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Inhalation of Chrysotile Asbestos Induces Rapid Cellular Proliferation in Small Pulmonary Vessels of Mice and Rats

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Asbestos inhalation in mice and rats causes a rapid proliferative response in epithelial and interstitial cells, followed by the development of an interstitial lesion at the first alveolar duct bifurcations where fiber deposition and alveolar macrophage accumulation occur. Here we report that endothelial and smooth muscle cells of arterioles and venules near the bifurcations incorporated significantly increased levels of ^{3}H-TdR 19 to 72 hours after chrysotile exposure. As many as 28% of the vessels had labeled cells 31 hours after exposure. No labeled cells were observed in vessels from sham-exposed or iron-exposed controls. This proliferative response resulted in a doubling of both the number of smooth muscle cells and the thickness of the smooth muscle cell layer, determined by ultrastructural morphometry 1 month after exposure. The fact that a variety of cell types incorporates ^{3}H-TdR so rapidly after asbestos inhalation leads us to speculate that the response involves the release of diffusible growth factors. (Am J Pathol 1990, 136: 695–705)

Inhalation of chrysotile asbestos fibers causes progressive interstitial fibrosis in both humans[1] and experimental animals.[1-3] We have developed a rodent model for studying the basic mechanisms of asbestos-induced interstitial lung disease.[4-6] With this model, we have learned that inhaled fibers are deposited initially at the bifurcations of alveolar ducts and that alveolar and interstitial macrophages are attracted to these sites of deposition by a complement-dependent mechanism.[5,7-10] One of the earliest sequelae of particle deposition is an increase in tritiated thymidine (^{3}H-TdR) incorporation by a variety of cell types in the bronchiolar-alveolar regions.[6] These cells include nonciliated epithelial cells of the terminal bronchioles, as well as type II epithelial and interstitial cells of the alveolar duct bifurcations. The percentage of cells that incorporates ^{3}H-TdR 19 to 48 hours after a 5-hour asbestos exposure is increased up to 18-fold over normal unexposed animals.[6] These findings correlate with increases in numbers of epithelial and interstitial cells shown by ultrastructural morphometry.[5] Increases in ^{3}H-TdR incorporation and cell numbers in asbestos-exposed rats were found only in the regions of the bronchiolar-alveolar junctions. Alveolar ducts distal to the first bifurcation and parenchymal tissue in sections cut randomly throughout the lung showed no measurable changes.[5,6] Ultrastructural morphometry demonstrated no change in the number or volume of capillary endothelial cells at the bifurcations,[5] but the responses of the cells of arterioles and venules had not been studied in this model of interstitial lung disease.

In the paper presented here, we extend our observations on the pathogenesis of the earliest asbestos-induced lung lesions and show that endothelial and smooth muscle cells of small vessels of the bronchiolar-alveolar regions exhibit dramatic increases in incorporation of ^{3}H-TdR. Normal endothelial cells lining blood vessels are known to exhibit a remarkably low turnover rate, perhaps best expressed in terms of years.[11-14] Smooth muscle cells in the medial walls of normal small vessels also exhibit a very low proliferative rate, with less than 1% of the cells in S phase of the cell cycle at any point in time.[14,15] Although the proliferation of these cells is an important part of embryonic tissue growth and wound repair,[16,17] as well as the pathologic processes of diabetic retinopathy,[18] atherosclerosis,[19] and tumor growth,[20] there is no evidence that the cells of small pulmonary vessels respond in any way after inhalation of a fibrogenic dust such as asbestos.

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The striking increase in ^{3}H-TdR incorporation reported here may be significant because this proliferative response resulted in increased numbers of smooth muscle cells and concomitant thickening of the vessel walls. These findings support the view that diffusible, perhaps macrophage-derived, mitogenic factors are elaborated very soon after the deposition of asbestos fibers at the alveolar duct bifurcations and provide an anatomic basis for ongoing *in vitro* studies designed to characterize the factors at biochemical and molecular levels.

Methods

Asbestos Exposures

Male mice, strain B10.D2/nSn (The Jackson Laboratory, Bar Harbor, ME), which were 8 weeks old, were exposed in two separate experiments to an aerosol of chrysotile asbestos (4 mg/m^3 respirable mass) for 5 hours in open cages as previously described.[8,9] Control animals were sham exposed to room air.

^{3}H-thymidine (6.7 Ci/mmol; Amersham, Arlington Heights, IL) was administered (2 μCi/g) to the animals intraperitoneally after recovery periods of 0, 19, 24, 31, and 48 hours, 8 days, and 1 month for the first exposure, 24, 48, and 72 hours for the second, and 24, 48, 72 hours, and 1 month for the controls in both exposures after asbestos inhalation. Four hours after the administration of ^{3}H-TdR, the animals were killed by intraperitoneal injection of 25 mg/ml sodium pentobarbitol.

Tissue Preparation

Lungs from mice were fixed by vascular perfusion *via* the pulmonary artery with a fixative of 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M sodium cacodylate buffer with 5 mmol $CaCl_2$, at 23 cm H_2O pressure for 5 minutes. Whole lungs were removed from the animal and immersed in fixative overnight. After overnight fixation, the volume of fixed lungs was determined by water displacement and a portion of lung tissue was taken for histologic examination. After histologic verification that the animals showed no evidence of chronic lung disease or infection, slices of fixed tissue approximately 2 × 5 × 6 mm in size, adjacent to the slices taken for histology, were taken from the left lung, at right angles to each mainstem bronchus. The slices were postfixed with 0.5% osmium tetroxide in veronal acetate buffer, dehydrated with ethanol and propylene oxide, and embedded in soft epox 812 plastic resin, which was then polymerized at 60 C for 15 hours. The blocks of embedded tissue were softened on a warming tray and slices approximately 0.5 mm thick were cut parallel to the large tissue face with a razor blade. Terminal bronchioles with their attached alveolar ducts and alveolar duct bifurcations were randomly selected under a dissecting microscope using a bright light reflected from the surface of the cut block face, which clearly revealed the orientation of the lung tissue. The selected bifurcations were cut from the warmed plastic block and glued on to a blank epoxy capsule with epoxy glue, as previously described by Warheit et al.[8]

Microscopic Analysis and Autoradiography

Thick sections (0.5 μm) were cut with a diamond knife on a MT5000 microtome (Dupont, Wilmington DE), wet mounted on glass slides, and coated with a 1:1 dilution of Ilford L4 emulsion and distilled water. The sections were exposed in dessicated, light-tight boxes kept in total darkness at 4 C for 3 weeks, developed in Kodak D-19, fixed in Kodak fixer, washed for 45 minutes, and allowed to air dry. Sections were stained with toluidine blue-azure II.

Cell nuclei of all vessels in each section were counted by light microscopy at 100× magnification. A nucleus with six or more grains was considered labeled. Radiolabeled endothelial and smooth muscle cell nuclei were counted and expressed as a percentage of total cell nuclei (labeling index). Vessels were randomly selected because the tissue was originally dissected to expose terminal bronchioles and first alveolar duct bifurcations and the vessels were not a concern in selecting these tissues. The diameter of vessels cut in cross section were measured from photographs. The relationship between 3H-thymidine incorporation and the proximity of the vessel to the airway was determined by comparing the labeling index in vessels placed in three groups according to anatomical location: 1) adjacent to the terminal bronchiole, 2) within 100 μm of the terminal bronchiole, and 3) peripheral to, or further than 100 μm from the terminal bronchiole. Examples of vessels in the three groups are shown in Figure 1.

Additional tissues from 8-week-old male CD(SD)BR rats (Charles River, Raleigh, NC) exposed for 5 hours to asbestos ($\approx$10 mg/m^3 respirable mass) or carbonyl iron (30 mg/m^3), and prepared for autoradiography as described above, were available from earlier studies,[6] and vessel labeling was determined in these rat lungs 19, 24, 33, and 48 hours after exposure.

Morphometry

Morphometric measurements were carried out on vessels in the bifurcation regions of asbestos and sham-exposed mice 72 hours, 8 days, and 1 month after exposure. Thin sections adjacent to thick sections taken for autoradiogra-

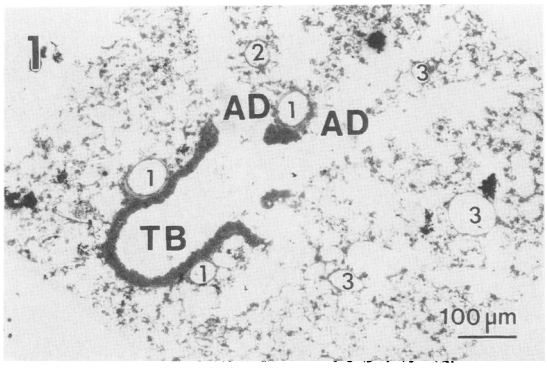

Figure 1. *Section of lung tissue from a mouse illustrating a terminal bronchiole (TB) and its alveolar ducts (AD), as well as small parenchymal vessels. The vessels were placed into three groups according to their anatomical location in the section relative to the terminal bronchiole. The three groups are: 1) adjacent to the terminal bronchiole. 2) not directly adjacent to, but within 100 µm of the terminal bronchiole, and 3) peripheral to (further than 100 µm from) the terminal bronchiole.*

phy were put on parlodion-coated one-hole grids and stained with uranyl acetate and lead citrate for examination by transmission electron microscopy (JOZEL 100CX electron microscope). A series of overlapping micrographs of each vessel was taken at 2600× and a montage of each vessel was constructed from the micrographs. The length of the endothelial cell basement membrane around each vessel and the length of the periphery of the smooth muscle cell layer were measured from the montage using a Hewlett-Packard 9830 computer and model 9864A digitizer. The thickness of the smooth muscle cell layer was calculated as the average distance between the basement membrane and the periphery of the smooth muscle cells. In addition, the number of endothelial and smooth muscle cell nuclei were counted and expressed as the number of nuclei per length of the basement membrane around each vessel. Cells of the vessels were also examined for the presence of asbestos fibers.

Statistical Analysis

A one-way analysis of variance on the mean labeling indices, transposed using the arcsine square root transformation, was used to determine if differences in vascular and interstitial cell incorporation of ^{3}H-TdR among the eight time points were significant.[21] Fisher's Least Significance Difference test was used to determine at which time points incorporation of ^{3}H-TdR was significantly elevated relative to the sham-exposed animals and to animals examined zero hours after exposure.[22] Fisher's Exact test was used to determine the significance of differences in labeling of vessels at different locations relative to the terminal bronchiole, and the Chi-square test was used to determine whether differences in the size of labeled and unlabeled vessels were significant. Morphometric measurements of smooth muscle cell number and thickness were compared using a two-sided *t*-test.

Results

Autoradiography

Sham-exposed Controls

We examined 690 endothelial and 874 smooth muscle cells in 90 vessels from 21 control animals and found that no endothelial or smooth muscle cells had incorporated ^{3}H-thymidine at any point in time (24, 48, 72 hours, or 1 month) after sham exposure.

Asbestos-exposed Animals

Endothelial, smooth muscle, and interstitial cells of small vessels near the first alveolar duct bifurcations (Figure 1) in the lungs of mice and rats, exhibited dramatic increases in incorporation of ^{3}H-TdR, 19 to 72 hours after chrysotile asbestos inhalation. In this distal region of the lung parenchyma it proved difficult to accurately and consistently establish whether the labeled vessels were arterioles or venules based on wall thickness. However, it appeared that both venules and arterioles exhibited labeled cells and light microscopy showed unequivocal evidence of many labeled cells (Figure 2).

Time Course of Labeling

A total of 366 vessels from 72 asbestos-exposed animals were examined for labeled cells. The percentage of vessels with at least one endothelial or smooth muscle cell labeled at varying times after exposure is shown in Figure 3. Labeling was greatest (28%) 31 hours after exposure, decreasing after 72 hours, and remaining low 1 month after. A similar time course was exhibited by endothelial cells of the small vessels. These cells had increased levels of ^{3}H-TdR incorporation 19 to 72 hours after exposure (Figure 4) and the data were statistically increased significantly 31 and 48 hours after exposure. Endothelial cells from an average of 5 vessels per animal were counted from 6 to 18 animals per time group. Although the variation among animals is large, there were statistically significant increases in labeling 31 and 48 hours after asbestos exposure, compared to sham-exposed controls and the zero-hour time group, both of which exhibited no labeling of endothelial cells. Labeling of smooth muscle cells followed a timecourse similar to the endothelial cells with

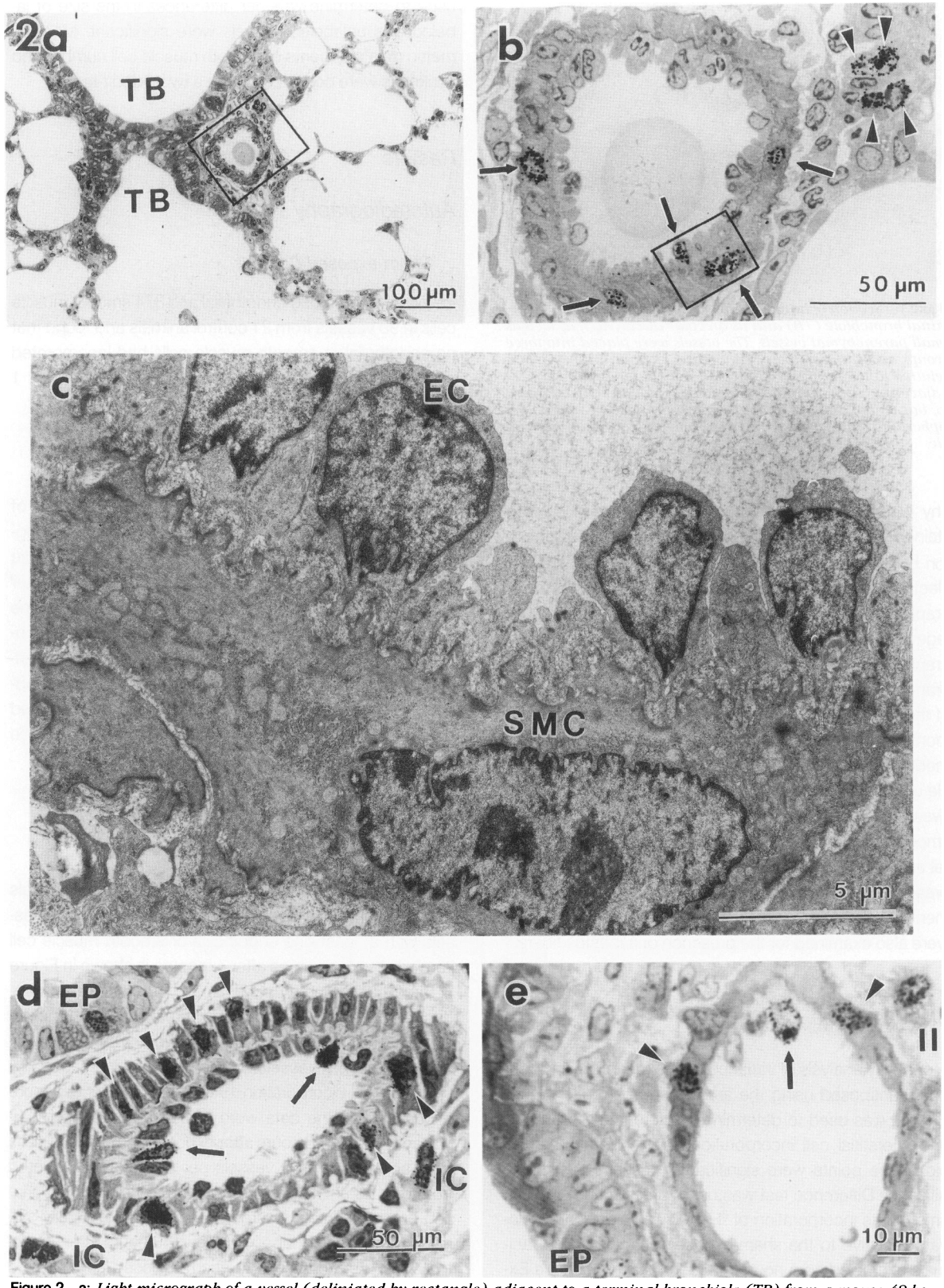

Figure 2. **a:** *Light micrograph of a vessel (deliniated by rectangle) adjacent to a terminal bronchiole (TB) from a mouse 48 hours after chrysotile exposure. The vessel is enlarged in* **b** *to show the labeled cells.* **b:** *Arrows point to the endothelial and smooth muscle cells that have incorporated ^{3}H-thymidine. Labeled interstitial cells (arrowheads) are also seen.* **c:** *Transmission electron micrograph that confirms that the ^{3}H-thymidine-labeled cells deliniated by the rectangle in* **b** *are indeed smooth muscle (SMC) and endothelial cells (EC).* **d and e:** *Autoradiographs of small pulmonary vessels from two mice examined 48 hours after exposure to chrysotile asbestos. Arrows point to endothelial cells and arrowheads to smooth muscle cells that have incorporated ^{3}H-thymidine. More than 30% of the smooth muscle cell nuclei in the vessel in panel* **d** *are labeled. There are labeled epithelial cells (EP) in the bronchioles. Labeled interstitial cells (IC) surround the vessel in* **d** *and a type II epithelial cell (II) is seen in* **e**.

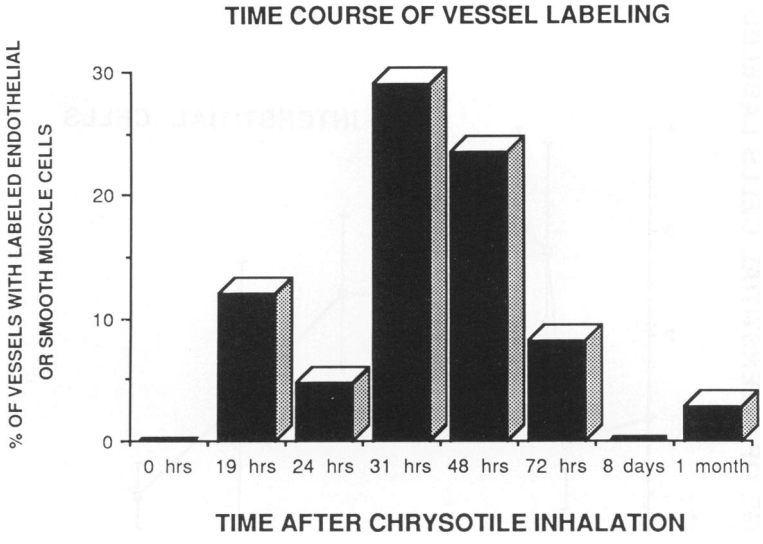

Figure 3. *The percentage of vessels, randomly selected from the bronchiolar-alveolar regions of mice with at least one endothelial or smooth muscle cell labeled at varying times after a brief exposure to chrysotile asbestos. Maximum labeling was seen 31 to 48 hours after exposure, decreasing by 72 hours, and remaining low 8 days and 1 month after exposure. Labeling of vessels in sham-exposed controls examined 24, 48, 72 hours and 1 month after exposure was zero.*

increases averaging 4.8 ± 2.5% of cells labeled 48 hours after exposure. The variability in smooth muscle cell labeling among animals was sufficiently large to preclude demonstration of statistically significant differences in ^{3}H-thymidine incorporation over time. However it is clear that smooth muscle cells of some vessels showed a dramatic increase in ^{3}H-TdR incorporation, as high as 30% in some cases (Figure 2).

Similar to observations in previous studies with asbestos-exposed rats,[6] the mice studied here had increased numbers of interstitial cells labeled with ^{3}H-TdR (Figure 2). Sham-exposed animals never exhibited more than 1% labeling at any time point, while between 24 to 72 hours after a mean labeling index of 2.3 ± 0.8% labeled interstitial cells was maintained (Figure 5).

As noted above, asbestos-exposed rats have been studied to establish the timecourse of ^{3}H-TdR incorporation by epithelial and interstitial cells of the first alveolar duct bifurcations.[6] We have used these same tissues in a retrospective fashion and report here that the asbestos-exposed rats exhibited a similar timecourse of labeling of the small vessels near first alveolar duct bifurcations, with 9.1% of vessels labeled in 16 animals (4 per time point) examined 19, 24, 33, and 48 hours after chrysotile inhalation. No labeled endothelial or smooth muscle cells were found in sham-exposed controls or in rats that had

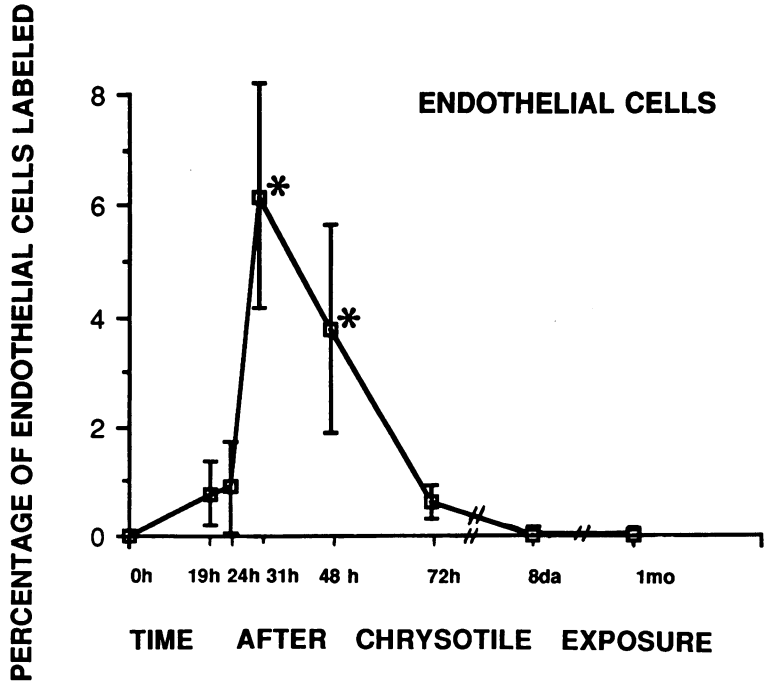

Figure 4. *The timecourse of ^{3}H-thymidine incorporation by endothelial cells of small parenchymal vessels in asbestos-exposed mice. The values represent the means and standard deviations of the percentage of labeled cells (labeling index) among animals at each time point. There was an average of 5 vessels from one or two bifurcations per animal and an average of eight animals per time point. Labeling of endothelial cells in control animals examined 24, 48, 72 hours, and 1 month after sham exposure was zero. ^{3}H-thymidine incorporation 31 and 48 hours after asbestos exposure was significantly different by Fisher's Least Significant Difference test,[22] from the value of zero incorporation exhibited by both sham-exposed animals and those examined immediately after exposure.*

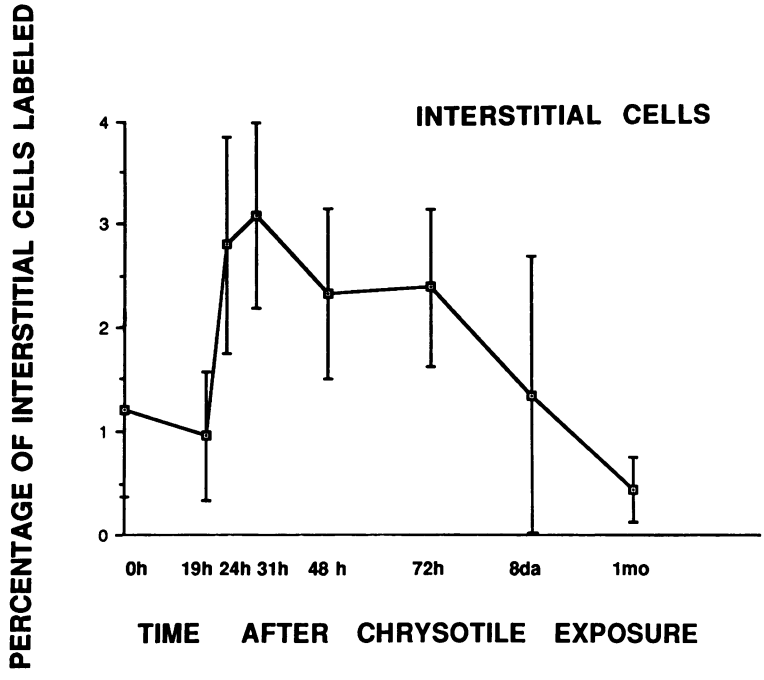

Figure 5. *The timecourse of 3H-TdR incorporation in interstitial cells surrounding the small parenchymal vessels in asbestos-exposed mice. Labeling of interstitial cells in sham-exposed control animals was less than 1%.*

been exposed to carbonyl iron, a nonfibrogenic control particle.[6]

Location of Labeled Vessels

Vessels were grouped into three categories according to their relative location to terminal bronchioles (Methods and Figure 1). Figure 6 shows the percentage of labeled vessels from mice between 19 and 72 hours after asbestos exposure. Vessels adjacent to the bronchioles exhibited significantly higher levels of labeling than those that were more peripheral.

Size of Labeled Vessels

We found no evidence that the presence of labeled endothelial or smooth muscle cells correlated with vessel size. The majority of vessels in the bifurcation region were between 30 and 90 μm in diameter. The mean diameter of all vessels measured was 59.3 ± 35.6 (SD) μm (n = 135).

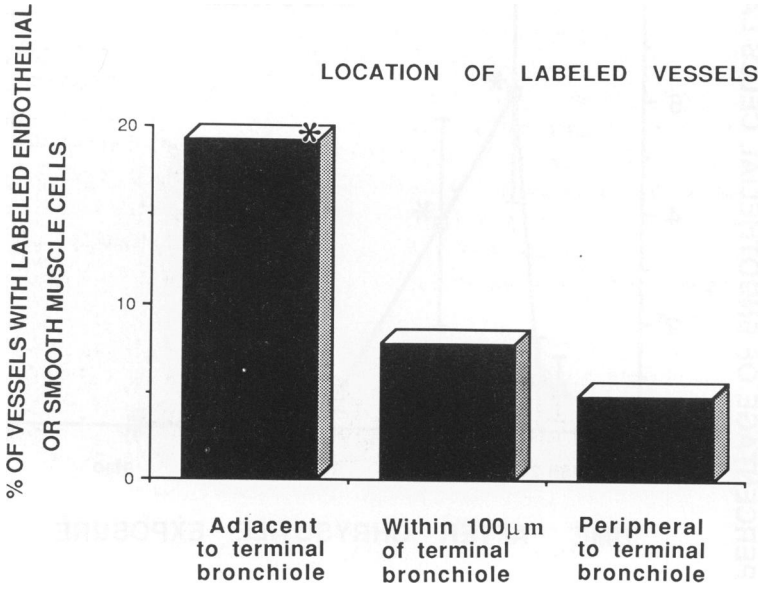

Figure 6. *The percentage of vessels with labeled endothelial or smooth muscle cells in three anatomical locations relative to the terminal bronchiole in asbestos-exposed mice, 19 to 72 hours after chrysotile exposure. Differences in labeling between vessels adjacent and vessels peripheral to the terminal bronchiole are statistically significant (*Fischer's Exact test, P < 0.05).*

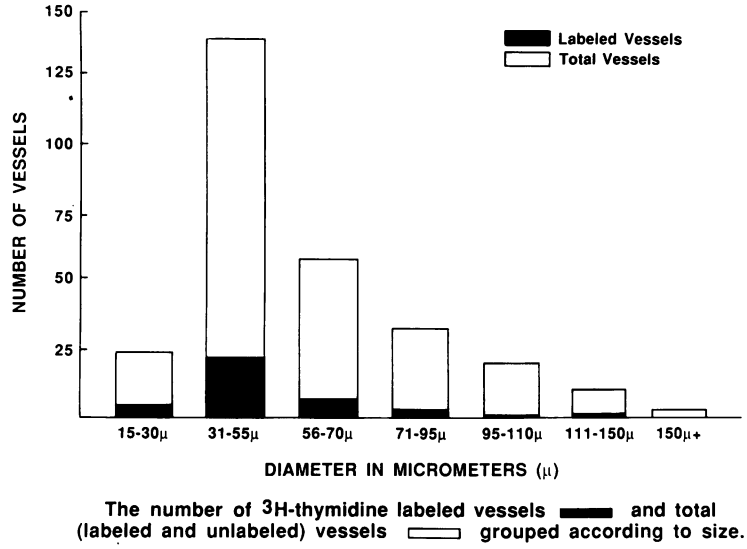

Figure 7. *The size distribution of labeled vessels (solid bars) and total vessels (white bars) in animals 19 to 72 hours after asbestos exposure. There is no difference in the size distribution between labeled and total vessels (Chi-square test).*

Figure 7 shows that the size distribution of labeled vessels was not significantly different (Chi-square test) from the distribution of all the vessels from mice examined 19 to 72 hours after exposure. The reaction appeared to be restricted to vessels larger than 20 μm. Labeling of capillary endothelial cells was not increased in the asbestos-exposed animals.

Morphometry

There was no difference in the number of endothelial or smooth muscle cells nor in the thickness of the smooth muscle cell layer in any animals examined 72 hours or 8 days after exposure. Endothelial cell number appeared to be slightly elevated in vessels from mice studied 1 month after exposure (0.05 ± 0.01 endothelial cells per length of basement membrane compared to 0.04 ± 0.02 cells in control animals), but this difference did not reach statistical significance. However, there were significant differences in both the thickness of the media and the number of smooth muscle cells per length of basement membrane 1 month after exposure (Figure 8). Both the number of smooth muscle cells and the thickness of the smooth muscle cell layer were nearly doubled in asbestos-exposed mice compared to sham-exposed controls.

We have seen no asbestos fibers in the vessel lumens or walls, even 1 month after exposure, when fibers commonly are observed in interstitial cells and tissues of the alveolar duct bifurcations.[4,5,23,24]

Discussion

An increase in ^{3}H-thymidine incorporation by endothelial and smooth muscle cells of small vessels adjacent to the first alveolar duct bifurcations in the lungs of mice has been demonstrated 19 to 72 hours after inhalation of chrysotile asbestos. Both arterioles and venules between 20 and 150 μm in diameter were affected. Labeled nuclei of capillary endothelial cells rarely were encountered, and this is consistent with the findings of Chang et al.,[5] who showed that the number and volume of capillary endothelial cells were not significantly altered in rats 48 hours or 1 month after a single exposure to asbestos.

Interestingly, more of the vessels next to terminal bronchioles were labeled than those peripheral to the small airways. This may be because the adjacent vessels are closer to the asbestos fibers, which have been translocated and retained in interstitial cells and tissues.[4-6,23,24] We know that the labeling of alveolar type II and interstitial cells,[6] as well as the interstitial changes determined by morphometry,[5] are confined to the first alveolar duct bifurcations. Therefore, it is not unreasonable to expect that the cellular reaction in the vessels would follow a similar pattern.

To our knowledge, there are no other reports demonstrating incorporation of ^{3}H-TdR simultaneously by both endothelial and smooth muscle cells in the small vessels, other than during wound healing or neoplastic growth (oral personal communication, November 1988, Dr. J. Folkman, Department of Surgery, Harvard University Childrens Hospital). However, simultaneous ^{3}H-TdR incorporation has been demonstrated in larger vessels, in models of arterial injury[25,26] and pulmonary hypertension.[27] Apparently, a brief asbestos exposure can cause a mitogenic response in both smooth muscle and endothelial cells of small pulmonary vessels. This seems particularly significant when considering the extremely low replication rate of these cells in normal adult humans and animals[11-14] and that labeling in sham-exposed animals is zero. The

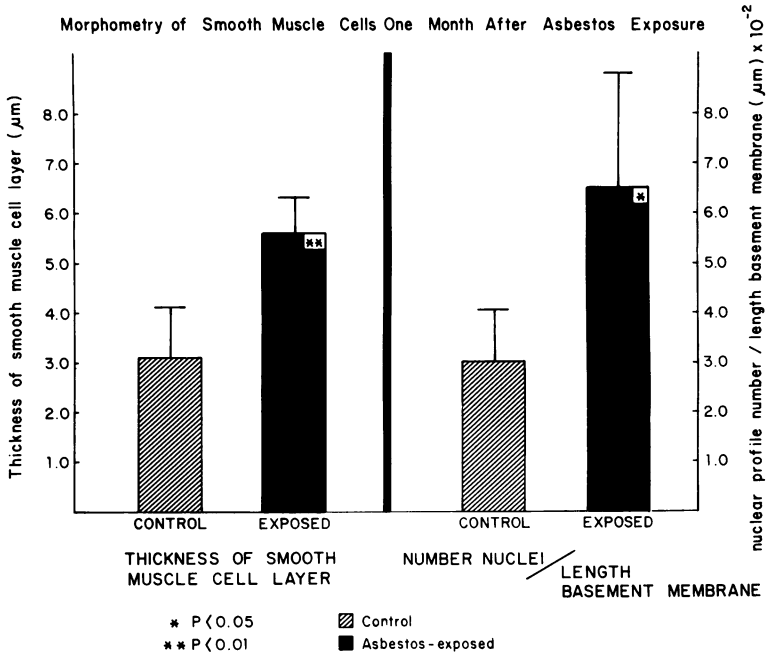

Figure 8. *Ultrastructural morphometry demonstrated that the thickness of the smooth muscle cell layer and the number of smooth muscle cells per length of basement membrane were approximately doubled in vessels of asbestos-exposed mice compared to sham-exposed controls 1 month after exposure. Statistical comparisons were made using a two-sided* t*-test.*

responses in the vessels 19 to 72 hours after exposure are likely to be biologically significant because this time course coincides with that of bronchiolar and alveolar epithelial and interstitial cell labeling observed at first alveolar duct bifurcations in asbestos-exposed rats and mice.[6,28] These initial changes then progress to focal fibrotic lesions.[5] The parallel time course of the early proliferative responses suggests that similar stimuli could be responsible for ^{3}H-TdR incorporation by the different cell types. Labeling of interstitial cells in sham-exposed control animals was less than 1%, in accordance with control levels of interstitial cell labeling reported in other studies.[6,29,30] Incorporation of 3H-TdR in interstitial cells surrounding the vessels was no greater than that observed in interstitial cells surrounding the terminal bronchioles in rats and mice 24 to 48 hours after a brief asbestos exposure.[6,28] As we have seen in other studies,[8,28] rats and mice show a similar reaction to asbestos inhalation. It is interesting that carbonyl iron, which has been found to cause no ^{3}H-TdR incorporation and no fibrotic lesions 1 month after chrysotile exposure in this rodent model,[6] also caused no response in the vessels.

The thickness of the smooth muscle cell layer and the number of smooth muscle cells is significantly increased 1 month after exposure. The physiologic significance of this hyperplastic response of the smooth muscle cell layer in these animals is not clear. The abnormal growth of blood vessels often is associated with damage to endothelial cells[25] and changes in the basement membrane.[31,32] We have observed no morphologic changes indicating cytotoxicity or the death of endothelial or smooth muscle cells, nor was there any evidence that asbestos inhalation caused an alteration in the basement membrane. In studies of hypoxia and crotalaria-induced hypertension, Meyrick and Reid[26,27] showed that 3H-TdR uptake in endothelial cells after cellular injury occurred 7 to 14 days after the exposure. Also, Koo and Gotlieb[33] recently showed that peak incorporation of ^{3}H-TdR by vascular cells occurred 5 to 7 days after mechanical denudation of the endothelium in cultures of aortic tissue. The proliferative response to injury observed in these studies occurred days to weeks later than the peak incorporation seen here in the asbestos-exposed animals. On the other hand, Polverini and coworkers[34] showed peak labeling of endothelial cells 48 hours after the induction of delayed hypersensitivity reactions. Interestingly, this coincided with the time of peak monocyte infiltration and the endothelial response was attributed to the release of unidentified mitogenic factors.

A mechanism to explain the rapid mitogenic response of the vascular cells to asbestos is lacking. Although deposited asbestos fibers are taken up by the type I epithelium and are translocated to the alveolar interstitium,[4-6,24] fibers do not appear to be translocated to the vessels. A lack of fibers and no apparent injury to cells in the vasculature suggest an indirect mechanism of asbestos-induced proliferative response. The fact that many different cell types in closely apposed anatomic regions simultaneously respond so rapidly after asbestos inhalation suggests that diffusible growth mediators are released, rather than the occurrence of mechanical or toxic injury, which appears to cause a more delayed ^{3}H-TdR incorporation by vascular cells, as discussed above.[26,27,33]

There are many growth factors that have the capacity to stimulate the proliferation of endothelial and smooth

muscle cells in culture[35] and induce new blood vessel growth in angiogenesis assays.[36] These same factors could influence cells in the lung. Possible sources of mitogenic stimuli are the alveolar and/or interstitial macrophages that accumulate in response to asbestos deposition and complement activation.[5,8] The anatomic distribution of macrophages and the timecourse of macrophage accumulation correspond to the location and timecourse of the mitogenic response of cells in the vessels and in adjacent bifurcations and terminal bronchioles.[5,6,8-10] Activated macrophages are known to secrete a variety of factors that can mediate the growth of endothelial, smooth muscle, and interstitial cells in culture.[35,37,38] Macrophage-derived growth factors that induce the proliferation of fibroblasts[39-41] and increase ^{3}H-TdR incorporation by type II epithelial cells[42] *in vitro* have been clearly demonstrated. Macrophages and macrophage-conditioned medium cause endothelial cell proliferation in a variety of assays,[16,43-48] and can stimulate proliferation of endothelial cells *in vitro*. Tumor-associated macrophages similarly have been shown to secrete substances that are angiogenic and induce endothelial cell proliferation *in vitro*.[49] In addition, macrophages secrete transforming growth factor-α,[50] an angiogenic peptide that is a potent growth factor for epithelial cells.[51] Macrophages also release fibroblast growth factors,[52,53] tumor necrosis factor,[54-56] interleukin-1,[57-59] prostagladin E_2,[60] and leukotrienes,[61] all of which are angiogenic or mitogenic for endothelial cells and/or smooth muscle cells. Macrophages also make transforming growth factor-β,[62] which does not stimulate endothelial cells *in vitro* but is active in angiogenesis assays.[63] Platelet-derived growth factor, a potent mitogen for smooth muscle cells and fibroblasts,[64,65] is made by both macrophages[66-69] and endothelial cells.[70] Thus there are likely to be at least several cell types involved in a complex system of paracrine regulation of the rapid response observed in the small pulmonary vessels. We have not observed any accumulation of neutrophils or platelets at the sites of ^{3}H-TdR incorporation[3,5,8] and have no reason to suspect that these cells are involved in the response.

Presently there is not enough information to allow an understanding of which cell types and growth factors are interacting to cause the vascular response *in vivo*. The candidates for the factors that could cause the increase in ^{3}H-TdR incorporation and hyperplasia obviously are numerous and complex, as discussed above. Because the asbestos-induced response occurs so rapidly after exposure and in the absence of overt signs of inflammation, other than macrophage accumulation, we postulate that these cells release growth factors that at least initiate the pathobiologic responses of hyperplasia and local fibrogenesis. This model provides an anatomic basis for choosing the cell types and time periods for ongoing *in vitro* studies designed to characterize the factors involved and the cells that produce them.

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