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Immunohistochemical localization of transforming growth factor-β and insulin-like growth factor-I in asbestosis in the sheep model

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Abstract Asbestosis is characterized by increased collagen deposition along the walls of terminal respiratory bronchioles that extends into the alveolar ducts and septae. Alveolar macrophages are activated and release growth factors that stimulate mesenchymal cell proliferation and enhanced formation of extracellular matrix. Both insulin-like growth factor-I (IGF-I), and transforming growth factor β (TGF-β) regulate cellular growth and promote matrix accumulation and are hypothesized to play important roles in asbestosis. We performed immunohistochemistry using polyclonal antibodies to specific synthetic peptides of the three mammalian isoforms of TGF-β (TGF-β1, -β2, -β3) and to IGF-I on lungs of sheep treated intratracheally with chrysotile asbestos. All three TGF-β isoforms were found in bronchial and bronchiolar epithelium, macrophages, and bronchial and vascular smooth muscle in control lungs. The distribution of TGF-β was increased in these lung constituents as fibrotic lesions developed. Fibrotic lesions additionally demonstrated intense immunostaining of all three TGF-β isoforms that localized to the extracellular matrix zones with little staining of interstitial cells. In the control sheep lungs, IGF-I staining was detected in bronchial and bronchiolar epithelium, bronchial glands, bronchial and vascular smooth muscle, endothelium, and macrophages. IGF-I immunostaining was detected in macrophages in peribronchial fibrosis and in fibroblasts along the periphery of and within lesions, but not in the extracellular matrix. Metaplastic proliferating epithelium and macrophages were strongly immunoreactive for IGF-I in advanced lesions. Our data demonstrate different immunostaining patterns for IGF-I and TGF-β in asbestosis, with IGF-I in the cellular periphery and TGF-β in the extracellular matrix consistent with a complementary role in stimulating interstitial fibroblast proliferation and new collagen deposition in areas of active fibrosis.

Key words Insulin-like growth factor I · Transforming growth factor-β · Pulmonary fibrosis

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Introduction

Pulmonary fibrosis results from an excessive accumulation of connective tissue in the lung interstitium. Research on animal models and bronchoalveolar lavage studies in human subjects with interstitial lung disease suggests that pulmonary injury and the recruitment of inflammatory cells, primarily macrophages, initiates a cascade of events mediated by a variety of cytokines leading to lung remodeling and the accumulation of extracellular matrix [1, 3, 4, 6–9, 11, 15, 27, 29, 41].

Asbestosis results from inhalation of fibers that penetrate deep into the lung, reaching the peripheral air spaces where they are phagocytosed by and activate alveolar macrophages (AMs), initiating an inflammatory response [39, 41]. AMs develop morphologic alterations and release mediators that potentiate an inflammatory response [40, 41, 43, 44, 47]. This initial inflammatory response consists of an accumulation of alveolar macrophages in the alveolar ducts and peribronchiolar regions of the terminal respiratory bronchiole, followed by an influx of interstitial macrophages and fibroblasts, which results in interstitial thickening [12, 15, 46]. Migrating asbestos fibers and oxidants released by activated macrophages also injure adjacent cells, including type I alveolar epithelial cells [15, 31, 39, 41], which results in compromise of epithelial integrity and allows access of growth factors and cytokines to the interstitium. As part of the healing process, type II epithelial cell

hyperplasia develops and is accompanied by interstitial fibrosis with deposition of extracellular matrix proteins. Ultimately, fibrosis of the peribronchiolar and interstitial lung tissues becomes the predominant pathologic finding as advanced lung fibrosis occurs [41].

The sheep model of asbestosis has contributed significantly to our understanding of the basic mechanisms of this pneumoconiosis [3, 4, 7–9, 27]. Well-established clinical techniques such as pulmonary function tests, chest radiography, gallium lung scanning, and bronchoalveolar lavage have been applied to this experimental animal model and have shown that this model closely simulates human disease [3–5, 7–9, 27]. Thus, the sheep model of asbestosis is an excellent model to elucidate the role of various cytokines and growth factors in the development of pulmonary fibrosis.

A number of well-characterized cytokines have been found to be produced by inflammatory cells of fibrotic lungs. Activated alveolar macrophages derived from patients with idiopathic pulmonary fibrosis (IPF), a prototypic interstitial lung disorder, synthesize and release exaggerated amounts of mesenchymal cell growth factors and cytokines, including platelet-derived growth factor (PDGF), insulin-like growth factor I (IGF-I), and interleukin-8 (IL-8) [2, 14, 29, 38, 41, 47]. Recently, a study using a bleomycin-induced lung inury in rats as a model for human IPF demonstrated that increased mRNA and protein for transforming growth factor (TGF)-β isoforms, especially TGF-β3, were evident in both the inflammatory and the reparative phases of the disease [42]. TGF-β regulates the growth and differentiation of a variety of cells and induces the synthesis and release of proteins that compose the extracellular matrix [30]. For this reason, they have been implicated in the cause of a variety of fibrotic disorders [10, 34]. There are three TGF-β isoforms that share 76–80% amino acid sequence homology. Each isoform is nearly 100% conserved within the mammalian species examined [30]. TGF-β is secreted as a latent precursor that requires activation for biological activity. Once released from the latent complex the mature dimer of 25 kDa can bind to receptors to elicit function [30]. Thus, TGF-β potential activity is regulated at the level of activation and receptor binding. We have previously demonstrated that asbestos elicits the release of oxidants, fibronectin, PDGF, AM-derived IGF-I, interleukin1β (IL1β) and tumor necrosis factor-α (TNF-α) in short-term in vitro culture of alveolar macrophages lavaged from individuals with asbestosis [39–41, 47]. We report here that IGF-I and TGF-β have different but complementary immunostaining patterns in active fibrotic lesions of asbestosis, which suggests that they may have important cell–cell and cell–matrix interactions contributing to different components of inflammatory and fibrotic lung disease.

Materials and methods

Tissues

Sheep were exposed to chrysotile asbestos fibers as previously described [9]. Briefly, sheep (25–40 kg) were exposed to phosphate-buffered saline (PBS n = 10) only or to 100 mg UICC Canadian chrysotile asbestos fibers (n = 15) in 100 ml PBS every 2 weeks by installation intratracheally after nasotracheal intubation. After 1 year, the exposed sheep were divided into three groups of five sheep: “low” received no further asbestos, “intermediate” continued to receive 10 mg UICC chrysotile every 10 days, and “high” continued to receive 100 mg UICC chrysotile every 10 days until the end of the 2nd year, at which time the sheep were sacrificed. Sheep were sacrificed by standard CO_2 asphyxiation, and the lungs removed and processed according to the recommended protocol of the Pneumoconiosis Committee of the American College of Pathologists [17]. Appropriate animal committee approvals were obtained from the Animal Care Utilization Committee. The asbestos fibers were uniform and well-characterized, with 92% less than 0.25 mm in diameter and 20 mm in length [9].

Silver nitrate staining of asbestos fibers

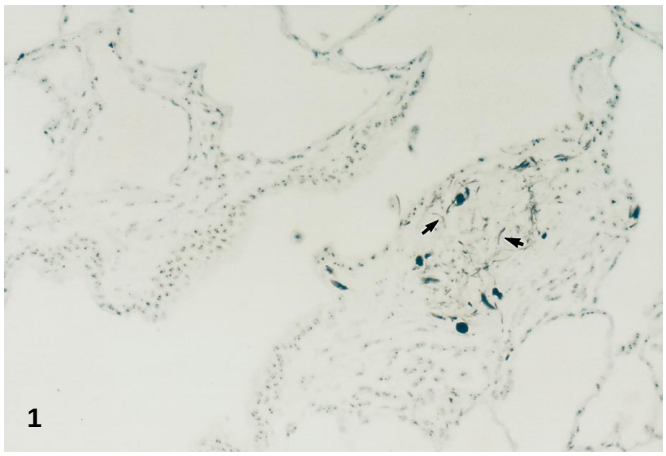
Silver nitrate staining to detect asbestos bodies was performed on tissue sections as previously described [23]. Scoring of tissue fiber burden was performed by one of us (J.J.), who was blinded to the degree of asbestos exposure.

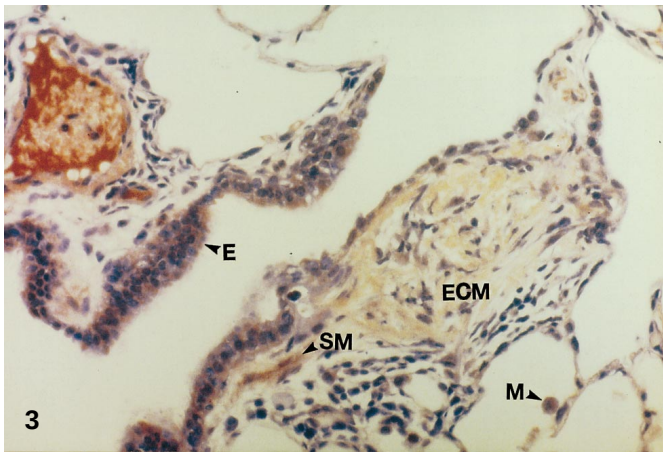
Antibodies

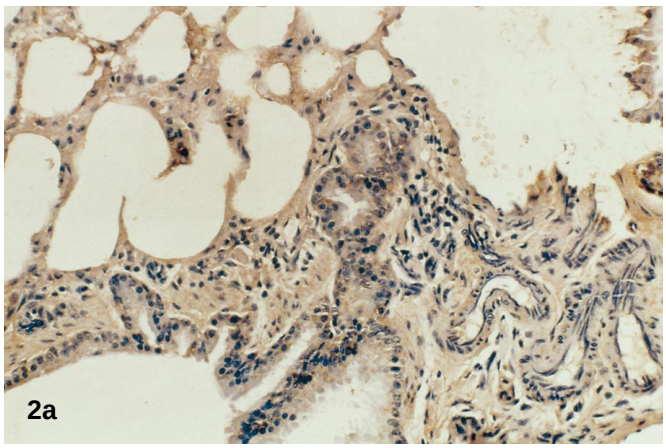
Isoform-specific antibodies to TGF-β1, TGF-β2, and TGF-β3 were produced as previously described [32]. Briefly, peptides of each TGF-β isoform were synthesized using a 430A peptide synthesizer (Applied Biosystems, Foster City, Calif.). The following amino acid residues were used: TGF-β1 and TGF-β2, residues 4–19; TGF-β3, residues 9–20. The amino acid sequence for all three isoforms is identical in sheep and humans. Each rabbit polyclonal antiserum was purified by peptide affinity chromatography and was shown to be specific for the corresponding mature TGF-β isoform and not to cross-react with the other TGF-β isoforms by Western blot analysis [32]. The antibodies recognize both the latent and active forms of TGF-β isoforms, since the epitopes on the mature molecule are available as TGF-β exists as a latent molecule. Rabbit polyclonal anti-human IGF-I antibody, which cross-reacts with sheep and other mammalian sources, was purchased from Incstar Corp. (Stillwater, Minn.).

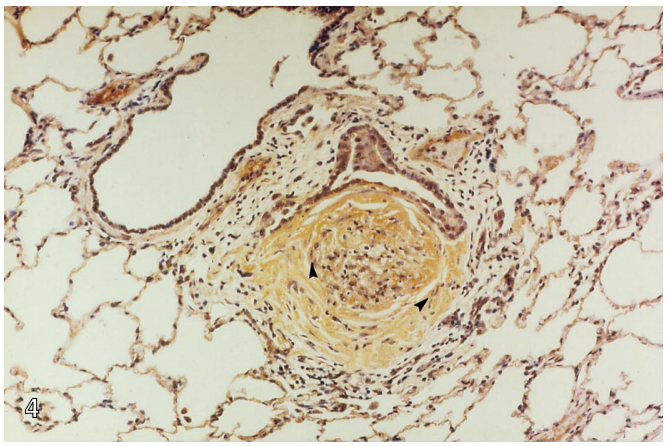
Immunohistochemistry

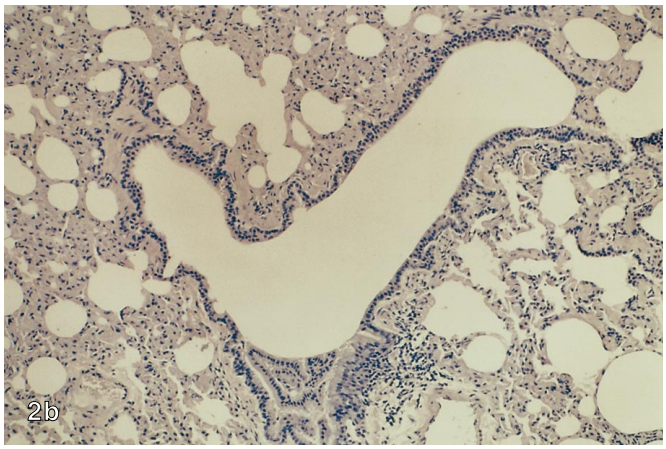
Sheep lungs were fixed overnight in 10% neutral-buffered formalin, dehydrated in increasing concentrations of ethanol, embedded in paraffin wax, and sectioned at 5 μm. The sections were incubated in Tris-buffered saline (TBS, 0.01 M Tris, 0.15 M NaCl, pH 7.4)/0.3% Triton X-100 for 15 min, TBS for 5 min, absolute methanol for 2 min, and methanol/0.6% hydrogen peroxide (vol/vol) for 30 min to quench endogenous peroxide. The slides were then rinsed briefly in methanol followed by three washes in TBS/0.1% (wt/vol.) bovine serum albumin (BSA) for 5 min each. The sections were treated with hyaluronidase [1 mg/ml in 100 mM sodium acetate buffer, pH 5.5, with 0.85% (wt/vol) NaCl] for 30 min at 37°C, then rinsed three times in TBS/0.1% BSA. Nonspecific protein staining was blocked by 1.5% goat serum for 20 min. The sections were incubated with 100–200 μl of primary antibody to give a final concentration of 2.5 μg/ml in blocking solution overnight at 4°C. The sections were rinsed at room temperature with TBS and incubated with biotinylated goat anti-rabbit immunoglobulin for 1 h in a humid staining box at room temperature (Elite Vectastain Kit, Vector Laboratories, Burlingame, Calif.). After extensive washing, the sections were incubated with avidin-biotin-peroxidase complex for 1 hr at room temperature and developed according to the manufacturer’s recommendations. The slides were counterstained with Gill’s hematoxylin #2.











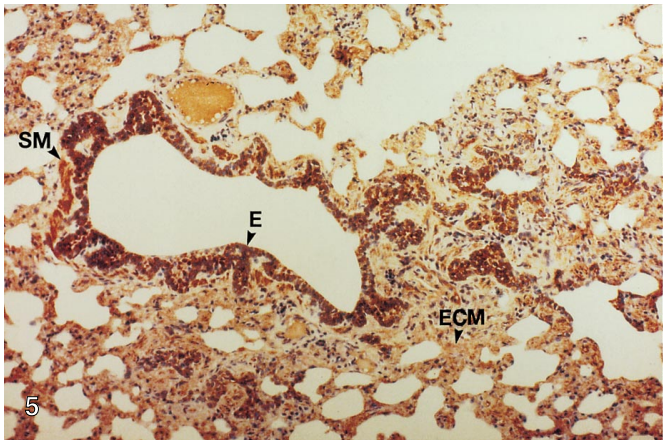


Fig. 1 Peribronchiolar lesion of asbestosis in sheep. Silver nitrate staining demonstrates serpentine chrysotile asbestos fibers *(arrows)* deposited in the extracellular matrix of the lesion. × 200

Fig. 2 A, B Control sheep lung. **A** TGF-β1 staining of PBS-exposed control sheep. Positive staining can be noted in rare epithelial cell. × 200 **B** Fibrotic sheep lung stained with nonimmune sera followed by second antibody shows no immunostaining

Fig. 3 TGF-β1 immunostaining of a serial section shows immunoreactivity in the extracellular matrix *(ECM)* of the fibrotic lesion. TGF-β1 immunoreactivity was also detected in the bronchiolar epithelium *(E)*, macrophages *(M)*, and smooth muscle *(SM)*. × 200

Fig. 4 TGF-β1 immunohistochemistry pattern in peribronchiolar fibrosis from "intermediate"-dose asbestosis in sheep. A nodular fibrotic lesion containing a few interstitial cells is seen surrounded by interstitial fibroblasts. Intense TGF-β1 immunoreactivity for all isoforms was detected predominantly in the extracellular matrix and in occasional interstitial fibroblasts *(arrows)*. TGF-β2 and TGF-β3 showed similar immunostaining. × 40

Fig. 5 TGF-β2 immunoreactivity in intermediate-chrysotile-dose sheep asbestosis. There is interstitial thickening and fibrosis stained for TGF-β2. Marked immunoreactivity was detected in bronchiolar epithelium *(E)*, smooth muscle *(SM)*, and extracellular matrix *(ECM)* deposits in the thickened interstitium

Table 1 Localization of TGF-β isoforms and IGF-I in lungs of sheep exposed to asbestos (*IGF* insulin-like growth factor, *TGF* transforming growth factor, *nt* not tested)

Sheep no.	Pathological description	$AgNO_3$ stain for asbestos fibers	Distribution of TGF-β isoforms and IGF-I															
			Bronchiolar epithelium				Type II pneumocytes				Honeycomb cysts				Macrophages			
			β1	β2	β3	IGF-I	β1	β2	β3	IGF-I	β1	β2	β3	IGF-I	β1	β2	β3	IGF-I
Low dose																		
1	No fibrosis or inflammation	0	+	±	±	nt	–	–	–	nt	–	–	–	nt	–	–	–	nt
2	Peribronchiolar fibrosis, no interstitial fibrosis	+++	++	++	++	++	–	–	nt	–	–	–	nt	–	+	+	nt	+
3	No fibrosis, minimal inflammation	0	nt	±	+	nt	nt	–	–	nt	nt	–	–	nt	nt	±	+	nt
4	No fibrosis, minimal inflammation	+	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	±
5	Minimal inflammation	++	++	++	nt	nt	–	–	nt	nt	–	–	nt	nt	+	+	nt	nt
Intermediate dose																		
6	Peribronchiolar inflammation	++	++	++	++	+	–	–	–	–	–	–	–	–	±	±	±	+
7	Minimal inflammation and fibrosis	++	+	+	+	nt	–	–	–	nt	–	–	–	nt	+	+	+	nt
8	Mild peribronchiolar fibrosis	++	+	++	+	nt	–	–	–	nt	–	–	–	nt	–	–	–	nt
9	Peribronchiolar fibrosis, pleural fibrosis	++	+	+	+	nt	–	–	–	nt	–	–	–	nt	–	–	–	nt
10	Peribronchiolar fibrosis	++	+	++	+	+	–	–	–	–	–	–	–	–	+	+	+	++
High dose																		
11	Peribronchiolar inflammation and fibrosis	++++	+++	+++	+++	nt	–	–	–	nt	–	–	–	nt	+++	+	+++	nt
12	Peribronchiolar inflammation and fibrosis, focal honeycombing	++++	+	++	nt	+	+	++	nt	+	+	++	nt	+	++	++	nt	++
13	Peribronchiolar fibrosis	++	++	±	+++	nt	–	–	–	nt	–	–	–	nt	+	+	+	nt
14	Early honeycombing	++	+++	+++	+++	nt	+++	+++	+++	nt	++	+	++	nt	++	++	++	nt
15	Interstitial fibrosis	++++	++	+++	++	++	++	++	++	+	++	++	++	+	+	+	+	+
Control																		
1	No fibrosis or inflammation	–	+	++	+	+	–	–	–	–	–	–	–	–	–	–	–	+
2	No fibrosis or inflammation	–	+	+	+	nt	–	–	–	nt	–	–	–	nt	–	–	–	nt

Results

Lung histopathology

The lungs of sheep exposed to a “low” cumulative dose of chrysotile asbestos showed an occasional mild increase in inflammatory cells in alveoli and peribronchial regions. The lungs of sheep exposed to the “intermediate” cumulative dose of asbestos demonstrated peribronchiolar fibrosis and inflammation, alveolitis with an abundance of macrophages, and focal honeycombing. Lungs of sheep exposed to the “high” cumulative dose of asbestos showed pathologic changes of increased cellularity, interstitial fibrosis, and focal areas of honeycombing. The honeycomb changes consisted of cyst-like spaces with fibrotic walls lined with columnar epithelial cells and hyperplastic type II pneumocytes. These changes occurred on a background of fibrotic interstitium with collagen deposition. Control sheep showed normal lung histology.

Association of asbestos fibers with areas of fibrosis

Silver staining of histological sections demonstrated the presence of asbestos fibers varying in concentration with

the degree of exposure and pathology. Chrysotile asbestos fibers were detected in the peribronchiolar lesion, considered the earliest lesion to develop in asbestosis. The lesions contained extracellular matrix deposits in the subepithelial region adjacent to clusters of serpentine chrysotile asbestos fibers (Fig. 1). In sheep exposed to low cumulative doses of asbestos, asbestos fibers were visible in only 2 of the 5 animals, associated with minimal inflammation. The sheep exposed to intermediate cumulative doses of asbestos demonstrated a range of concentrations of asbestos fibers, with 2 of the 5 sheep showing fibers in most high-power fields. In the high-cumulative-dose group the sheep showed an intense presence of fibers in practically all fields.

Immunohistochemical localization of TGF-β isoforms

TGF-β isoform-specific antibodies applied to sections of lungs from unexposed sheep demonstrated the minimal presence of TGF-β1, -β2, and -β3 in bronchial and bronchiolar epithelium (Fig. 2 A), and rarely in macrophages, cartilage, bronchial and vascular smooth muscle. There were no consistent differences in the staining patterns of the individual of isoforms. No immunostaining was detected in fibrotic lung sections using nonimmune sera or saline followed by secondary antibody (Fig. 2 B). All three TGF-β isoforms showed similar cell-immunostaining patterns in fibrotic lungs; however, areas of fibrosis and hyperplastic type II pneumocytes showed more intense staining (Table 1). Peribronchiolar fibrosis in the sheep with intermediate asbestos exposure showed staining for TGF-β1 in the extracellular matrix zone, and the lesion contained a cluster of asbestos fibers (Fig. 3, same lesion as in Fig. 1, demonstrating silver stain of fibers). Bronchiolar epithelium and alveolar macrophages stained intensely for TGF-β1. TGF-β2 and TGF-β3 showed a similar pattern of immunostaining (data not shown). All three TGF-β isoforms were detected predominantly in the extracellular matrix of peribronchiolar fibrotic lesions, where occasional interstitial fibroblasts were also immunostained (Fig. 4, TGF-β1). The endothelium and smooth muscle cells of vessels were also immunopositive. Figure 5 demonstrates TGF-β (TGF-β2) immunostaining in intermediate-stage asbestosis, which involves interstitial thickening and fibrosis. Bronchiolar epithelium, smooth muscle, fibroblasts and extracellular matrix deposits in the thickened interstitium stained intensely. Advanced asbestosis is characterized by areas of honeycomb lung with hyperplastic type II pneumocytes and metaplastic epithelium lining cyst spaces. TGF-β isoform (TGF-β3) immunostaining in advanced asbestotic sheep lung was observed in the hyperplastic type II pneumocytes and epithelial cells that comprised the metaplastic epithelium lining bronchiolar duct proliferations in scar tissue (Fig. 6).

Immunohistochemical localization of IGF-I

IGF-I immunostaining in lungs of unexposed sheep was detected in bronchial and bronchiolar epithelium, bronchial glands, bronchial and vascular smooth muscle, endothelium, mesothelium, and in occasional macrophages. In lesions of peribronchiolar fibrosis where macrophages were more abundant, IGF-I staining was prominent in the macrophages. Interestingly, IGF-I was noted in fibroblasts residing mostly at the periphery of lesions and in some fibroblasts within the center of active fibrotic lesions, but not in the extracellular matrix of the lesions (Fig. 7). Figure 7 shows the same section as Fig. 4, where the extracellular matrix stained intensely for TGF-β1; in contrast, the matrix is negative for IGF-I and fibroblasts around the periphery are positive. In more advanced lesions, the metaplastic proliferating epithelium, fibroblasts, and macrophages stained heavily for IGF-I (data not shown).

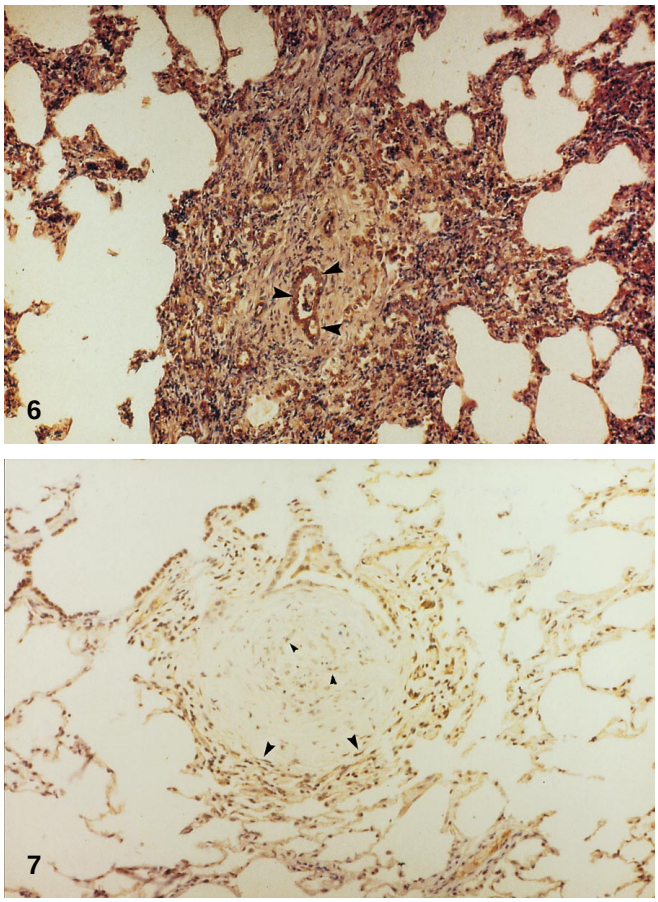


Fig. 6 Advanced asbestosis in sheep with honeycomb lung demonstrating TGF-β3 immunoreactivity. Immunostaining was detected in hyperplastic type II pneumocytes and epithelium lining bronchiolar duct proliferations in scar tissue *(arrows)*. × 40

Fig. 7 IGF-I immunostaining of intermediate-dose asbestosis in the sheep lung. The serial section is the same that as in Fig. 4 demonstrating IGF-I immunoreactivity in fibroblasts *(large arrows)* around the fibrotic lesion and in the few interstitial cell *(small arrows)* trapped within it, but not in the extracellular matrix. There is a striking difference from TGF-β1 staining in Fig. 4, where immunoreactivity was noted in the matrix. × 40

Discussion

Asbestosis is characterized by alveolitis, peribronchiolar fibrosis and, with greater exposure, interstitial fibrosis with honeycombing. We have found different distributions of immunoreactivity for IGF-I and the TGF-β isoforms in experimentally induced asbestos-related lesions in a sheep model. TGF-β isoforms were primarily found associated with the extracellular matrix and occasional fibroblasts in sites of active pulmonary fibrosis. In contrast, IGF-I staining was associated with fibroblasts found at the periphery of fibrotic lesions as well as with the relatively fewer fibroblasts found enmeshed in the central matrix zone of these lesions.

IGF-I and TGF-β in these lesions are complementary and may be synergistic with regard to their function. IGF-I in fibrotic lung disordrs (idiopathic pulmonary fibrosis, asbestosis) was first detected as an alveolar macrophage-derived growth progression activity that stimulated "competent" cells late in G1 to synthesize DNA and proliferate [40]. Lung fibroblasts synthesize DNA and proliferate in response to IGF-I [21, 40]; this effect was blocked in the presence of a specific antibody to the IGF-I receptor [21]. The IGF-I immunoreactivity noted in the resident fibroblasts at the periphery and in the central zones of active peribronchiolar fibrotic lesions in the sheep model of asbestosis shown by our studies may reflect synthesis of IGF-I by these fibroblasts, which may in turn exert autocrine/paracrine stimulation of fibroblast proliferation. IGF-I can also increase human lung fibroblast collagen production [21]. These observations are consistent with our histopathological description, which adds more supporting evidence to the potential importance of IGF-I in the pathogenesis of pulmonary fibrosis mediated through the resident fibroblast and its microenvironment.

TGF-β is a potent chemoattractant for monocytes and neutrophils [37] and induces monocytes to express IL-1 [45], which contributes to fibroblast proliferation by inducing endogenous PDGF expression [36]. Activation of alveolar macrophages results in TGF-β and PDGF secretion [29]. TGF-β is mitogenic for immature fibroblasts [22, 24]. TGF-β stimulates the synthesis of mRNA and protein for matrix components by human lung fibroblasts [18, 25, 35] and also suppresses secretion of proteases that degrade the extracellular matrix and increases the production of protease inhibitors, thereby maintaining its integrity [25, 28]. Therefore its presence results in matrix accumulation.

The immunohistochemical staining pattern in our study showed that all three TGF-β isoforms stained extracellular matrix intensely, with fainter staining of interstitial fibroblasts trapped within or around early active fibrotic lesions. There was strong TGF-β immunoreactivity in the bronchial and bronchiolar epithelium, and alveolar macrophages in all pathologic stages of asbestosis, suggesting these cells as the sources of the TGF-β. These data are consistent with previous reports demonstrating that TGF-β1 immunostaining co-localized to fibrotic foci containing fibroblasts actively expressing procollagen type I and fibronectin mRNAs and bronchiolar and alveolar epithelial cells [13, 26]. In situ hybridization studies have shown TGF-β1 mRNA not in fibroblasts but in clusters of alveolar macrophages adjacent to fibrotic foci [13], suggesting that alveolar macrophages are a major source of TGF-β and that the collagen and ECM proteins secreted by fibroblasts may be induced by a paracrine mechanism of TGF-β action. Future studies of our sheep asbestosis model incorporating in situ hybridization or quantitative PCR are necessary to prove that there is increased synthesis of TGF-β as well as immunohistochemical localization. In a rat model of early asbestosis, TGF-β1 was localized in alveolar macrophages and in some alveolar epithelial cells at alveolar septal tips and bifurcations where paracrine/autocrine action was postulated to contribute to fibronectin deposition near these sites [33]. Marked TGF-β immunostaining was also observed in hyperplastic type II cells adjacent to granulomas. We have also shown prominent immunostaining of epithelial cells in all stages of asbestosis and demonstrated that active early fibrotic foci in our sheep model contain occasional interstitial cells, fibroblasts and macrophages displaying moderate TGF-β immunoreactivity.

In a study of human IPF, bronchiolar epithelial cells, macrophages and type II pneumocytes contained abundant TGF-β mRNA and protein [20]. Direct evidence for the role for TGF-β in the collagen deposition associated with injury-induced fibrosis is shown by the fact that antibodies to $TGF\beta_1$ and $TGF\beta_2$ significantly reduced the collagen accumulation in the bleomycin mouse model of pulmonary fibrosis [19].

Our results also showed intense immunostaining for all three TGF-β isoforms in alveolar macrophages, bronchial and bronchiolar epithelium in all pathologic stages of asbestosis in the sheep model. TGF-β was present, albeit in lesser amounts, in lungs of unexposed sheep, implying that it has an important homeostatic function in nonfibrotic lungs as well as a role in the regulation of extracellular matrix accumulation. The cell types that were immunoreactive were similar to those in the asbestos-exposed lungs; however, the areas of fibrosis and remodelled epithelium in asbestos-exposed lungs showed striking increases in TGF-β expression. The fact that TGF-β immunostaining was largely found in the matrix is consistent with recent reports indicating that TGF-β is converted from a latent to an active molecule while bound to the extracellular matrix by the latent TGF-β-binding protein [43]. Thus, the extracellular matrix may act as a storage reservoir for latent TGF-β molecules. In this regard, fibroblasts have been shown to produce an IGF-dependent protease that cleaves IGF-binding proteins, and although these ideas are speculative, IGF release in the inflammatory microenvironment may have a role in release of bioactive TGF-β [16]. In conclusion, our study suggests that the complementary pattern of immunoreactivity for IGF-I and TGF-β in areas of active fibrosis early in the disease process may represent a functional synergism by these growth factors in the development of fibrosis. IGF-I may stimulate fibroblast proliferation and contribute to collagen secretion, whereas TGF-β

may expand and preserve the volume of extracellular matrix deposited by fibroblasts.

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