providing Figure 6.

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