Cellular and Molecular Mechanisms of Asbestosis*

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Occupational and experimental exposure to asbestos is associated with the development of peribronchiolar and interstitial pulmonary fibrosis.[1] Like other asbestos-associated diseases of the respiratory tract (*ie*, mesothelioma and bronchogenic carcinoma), asbestosis appears to involve alterations in differentiation and proliferation of affected cell types. Whether the disease process involves either an increase in replication of fibroblasts in the lung or an enhanced ability of individual fibroblasts to produce more collagen or both is uncertain. To address this question, we used a combination of *in vitro* and *in vivo* approaches.

Experiments Using Lung Fibroblasts Exposed to Asbestos *in Vitro*

A normal fibroblast cell line derived from Fischer 344 rat lung (RL-82 obtained from Dr Marlene Absher, Department of Medicine, University of Vermont) was maintained in Minimal Essential Medium (MEM, GIBCO) containing 10% fetal calf serum and exposed to crocidolite asbestos (UICC reference sample) at various concentrations in medium (*ie*, 1, 2.5, and 5 μg/cm² dish) when cells reached approximately 80% confluency. At 24-hour intervals, 10^{-4}M ascorbate was added to all plates. Cells then were pulsed with ^{3}H-proline (10 μCi/ml medium) for 2 hours prior to assays. After homogenization of cells and boiling for 10 minutes to inactivate proteases, cell samples were assayed in the presence and absence of purified bacterial collagenase (Advance Biofactures Corp; ~40 U/assay tube) to determine the ratio of newly synthesized collagen to noncollagen protein.[2] At each period, ^{3}H-thymidine, or ^{3}HT (1 μCi/ml medium) was added to additional plates for 1 hour before preparation for autoradiography.[3] Control (nonasbestos exposed) cells were treated identically. Results show an increase in total cell layer-associated collagen at 48 and 72 hours after addition of asbestos to RL-82 cells (Table 1). Increased replication of fibroblasts as determined by numbers of cells incorporating ^{3}HT did not occur under these circumstances (data not shown).

To determine whether the enhanced synthesis of total cellular collagen per nanogram of DNA reflected an increased accumulation of collagen type I mRNAs in fibroblasts exposed to asbestos, three recombinant plasmids containing type I collagen-specific sequences ($p\alpha_1R_1$, $p\alpha_1R_2$, $p\alpha_2R_2$) were obtained from C. Genovese, Ph.D. (Department of Pediatrics, University of Connecticut).[4] Quick-blot analysis consistently failed to show a difference between the amounts of procollagen type I mRNAs in control and asbestos-exposed cells. Subcellular fractionation studies now are in progress to determine whether asbestos-induced synthesis of procollagen reflects a partitioning of procollagen mRNAs into polysomes, a phenomenon occurring after exposure of lung fibroblasts to bleomycin.[5]

Table 1—*Amounts of Cell Layer-Associated Collagen in Normal Rat Lung Fibroblasts (RL-82) Exposed to Crocidolite Asbestos (N = 4 per group)*

	Total Collagen (cpm/ng DNA)		
Group	24 hr	48 hr	72 hr
No asbestos	8.52 ± 1.13	18.38 ± 0.94	23.66 ± 1.34
Asbestos			
1.0 μg/cm²	6.39 ± 0.90	25.59 ± 1.89*	21.92 ± 1.58
2.5 μg/cm²	5.7 ± 1.15	24.75 ± 2.18*	19.55 ± 2.88
5.0 μg/cm²	8.95	24.05 ± 2.06†	37.69 ± 1.66‡

*p<.02 (Student's *t* test adjusting for multiple comparisons between groups).
†p<.05.
‡p<.001.

Inhalation Experiments

The work described above indicates increased synthesis of procollagen by individual cells after exposure of rat lung fibroblasts to asbestos. To determine whether abnormal proliferation of fibroblasts in the lung also occurs after inhalation of asbestos, 6–8-week-old male Fischer 344 rats (n = 3–5/group) were exposed to crocidolite asbestos for 30 days (5 hr/day, 5 days/week) using a modified Timbrell generator. Twenty-four hours before removal of the lungs for vascular perfusion, the rats were injected with ^{3}HT (2 μCi/g). After the lungs were embedded in paraffin, alternative 3-m tissue sections were prepared for histology and stained with Masson's trichrome for detection of collagen. Additional slides were processed for immunochemistry using an antibody to copper-zinc superoxide dismutase (SOD) developed in this laboratory.[6] This allowed us to differentiate macrophages (SOD-positive) from fibroblasts (SOD-negative) in the interstitium of the lung. After dipping and development for autoradiography, slides were counterstained with hematoxylin before counting by light microscopy using 1,000× magnification. Eight to 12 fields on each slide were evaluated to determine the percentage of labeled bronchiolar epithelial, alveolar epithelial and interstitial cells (SOD-negative). In contrast to those of control subjects, the lungs of asbestos-exposed animals exhibited fibrotic changes as determined by increased deposition of trichrome-positive mate-

Table 2—*Quantitation of Numbers of Cells Incorporating ^{3}H-Thymidine in Fischer 344 Rat Lungs Exposed to Asbestos for 30 Days (N = 8-12 fields/slide)*

	Labeling Index, %	
	Control	Asbestos-exposed
Bronchiolar epithelial	.78 ± .17	1.26 ± .25
Alveolar epithelial	.8 ± .2	4.13 ± .87†
Interstitial (SOD −)*	1.13 ± .2	3.07 ± .46†

*As detected with an antibody to copper-zinc SOD prepared in this laboratory (Mossman et al, personal communication).
†p<.001 (Student's *t* test adjusting for multiple comparisons between groups).

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rial originating at the alveolar duct.[7] Autoradiography revealed that numbers of labeled alveolar epithelial cells and interstitial fibroblasts were increased significantly in asbestos-exposed lungs (Table 2).

In contrast, the labeling indices of bronchiolar epithelial cells in both control and asbestos-exposed lungs were comparable.

Conclusions

Our work suggests that at least two mechanisms result in asbestosis. On one hand, asbestos stimulates increased synthesis of total cellular procollagen by individual fibroblasts. This phenomenon does not reflect an increase in steady-state levels of procollagen type I mRNAs. In addition, interstitial fibroblasts have an enhanced replicative potential in the lung after inhalation of asbestos by rats.

References

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Asbestos-induced Modulation of Release of Regulatory Molecules from Alveolar and Peritoneal Macrophages*

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Asbestos inhalation causes several immunologic abnormalities in exposed individuals and experimental animals,[1-3] and it has been suggested that these phenomena have a role in the induction of pulmonary fibrosis and could be related to alterations of macrophage-mediated regulatory activities.[4] To investigate whether asbestos could affect the release of immunoregulatory molecules from macrophages, we have studied the effect of the exposure *in vitro* of mouse resident alveolar (AMø) and peritoneal macrophages (PMø) to nontoxic concentrations of asbestos on their suppressive capacity on the mitogen-induced proliferation of syngeneic spleen lymphocytes, on their ability to release arachidonic acid metabolites and superoxide anion (O_2^-) in response to zymosan and on the spontaneous release of interleukin 1 (Il-1). PMø purified by adherence and AMø obtained by bronchoalveolar lavage from 8-16-week-old C3H/HeN mice were cultivated in flat-bottom wells in the presence of control medium, latex beads, or UICC asbestos amosite, 80 μg/ml, for 21 hours. The medium was then carefully aspirated and the Mø further processed. Suppressive capacity was evaluated as previously described[5] by adding syngeneic spleen cells stimulated with an optimal dose of Con-A and evaluating the incorporation of tritiated thymidine after 72 hours of culture. The release of O_2^- was measured by ferricytochrome c reduction after stimulation with opsonized zymosan. Prostaglandin E_2 (PGE_2) and $F_{2\alpha}$, ($PGF_{2\alpha}$), and leukotriene C_4 (LTC_4) were evaluated by radioimmunoassay after stimulation with zymosan 200 μg/ml in medium without serum. Il-1 was evaluated by the C3H/HeJ thymocyte proliferation assay. The presence of interleukin-2 (Il-2) activity in the supernatants was assayed by the ability to support the proliferation of the CTLL cell line. In agreement with previous experiments[6] the exposure of AMø and PMø *in vitro* to amosite caused a dose-dependent decrease in their suppressive capacity, whereas phagocytosis of latex had no effect. This phenomenon was not due to loss of cell viability as judged by LDH release and trypan blue exclusion, and was paralleled by a marked reduction of the ability to produce O_2^-. Asbestos exposure caused a small increase of spontaneous PGE_2 production from both Mø populations; however, it had different effects on the zymosan-induced release of PGE_2 and $PGF_{2\alpha}$ from AMø and PMø. In fact, while PMø showed a reduction of the release of these metabolites after exposure to asbestos compared with untreated cells, AMø showed a marked increase. The release of LTC_4, however, was decreased in both Mø populations. Latex beads had minimal effects on these activities. In addition, amosite but not latex beads induced the release of Il-1 in cultures of AMø and PMø. Il-1 activity was present in the supernatants during the first 24 hours of culture, and the release continued during further incubation, even in serum-free medium. No Il-2 activity or dialyzable inhibitors were found in the supernatants.

These data, summarized in Figure 1, confirm previous

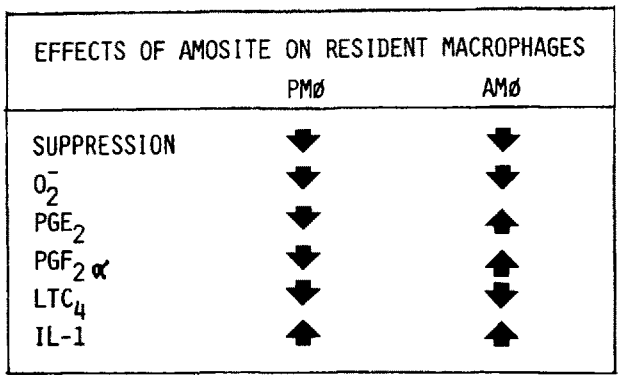

Figure 1

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