Alterations in the Cellular Population of the Alveolar Wall in an Animal Model of Fibrosis

A Morphometric Study

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A reproducible animal model of interstitial pulmonary fibrosis is generated in rodents by intratracheal injection of the antineoplastic drug, bleomycin sulfate. Pulmonary lesions resemble closely those observed in human patients with fibrosis. Fibrotic lesions are most pronounced 3–4 weeks after the drug's instillation. Histologically, increased interstitial connective tissue and hypercellularity are observed. Biochemical assays reveal increased lung collagen and elastin, and physiologic measurements show reduced compliance and decreased lung volumes, as those observed in humans with the disease.

Apparent increases in nonmuscle contractile cells in interstitial regions of humans with fibrosis and in rats with fibrosis induced by intratracheal injection of bleomycin have been reported. These observations correlate well with increased contractile capability of parenchymal strips of lung isolated from fibrotic humans (biopsy and surgical tissue) and bleomycin-treated rats and increased content of nonmuscle contractile proteins in bleomycin rat lung tissue. This study used ultrastructural stereologic methods to examine quantitative alterations in nonmuscle contractile interstitial cells (CIC) in lungs of rats 4 weeks after instillation of bleomycin.

Methods

Four specific pathogen-free (SPF) male Fischer 344 rats (Charles River Breeding Laboratories), approximately 300 g, were instilled intratracheally with bleomycin sulfate (1.2 mg/ml/rat) in saline as described previously. Four other rats were instilled with an equal volume of saline (0.9%) and served as controls. At 28 days postbleomycin treatment, lungs were airway perfusion-fixed in situ with 2% glutaraldehyde in 0.2M sodium cacodylate buffer, pH 7.4, at 350 mOsm, instilled at a pressure of 20 cm H2O and a flow rate of 110-120 ml/minute. After fixation for 30 minutes, the heart and lungs were removed in toto, the trachea clamped immediately with a hemostat, and the free organs immersed in 2% cacodylate-buffered gluteraldehyde at 20°C for 72 hrs.

Lung volumes were determined by water displacement. The right middle lobe was used for calculation of the alveolar fraction of total lung volume. The entire left lung was processed for ultrastructural morphometric analysis using a modification of Weibel's stratified random sampling technique.

The following cell types were classified: fibroblast, CIC (myofibroblast), smooth muscle, type I and II epithelium, endothelium, macrophage, lymphocyte, mast cell, and "indeterminate cell." CICs were identified by the presence of prominent bundles of cytoplasmic microfilaments. A lack of cytoplasmic filament bundles distin-

RESULTS

Cellular changes at 28 days are described in detail elsewhere. Briefly, the most significant finding was a dramatic increase in the population of CIC in fibrotic lungs, almost fourfold in volume and tenfold in number, over control subjects. These changes appeared to occur at the expense of nonfilament-containing fibroblasts, which were essentially the same as those in control subjects in both number and volume.

Table 1—Volume Densities of Noncellular Elements

<table>
<thead>
<tr>
<th></th>
<th>Control†</th>
<th>Bleomycin (28 Days)†</th>
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<tbody>
<tr>
<td>Interstitial matrix</td>
<td>0.399 ±0.018</td>
<td>0.455 ±0.018†</td>
</tr>
<tr>
<td>Collagen</td>
<td>73 ±8</td>
<td>132 ±91</td>
</tr>
<tr>
<td>Elastin</td>
<td>63 ±11</td>
<td>94 ±54</td>
</tr>
<tr>
<td>Other</td>
<td>36 ±5</td>
<td>39 ±4</td>
</tr>
<tr>
<td>Total tissue</td>
<td>729 ±43</td>
<td>956 ±631</td>
</tr>
<tr>
<td>Total interstitium</td>
<td>291 ±20</td>
<td>487 ±433</td>
</tr>
</tbody>
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*All values are expressed as mm, except interstitial matrix, which is a ratio of tissue to interstitium.
†All values are means ± 1 SE.

FIGURE 1. Numerical density of CIC vs degree of fibrosis for bleomycin and control lungs combined. Increase in the numbers of CIC with increasing fibrosis is apparent.
Values for volume density of noncellular interstitium, total tissue, total interstitium, and "interstitial matrix" are listed in Table 1. Noncellular interstitium, composed of collagen, elastin, and "other," increased significantly in the bleomycin-treated lungs. Of this, the volume occupied by collagen and elastin increased significantly, while that occupied by other noncellular interstitial material did not change quantitatively but decreased as a percentage of the interstitium.

In Figure 1, the numerical density of CIC is compared with the "fibrotic index" of the lungs, based on a grading system described previously in which fibrosis is graded from 0 (no fibrosis) to 4+ (severe fibrosis).

**Discussion**

These quantitative results confirm previous qualitative observations, at the light and electron microscopic levels, of increases in CIC in fibrotic human and animal lungs.\(^7\) It is probable that the increase in polymerized actin in these lungs also results directly from increased CIC, since this actin was found to be of the nonmuscle type.\(^6\) One may speculate the increased contractility of strips of parenchyma isolated from lungs of 28-day bleomycin-treated rats could relate to the augmented CIC compartment.

Of special interest is the correlation between the degree of fibrosis and the numbers of CIC (Fig 1). Although these data represent only 8 different animals (4 control and 4 bleomycin), a correlation between increased numbers of CIC and increasing fibrosis is apparent.

What is the significance of large numbers of nonmuscle contractile cells within the interstitium of fibrotic lungs? Other remodeling tissues such as granulation tissue\(^8\) also contain increased numbers of myofibroblasts. These tissues are capable of contraction due to the presence of these cells.\(^9\) Indeed, isolated fibrotic lung tissue from both human patients\(^10\) and bleomycin-treated rats\(^11\) show increased capability for contraction. Within the microenvironment of the fibrotic lung in vivo, one would expect the presence locally of increased levels of inflammatory and/or immunologic mediators, such as histamine, serotonin, platelet-activating factor, macrophage-derived factors, and arachidonate metabolites. Many of these compounds also are potent stimulators of smooth muscle contraction and might also affect nonmuscle contractile cells. Patients with interstitial pulmonary fibrosis and animals with fibrosis induced experimentally demonstrate decreased compliance or increased lung "stiffness." It generally is assumed that this is a passive phenomenon, related to increased interstitial connective tissue. If, on the other hand, nonmuscle contractile cells are responding to the presence of increased local levels of various mediators of inflammation which provoke contraction, it is possible that a portion of the reduced compliance characterizing fibrosis could be based on this action. Thus, pharmacologic intervention with agents that relax CIC or antagonize various inflammatory mediators might alleviate an active component of increased lung stiffness.

**References**