Oc
cupational and experimental
exposure to asbestos is asso-
ciated with the development of
eribronchial and interstitial
pulmonary fibrosis. Like other
asbestos-associated diseases of
the respiratory tract (ie,
mesothelioma and bronchogenic
carcinoma), asbestos appears to
involve alterations in differen-
tiation and proliferation of af-
cected cell types. Whether the
disease process involves 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, 10⁻³ M ascorbate was
added to all plates. Cells then were pulsed with ³H-proline
(10 µCi/ml medium) for 2 hours prior to assays. After
homogenization of cells and boiling for 10 minutes to inacti-
vote proteases, cell samples were assayed in the presence and
absence of purified bacterial collagenase (Advance Biofac-
tures Corp; —40 U/assay tube) to determine the ratio of
collagen or both is uncertain. To address this question, we
used a combination of in vitro and in vivo approaches.

*As detected with an antibody to copper-zinc SOD prepared in this
laboratory (Mossman et al, personal communication).

tp<.01 (Student's t test adjusting for multiple comparisons be-
between groups).

Collagen reflects a partitioning of procollagen mRNAs into
polysomes, a phenomenon occurring after exposure of lung
fibroblasts to bleomycin.¹

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 ³H thymidine
(2 µCi/g). After the lungs were embedded in paraffin, alternative
3-mm tissue sections were prepared for histology and stained
with Masson's trichrome for detection of collagen. Additional
slides were processed for immunohistochemistry using an
antibody to copper-zinc superoxide dismutase (SOD) developed
in this laboratory. This allowed us to differentiate macro-
phages (SOD-positive) from fibroblasts (SOD-negative) in
the interstitium of the lung. After dipping and development
for autoradiography, slides were counterstained with hema-
toxylin 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 epider-
ithelial, alveolar epithelial, and interstitial cells (SOD-nega-
tive) in the lungs of control subjects, the lungs of
asbestos-exposed animals exhibited fibrotic changes as deter-
mined by increased deposition of trichrome-positive mate-
rial.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>No asbestos</td>
<td>8.52 ± 1.13</td>
<td>18.38 ± 0.94</td>
<td>23.66 ± 1.34</td>
</tr>
<tr>
<td>Asbestos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 µg/cm²</td>
<td>6.39 ± 0.90</td>
<td>25.59 ± 1.86*</td>
<td>21.92 ± 1.58</td>
</tr>
<tr>
<td>2.5 µg/cm²</td>
<td>5.7 ± 1.15</td>
<td>24.75 ± 2.18*</td>
<td>19.35 ± 2.38</td>
</tr>
<tr>
<td>5.0 µg/cm²</td>
<td>8.95</td>
<td>24.05 ± 2.06†</td>
<td>37.69 ± 1.66†</td>
</tr>
</tbody>
</table>

*tp<.02 (Student's t test adjusting for multiple comparisons be-
between groups).
†tp<.001.

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Asbestos-exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchiolar epithelial</td>
<td>0.78 ± 0.17</td>
<td>1.26 ± 0.23</td>
</tr>
<tr>
<td>Alveolar epithelial</td>
<td>0.8 ± 0.22</td>
<td>4.13 ± 0.85</td>
</tr>
<tr>
<td>Intersitial (SOD -)†</td>
<td>1.13 ± 0.22</td>
<td>3.07 ± 0.46†</td>
</tr>
</tbody>
</table>

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

*From the Departments of Pathology and Biochemistry, University of
Vermont, Burlington.
Supported by Pulmonary SCOR grant P50HL4212 from the
National Heart Blood and Lung Institute. Dr. Shatos is a Parker B.
Francis Fellow of the Puritan-Bennett Foundation.
Asbestos-induced Modulation of Release of Regulatory Molecules from Alveolar and Peritoneal Macrophages*

P. Sestini, M.D.; A. Tagliabue, Ph.D.; M. Bartalini; and D. Boraschi, Ph.D.

Asbestos inhalation causes several immunologic abnormalities in exposed individuals and experimental animals, and it has been suggested that these phenomena have a role in the induction of pulmonary fibrosis and could be related to alterations in macrophage-mediated regulatory activities. 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 (AMφ) and peritoneal macrophages (PMφ) 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 (O2-) in response to zymosan, and on the spontaneous release of interleukin 1 (IL-1). PMφ purified by adherence and AMφ obtained by bronchoalveolar lavage from 8-to-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 Mφ further processed. Suppressive capacity was evaluated as previously described 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 O2- was measured by ferricytochrome c reduction after stimulation with opsonized zymosan. Prostaglandin E2 (PGE2) and Fαα (PGFαα), and leukotriene C4 (LTC4) 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, the exposure of AMφ and PMφ 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 O2-. Asbestos exposure caused a small increase of spontaneous PGE2 production from both Mφ populations; however, it had different effects on the zymosan-induced release of PGE2 and PGFαα from AMφ and PMφ. In fact, while PMφ showed a reduction of the release of these metabolites after exposure to asbestos compared with untreated cells, AMφ showed a marked increase. The release of LTC4, however, was decreased in both Mφ 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 AMφ and PMφ. IL-1 activity was present in the supernatants during the first 24 hours of culture, and the release continued 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

CONCLUSIONS

Our work suggests that at least two mechanisms result in asbestososis. 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

4 Genovese C, Rowe D, Kream B. Construction of DNA sequences complementary to rat α1 and α2 collagen mRNAs and their use in studying the regulation of type I collagen synthesis by 1,25-dihydroxyvitamin D. Biochemistry 1984; 23:6210-16

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