MINIREVIEW

Control of Collagen Deposition in Mammalian Lung (43886)

ROBERT S. BIENKOWSKI*,†,[1] AND MARILYN G. GOTKIN†,‡

Pediatric Research Center, Schneider Children's Hospital, Long Island Jewish Medical Center, The Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, New York 11040; Department of Biology,† Queens College of the City University of New York, Flushing, New York 11367; and Department of Biology,‡ Nassau Community College, Garden City, New York 11530*

Abstract. **This review considers the mechanisms controlling collagen deposition in mammalian lung in five different states: normal development, fibrosis, erosion, pneumonectomy, and the steady state. Deposition is the net result of positive and negative processes. The major positive processes are control of cell number and type, regulation of transcription and translation, post-translational modifications, fibril formation, and covalent cross-linking. The negative mechanisms are intracellular degradation, collagenase-mediated degradation, and phagocytosis, and they are integral to the life cycle of collagen.**

Cytokines and growth factors have many and complex effects on all the processes that constitute collagen metabolism. Interleukin-1 and tumor necrosis factor-α can either stimulate or inhibit collagen accumulation, presumably depending on the immediate environment. Interleukin-6 inhibits collagen degradation, and γ-interferon inhibits collagen production. Platelet derived growth factor and fibroblast growth factor have powerful mitogenic effects on connective tissue cells in lung, and can also affect collagen production directly. Transforming growth factor-β activates a battery of processes that uniformly contribute to accumulation of collagen. Transforming growth factor-β may be the "master switch" for a fibrotic program in lung. Therapeutic approaches to controlling lung fibrosis by manipulating cytokine levels are promising.

Prostaglandin E has uniformly negative effects on net collagen accumulation and may play a central role in an erosion program. [P.S.E.B.M. 1995, Vol 209]

Collagen deposition in mammalian lung is determined by various processes operating at different levels. Our aims in this essay are to consider these processes within a global view of deposition in normal and pathologic states; to highlight gaps in existing knowledge, as well as areas of frank disagreement, and to present speculations that may stimulate future investigations.

We concern ourselves here with collagen deposition in five different states: normal development, fibrosis, erosion, pneumonectomy, and the adult steady state. Collagen in the lung is often considered synonymously with fibrosis, and although we started out trying to be evenhanded in discussing collagen in the five states, the lion's share of the treatment goes to fibrosis. And this reflects the emphasis in the literature. In a sense, it is artificial to consider collagen deposition independently of the other components of extracellular matrix such as fibronectin, elastin, proteoglycans, and laminins; and mention should be made

[1] To whom requests for reprints should be addressed at Pediatric Research Center, Schneider Children's Hospital, Long Island Jewish Medical Center, New Hyde Park, NY 11040.

0037-9727/95/2092-0118$10.50/0

at the outset of the many reviews on various aspects of extracellular matrix in lung (1–5).

A great temptation in writing a review, indeed an important function of a review, is to seek generalizations. But one must be careful. For every generalization there is likely to be a counter-example—some cell type, some set of culture conditions, some experimental system—that does not fit. In seeking our own generalizations here we have employed the standard of civil law, "preponderance of evidence," rather than the stricter standard of criminal law, "beyond reasonable doubt." While we emphasize work with lung and lung-derived systems, we also consider the extensive literature describing work in other soft tissues such as skin.

Collagens

There are almost 20 genetically distinct collagens, numbered in approximately the order in which they were discovered. Our knowledge of how the metabolism of a given collagen type is regulated is inversely proportional to its number. Here, we consider mechanisms that affect deposition of collagen I, the major interstitial collagen, and, to a much lesser extent, collagen IV, the principal component of basement membrane. For a succinct discussion of collagens I through XIV, consult reference (6).

Collagen I. A collagen I molecule is made up of three polypeptides called α(I) chains. The secondary and higher order structures of the α-chains are largely determined by two characteristics: a very high content of proline (approximately 20%) that imparts a helical conformation to the chains, and an amino acid sequence in which glycine appears in every third position, $(Gly\text{-}X\text{-}Y)_n$, where n is approximately 300. A collagen I molecule usually consists of two α1(I) chains and one α2(I) chain; however, α1(I) homotrimers can form if α2(I) chains are not made. A collagen molecule is synthesized as a higher molecular weight precursor called procollagen, consisting of three proα-chains with extension peptides at the N and C termini. The most commonly held view of the folding process is that three proα-chains come into register at their C termini and wind about each other in the C to N direction to form a long, thin and rigid triple helix. The glycyl residues lie along the axis at the points of closest approach of the chains to each other. Hydroxyprolyl residues, formed by post-translational hydroxylation of approximately half the prolyl residues, stabilize the triple helix. The extension peptides are cleaved after the molecule is secreted. Collagen molecules can associate laterally and head-to-end to form fibrils. Collagen III is very similar to collagen I in structure and composition, a major difference being that collagen III is a homotrimer of α1(III) chains. Collagen III is also found in the interstitium, usually codistributed with collagen I. For a more extensive discussion of interstitial collagens in lung, consult reference (7).

The genes coding for the proα1(I) and proα2(I) chains, COL1A1 and COL1A2, are located on Chromosome 17 and 7, respectively (8). Although the message levels for proα1(I) and proα2(I) chains are usually maintained fairly tightly in a 2:1 ratio, transcription of the genes can be regulated independently (9, 10). The first intron of COL1A1 contains positive and negative elements (11). The Mov13 mutation in mice, in which a Moloney virus genome is inserted into the first intron (12, 13), provides a particularly instructive example of the importance of these regulatory elements. The insertion blocks transcription in all tissues except teeth, strongly suggesting the existence of tissue-specific *cis* regulatory elements (14, 15). The N and C extension peptides released when procollagen monomers are integrated into fibrils can inhibit (16) or stimulate (17) collagen synthesis. For general discussions of collagen gene regulation, consult reference (18–21).

Collagen IV. The collagen IV molecule is flexible and has numerous interruptions in the triple helical region. There are six genetically distinct proα(IV) chains which can combine in triplets (22). Genes for the proα1(IV) and proα2(IV) chains are arranged head-to-head on Chromosome 13 and transcribed in opposite directions under the control of the same promoter. The significance of the large repertoire of proα(IV) chains is not clear. For reviews, consult Ref. 23 and 24.

Components of Collagen Production

The amount of collagen deposited in a tissue is the algebraic sum of positive (productive) and negative (degradative) processes, and each process can be up- or downregulated. Figure 1 depicts the processes that constitute collagen production. Note that degradation processes, present at many steps along the way, are integral components of lung collagen metabolism.

Positive Processes

Increase in Cell Number and Clonal Activation of Cells with High Collagen Producing Potential. The amount of collagen made in the lung is a direct function of the number of collagen-producing cells. There are two ways to increase the number of cells at a site: replication and recruitment. Fibronectin, a major component of the extracellular matrix, is a potent fibroblast mitogen (25) and chemoattractant (26). Fibronectin fragments, such as might be released during initial stages of injury, are also highly stimulatory for fibroblasts (27, 28). Collagens I and III are synthesized by lung fibroblasts (29), mesothelial cells (30), and endothelial cells (31). Collagen IV is synthesized by type 2 epithelial cells (32, 33), fibroblasts (34), and mesothelial cells (30).

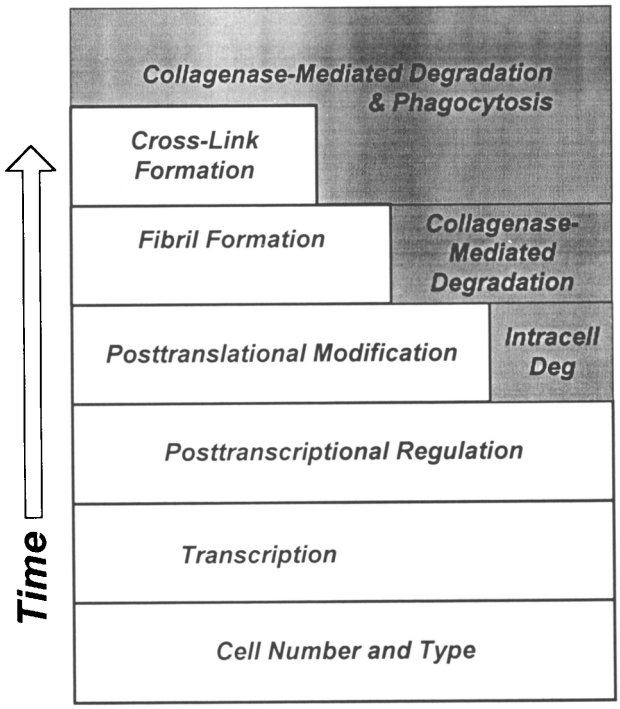

Figure 1. Components of collagen production. Note that degradation processes are integral parts of the general scheme of collagen metabolism.

Far from being undifferentiated "connective tissue cells," fibroblasts are a diverse group and can carry out a number of different functions. Fibroblast heterogeneity has been observed, and subpopulations have been separated and characterized, based on differences in collagen production, proliferation rates, morphology, response to specific inflammatory mediators, accumulation of intracellular lipid, and a variety of cell surface receptors including integrins. Classification of subpopulations based upon only one characteristic can be misleading because a given cell might be considered a member of more than one subpopulation. For comprehensive discussions of these points, consult references (35, 36).

Lipid-laden interstitial cells. A distinction has been made between lipid-laden interstitial cells (LIC) and non-lipid-laden (NLIC) fibroblasts in rat lungs (37). LIC containing abundant intracellular lipid droplets and bundles of myofilaments are found during alveolarization in the postnatal period and following inflammatory injury (suggesting that repair processes mimic development). LIC produce high levels of extracellular matrix components.

Myofibroblasts. Several investigators have described an interstitial cell, termed the myofibroblast, with morphologic and biochemical features of both contractile cells and fibroblasts. Myofibroblasts are prominent during the branching and alveolar phases of development (38, 39) and following injury (40–42). These cells may participate in contracting or organizing collagen matrices.

FI, FII, and FIII cells. Mollenhauer and Bayreuther described three separate classes of fibroblasts in rat skin and lung which they designated FI, FII, and FIII (43). These cells differ in morphology, proliferative properties, and rates of collagen production. FI are spindle-shaped and highly proliferative, and synthesize low levels of collagens I and III. FII are epitheloid in shape, proliferate more slowly than FI, and synthesize more collagen than FI cells. FIII are large, tetraploid, stellate cells that proliferate more slowly than FI or FII, but synthesize higher levels of collagen. The relative numbers of FI, FII, and FIII cells change with donor age.

$Thy1^+$ and $Thy1^-$ cells. Phipps and colleagues have characterized two distinct subpopulations of murine lung fibroblasts based on the presence ($^+$) or absence ($^-$) of a 25-kDa cell surface glycoprotein termed Thy1 (44, 45). Although these cell lines, designated $Thy1^+$ and $Thy1^-$, produce collagens I and III and proliferate at comparable rates, they exhibit morphologic and physiologic differences, particularly in their abilities to synthesize and respond to cytokines (46) and express cell surface markers. Spindle-shaped $Thy1^+$ fibroblasts have a distended endoplasmic reticulum and contain intracellular lipid, and may correspond to LIC cells (47). In contrast, $Thy1^-$ fibroblasts resemble myofibroblasts.

Transcription of Collagen Genes. Transcription of collagen genes can be modulated by a variety of agents such as prostaglandins of the E series (PGEs) and cytokines, especially interleukins-1α and -1β (IL-1α/β), and transforming growth factor-β (TGF-β).

Post-transcriptional Regulation. Collagen production can be controlled by modulating the stability and efficiency of utilization of mRNA (48). In skin fibroblasts, ascorbic acid induces a greater increase in collagen production than can be attributed to the change in mRNA levels alone (49). The N-terminal propeptide of procollagen inhibits translation of mRNA in a nonspecific manner (50). Cytokines can also affect collagen production by altering the stability of mRNA.

Post-translational Modification. Newly synthesized proα-chains associate in the rough endoplasmic reticulum to form triple helical procollagen molecules which are then subjected to several posttranslational processes including hydroxylation of prolyl and lysyl residues, and glycosylation of hydroxylysyl side chains. Several therapeutic approaches to inhibiting collagen synthesis in fibrosis target prolyl hydroxylase, which hydroxylates prolyl residues in proα-chains (51, 52).

Deposition. Collagen fibrils in the interstitium are copolymers of collagens I and III (53–55). There are two different, but complementary, views about how these structures grow. Collagen monomers can, under appropriate conditions, self-assemble *in vitro* to

form fibrils similar to those seen *in vivo* (56). In contrast to this physical chemistry approach, Trelstad and Birk have emphasized that cells synthesizing collagen can also participate in building fibrils. In this view, fibrillogenesis occurs in pockets or extracellular compartments formed by invaginations in the cell surfaces. This model is based on high resolution electron microscopic examination of fibrils in cornea (57), tendon (58) and dermis (59). Furthermore, the cells that participate in fibrillogenesis appear to be rearranged during the process (60). The N- and C-terminal extension peptides of procollagen appear to play important roles in incorporating individual molecules on the surface of a fibril (61), perhaps by orienting or aligning procollagen molecules in relation to the growth regions of the fibril. Fibrils are stabilized by covalent cross-links that form between α-chains in the same and adjacent molecules. The first part of this process is enzymatically mediated by lysyl oxidase. With time, progressively more complicated crosslinks form, uniting several α-chains (62, 63).

In most basement membranes, collagen IV monomers associate at their N- and C-termini to form an open lattice (similar to chicken wire) (6). It is not clear how this lattice is constructed. Chen and Little have described fibrils of collagen IV in organ cultures of embryonic lung (64, 65), but the significance of these structures has not been elucidated.

Negative Processes

Intracellular Collagen Degradation. A significant amount of the collagen synthesized by connective tissue cells is degraded rapidly rather than secreted (66). The basal level of degradation in lung fibroblasts is approximately 15% (67), but degradation increases when cells are induced to synthesize structurally abnormal collagen or exposed to PGE1 (68, 69). The location of this process is not known; some have reported degradation in lysosomes (70), but others have reported conflicting results (71). There are strong indications that intracellular collagen degradation occurs in a distal compartment of the secretory pathway (72). Whether newly synthesized collagen is also degraded in the endoplasmic reticulum, as has been shown for several other secretory proteins (73, 74), is not known.

Degradation Mediated by Collagenases. The triple-helical regions of collagens are refractory to most proteases, however matrix metalloproteinases (MMP) known as collagenases can degrade interstitial and basement membrane collagens. Collagenases are synthesized by fibroblasts and by migratory cells such as neutrophils and macrophages that can invade the lung in response to various chemoattractants. Neutrophil collagenase (MMP-8) is more active against collagen I than collagen III, whereas fibroblast (MMP-1) and macrophage collagenases are equally active against both collagens (75). The collagenases MMP-2 and MMP-9 are active against collagen IV, and they are synthesized by fibroblasts and macrophages (76). For comprehensive reviews, consult references of MMPs (77–79); and for work specifically dealing with lung, consult references (80, 81). Constituitive expression of collagenase is very low in most cells, but can be increased by phorbol ester or cytokines. Such stimulation induces expression of the proto-oncogenes c-*fos* and c-*jun* whose gene products bind to regulatory sites in the promoter regions of collagenase genes (78). Collagenases are secreted as latent proenzymes and must be activated either by mercurial compounds or—more physiologically—by proteases such as plasmin or kallikrein (78, 79). Alveolar macrophages and lung fibroblasts also synthesize inhibitors of collagenase and other MMPs known as tissue inhibitors of metalloproteinases (TIMPs) (82–84). Collagen accumulation might be controlled by modulating MMP production and activation and controlling TIMP levels.

Phagocytosis. Fibroblasts can engulf or phagocytose large pieces of collagen fibrils (85–89). After internalization, the fibrils are degraded, probably by lysosomal proteases.

Regulators of Collagen Synthesis and Degradation

Prostaglandins. Inflammatory cells and fibroblasts synthesize PGE2, which has generally negative effects on fibroblast growth and collagen production in soft tissues. Alveolar epithelial cells exposed to silica *in vitro* also synthesize PGE2 (90). PGE1 and PGE2 decrease collagen synthesis (91–94), increase intracellular collagen degradation (69), and may stimulate collagenase production directly (95). PGE2 inhibits transcription of COL1A1 in lung fibroblasts, but does not affect transcription of COL1A2 (96). One result of this "discordant regulation" would be a surplus of proα2(I) chains relative to proα1(I) chains, and it is possible that increased intracellular degradation (69) is a response to these redundant chains. PGE may regulate collagen production in cyclic AMP-dependent and -independent ways. Fine *et al.* reported that the inhibition of transcription of COL1A1 by PGE is independent of cyclic AMP (96). However, earlier work by Crystal and colleagues strongly indicated that PGE affects collagen production and intracellular degradation by cyclic AMP-dependent processes. The effects of PGE could be duplicated by agents that increase cyclic AMP and inhibited by agents that lower it (93, 94, 97).

It must be emphasized that the foregoing discussion is limited to soft tissue fibroblasts, and the effects of PGE in mineralized tissues seem to be very different. For example, PGE2 stimulates collagen synthesis in fetal rat calvariae (98).

Cytokines. Cytokines—interleukins, interferon, and polypeptide growth factors—are powerful modulators of cell metabolism and can affect every process shown in Figure 1. See references (99, 100) for comprehensive reviews. Cytokines are synthesized by connective tissue cells and by inflammatory cells such as macrophages, and many cytokines can stimulate their own production (101–104). Cytokines interact with cell surface receptors of varying specificity (105). These interactions can initiate signal transduction pathways by increasing cyclic AMP (102) and, very often, by activating tyrosine kinases (106–109). A cytokine can elicit opposite effects in different cells by activating different signal transduction pathways (110). Cytokine activity can be controlled in several ways (see Fig. 2).

Modulating the ratios of related cytokines. Many cytokines are members of families and exhibit significant homology and cross-reactivity. Examples are IL-1α/β, acidic and basic fibroblast growth factor (aFGF and bFGF), and the several homologs of TGF-β. Extensive cross-reactivity of ligands and receptors suggests considerable redundancy in activating signal transduction pathways (111). Alternatively, changes in the relative amounts of homologous ligands or receptors may elicit subtly different cellular responses.

Activating latent forms. Some cytokines such as IL-1β and TGF-β are synthesized as inactive precursors and must be activated in order to exert an effect (112–114). TNF-α is synthesized as a membrane-bound precursor which is liberated by the action of a metalloproteinase (115, 116).

Modulating receptor levels. Several cytokines (e.g., IL-1α/β and TNF-α) interact with multiple receptors and different receptor-ligand interactions may initiate different signal transduction pathways. Cell surface-associated heparan sulfate proteoglycans (HSPGs) can function as cytokine receptors in various ways. Some may have the ability to initiate signal transduction directly, while others may be low affinity receptors that "present" cytokines to high affinity receptors that, in turn, may activate signal transduction pathways. Other HSPGs may function as storage sites for growth factors that could be released to act locally (117).

Competing with antagonists for receptor binding. Soluble receptor antagonists can bind to cytokine receptors but not elicit a response (e.g., the IL-1 receptor antagonist [IL-1ra]) (118).

Modulating levels of soluble receptors and binding moieties. Soluble receptors (e.g., for IL-1) can bind cytokines, limiting their potential to interact with cell surface receptors (106). In addition, some cell surface receptors can be cleaved proteolytically and compete for cytokines (119). (The distinction between receptors, soluble receptors, and binding proteins is often blurred. Some investigators insist that in order to be classified as a receptor a binding moiety must elicit some response from a cell.) Proteins and glycosaminoglycans in serum and matrix proteins can bind cytokines and act either as stores or traps. Examples include the insulin-like growth factor-binding proteins (IGFBPs 1 through 6), which bind insulin like growth factors (IGFs) (120–122), and heparin and HSPG, which bind platelet-derived growth factor (PDGF) (123), FGF (117), and TGF-β (124, 125). Betaglycan is an HSPG that can exist as both a membrane receptor for TGF-β as well as a soluble binding moiety that can inhibit binding to membrane-bound receptors (126). α2-Macroglobulin (α2-MG) can bind several cytokines including TGF-β, TNF-α, IL-6, PDGF, and FGF. In some cases, binding is much stronger when the α2-MG has been activated by a protease such as plasmin. Activated α2-MG-cytokine complexes are cleared rapidly from the circulation (127).

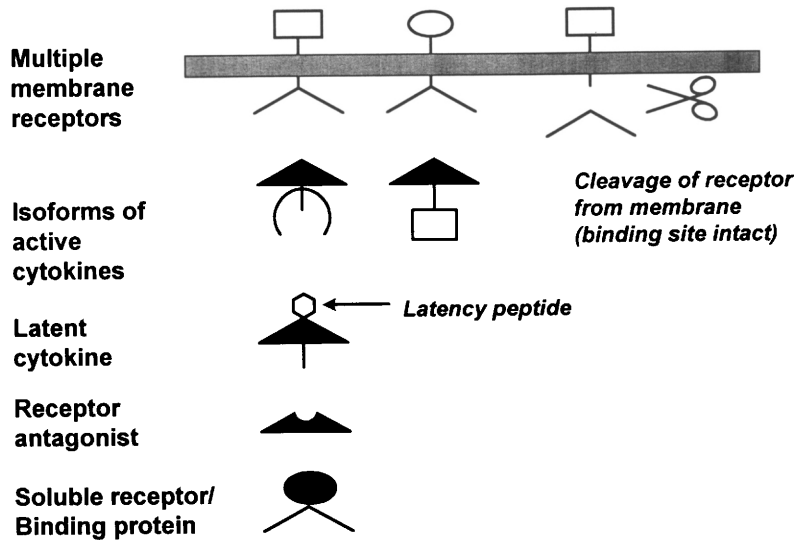

Figure 2. Interactions among cytokines, receptors, and antagonists. Each process represents a potential point at which cytokine activity can be modulated.

Effects of Cytokines on Collagen Accumulation. *Interleukin-1α and -1β and tumor necrosis factor.* IL-1 is synthesized by neutrophils and alveolar macrophages (102, 128). TNF-α is synthesized by alveolar macrophages (102, 129, 130) and is mitogenic for fibroblasts (131–133). There are conflicting data about whether TNF-α is a chemoattractant for fibroblasts (134, 135). IL-1 and TNF-α have been reported to enhance (136) and inhibit (137) collagen production in lung fibroblasts. In dermal fibroblasts, IL-1α/β enhance transcription of collagens I and III, but TNF-α reduces the stability of procollagen mRNA (138). TNF-α also enhances collagen synthesis in pleural mesothelial cells (139), and IL-1 stimulates production of collagen IV in mammary epithelial cells (140). The ability to stimulate production of collagen IV in lung could be part of a mechanism to repair damage to basement membrane.

IL-1α increases collagenase production in alveolar macrophages (141); and IL-1α/β and TNF-α increase collagenase production in lung fibroblasts (142) and cells from other soft tissues (142–144). Synovial fibroblasts do not normally synthesize type IV collagenase (capable of degrading basement membrane collagen), but they can be induced to synthesize it by IL-1β and TNF-α (145). This result has a clear implication for lung response during inflammation, because it shows that an interstitial cell can be reprogrammed to participate in remodeling basement membrane.

The existence of soluble receptor antagonists for IL-1 (IL-1ra) and soluble receptors indicates still another mechanism for modulating cytokine response (106, 118, 146). For example, IL-1ra inhibits the increase in collagenase production in alveolar macrophages normally elicited by IL-1 (147), and IL-1 stimulates TIMP production in many types of cells, including lung fibroblasts (142).

Interleukin-6. Interleukin-6 (IL-6), which can be synthesized by alveolar macrophages (103), endothelial cells (148), and fibroblasts (149), increase synthesis of TIMP in several different cell types including alveolar macrophages (150), lung fibroblasts (142), skin fibroblasts (151), synovial fibroblasts (142, 152–154), and endothelial cells (154). There is one conflicting report that IL-6 does not stimulate TIMP production in human skin fibroblasts (155).

γ-Interferon. γ-Interferon (IFN-γ) is synthesized by lymphocytes (156). It inhibits collagen production in fibrotic lungs (157) and in fibroblasts from normal and fibrotic lung tissue (158–160), and inhibits fibroblast migration in response to chemoattractants such as fibronectin (161). IFN-γ stimulates the incorporation of newly synthesized collagen into matrix by increasing the number of cell surface receptors for collagen (160). In addition, IFN-γ inhibits production in macrophages and fibroblasts of collagenases that can degrade collagens I and IV (150, 162–164). However, there are some conflicting results: Duncan *et al.* found that IFN-γ increased collagenase production in fibroblasts (165), whereas Scharffetter *et al.* found it had no effect (166). Elias *et al.* reported that IFN-γ has a bidirectional effect on lung fibroblast proliferation: it stimulates growth of quiescent cells and inhibits growth of proliferating cells (167). For a general discussion of the effects of IFN-γ on collagen production, consult reference (168).

Transforming growth factor-β. TGF-β is synthesized by fibroblasts and macrophages, and can stimulate its own production in an autocrine/paracrine fashion (169–171). There are several different isoforms, with β1, β2, and β3 being the most common (172, 173). TGF-β is distinguished from most other cytokines by its ability to activate a battery of processes that together increase matrix production (174, 175). We shall argue later that it qualifies as a "master switch" for a fibrotic program.

TGF-β increases collagen synthesis by stimulating expression of COL1A1 (10, 176). It stimulates expression of COL1A2 in mouse NIH/3T3 cells (10, 177) but not in lung fibroblasts (10). The effects of TGF-β on collagen gene transcription appear to be mediated by the binding of nuclear factor-1 or a similar protein to regulatory sites close to the promoter regions of COL1A1 and COL1A2 (177, 178).

TGF-β decreases production of interstitial collagenase (179), increases TIMP production (180, 181), and decreases intracellular collagen degradation (182). TGF-β also does the following: (i) stimulates cell replication in fibrotic lung tissue (183), and in lung fibroblasts in an *in vitro* wound model (184); (ii) increases production of integrins, which are cell surface receptors that anchor interstitial cells to the extracellular matrix (185); (iii) increases prolyl hydroxylase (in scleroderma skin fibroblasts [186]), which is crucial for collagen production; (iv) increases production of lysyl oxidase (187), which catalyzes formation of covalent crosslinks in fibrils between α-chains; (v) increases production of fibronectin (188), which binds to collagen in the extracellular matrix and is a growth factor and chemoattractant for lung fibroblasts (25, 26); and (vi) stimulates production of IL-6 which, in turn, stimulates TIMP production in fibroblasts and monocytes (180, 189, 190).

The extent to which TGF-β is a chemoattractant for lung fibroblasts is unclear. Several groups have reported that it is a powerful chemoattractant for fibroblasts in different tissues (161, 191–194). However, a recent paper by Osormo-Vargas *et al.* presents conflicting findings (195): different batches of TGF-β had no chemotactic effect on different preparations of low passage rat lung fibroblasts or Swiss 3T3 fibroblasts.

There are at least 3 different TGF-β receptors. The

type I and type II receptors are transmembrane proteins with serine/threonine kinase activity, suggesting that upon binding of TGF-β, the receptors activate signal transduction. The type III receptor is an HSPG (betaglycan) that can present TGF-β to a type II receptor (126). It is possible that different responses are elicited depending on which isoform interacts with which receptor (196, 197). However, it has recently been shown that interaction of only the type I and II receptors with either TGF-β1, β2, or β3 is adequate to elicit a range of effects on cell proliferation and gene expression (197).

Fibroblast growth factor. Fibroblast growth factor (FGF) stimulates mitogenesis in several types of cells (198, 199), and also stimulates fibroblast migration (200). It is produced by alveolar macrophages, and cells lavaged from lungs of patients with interstitial disease produce elevated amounts (201). FGF is also produced by lung fibroblasts (202) and endothelial cells (203). FGF decreases collagen synthesis in various cell types including normal and keloid skin fibroblasts (204, 205), and smooth muscle cells (206). In corneal endothelial cells, FGF stimulates synthesis of collagen I and inhibits synthesis of collagen IV (207). FGF stimulates production of interstitial collagenase in skin fibroblasts (208), heart fibroblasts (209), endothelial cells (148, 210), and articular chondrocytes (211). However, it must be stressed that the ability of FGF to stimulate collagen production by increasing the number of collagen-producing cells may be much more significant than whatever direct effect FGF may have on transcription of collagen genes.

Platelet-derived growth factor. Platelet-derived growth factor (PDGF) is a powerful mitogen (212, 213) and chemoattractant (195, 214) for fibroblasts. Different forms are synthesized by lung macrophages (141, 215) and epithelial cells (216). In general, cells lavaged from patients with significant lung disease are much more active in synthesizing this cytokine than cells from normal volunteers (217–219). PDGF increases collagen synthesis in dermal fibroblasts (220), vascular smooth muscle cells (221), and rat periodontal ligament fibroblasts (222). In an excisional wound model, topical application of PDGF increased the amount of collagen as assessed histologically (223). In a very interesting study, Okada *et al.* observed that PDGF suppressed synthesis of collagen IV and increased synthesis of collagen V (an interstitial collagen) in arterial smooth muscle cells (224). This result indicates that PDGF can modulate not only the amount of collagen but also the kind of collagen synthesized. PDGF has been reported to stimulate production of collagenase in dermal fibroblasts (225), and in normal and rheumatoid synovial fibroblasts (145, 226). However, inhibition of collagenase production by PDGF has also been reported (227). As noted for FGF, the powerful mitogenic effect of PDGF on fibroblasts may be much more important in increasing collagen production than any direct effect it has on regulating collagen synthesis at the transcriptional or translational levels.

Insulin-like growth factor-I. Insulin-like growth factor-I (IGF-I) is mitogenic for fibroblasts. It is produced by type 2 alveolar epithelial cells (228), alveolar macrophages (229), and fibroblasts (230). IGF-I stimulates collagen synthesis in lung and skin fibroblasts (228, 231, 232), but it inhibits collagen production and stimulates collagenase production in avian skin fibroblasts (233). Activated alveolar macrophages from patients with interstitial lung disease carry IGF-I receptors, suggesting that IGF-I may stimulate the macrophages to proliferate (229). In turn, proliferating macrophages could increase production of other cytokines that either modulate collagen production directly or stimulate fibroblast replication or migration.

Interactions among modulators. Many modulators interact to either enhance or inhibit each other's effects on collagen production. For example, Elias *et al.* reported that the combined effects of IL-1 and TNF-α on collagen synthesis in lung fibroblasts were either more or less inhibitory than either used alone depending on whether serum was present in the culture medium (136). The combined effects of IFN-γ and TNF-α on collagen synthesis in dermal fibroblasts are greater than the effects of either cytokine used alone (166).

We have already noted that there is some disagreement about whether TGF-β is chemotactic for fibroblasts. Interestingly, there is evidence that this cytokine inhibits the positive chemoattractant actions of other factors such as aFGF (234), fibroblast migration-stimulating factor (235), PDGF (236), and epidermal growth factor (237).

At certain concentrations, the effects of IL-1 and TNF-α on collagenase synthesis in synovial fibroblasts are synergistic (238). PDGF synergistically augments the IL-1-induced increase in collagenase synthesized by dermal fibroblasts (225), but inhibits the IL-1 effect in synovial fibroblasts (239). IFN-γ inhibits the IL-1-induced increase in collagenase production in synovial fibroblasts and alveolar macrophages (163, 238).

Figure 3 attempts to illustrate how interactions among IL-1, PGE2, and TGF-β affect transcription of collagen genes and production of collagenase. The main points are as follows:

- IL-1 and TGF-β can stimulate their own production.
- IL-1 enhances synthesis of PGE2 (137, 167, 240–242). In a very interesting study, Barr *et al.* showed that the effect of IL-1 on PGE2 synthesis was greater in monolayer cultures of dermal fibroblasts than in cells grown in a collagen gel (243). The stimulatory effect of IL-1 on PGE2 synthesis can be

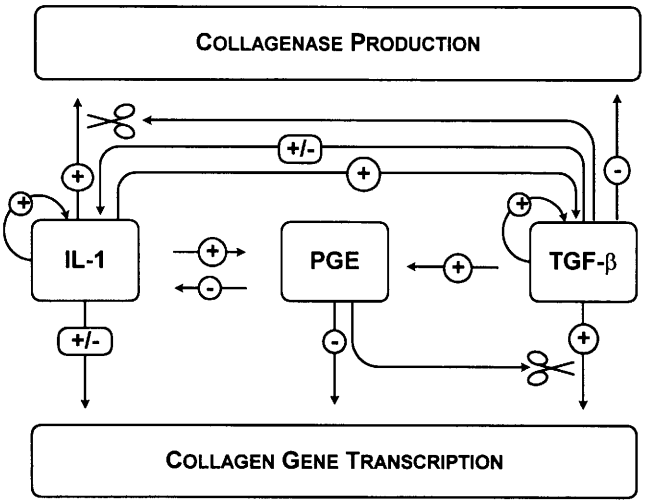

Figure 3. Modulation of collagen gene transcription and collagenase production by PGE, IL-1, and TGF-β. Note the extensive interactions among the modulators.

blocked by IL-1ra (244). There are several reports that PGE2 can inhibit IL-1 synthesis in monocytes and macrophages (245–248). In a separate study, Viherluoto *et al.* found that PGE2 inhibits secretion, but not synthesis, of IL-1 in monocytes (249). In contrast, Bailly *et al.* found that PGE2 had no effect on IL-1 production in monocytes (250). Interestingly, PGE2 can upregulate production of the IL-1 receptor (251).

- TGF-β enhances synthesis of PGE2 in lung fibroblasts (176, 242) and other cell types (252–254). It can enhance (255) and inhibit (256) PGE2 production in monocytes. Taylor *et al.* reported that TGF-β stimulates PGE2 synthesis in rheumatoid synovial cells (257), but Hamilton *et al.* reported no effect (258).
- IL-1 and TGF-β act synergistically to increase synthesis of PGE2 (253, 257).
- IL-1 can increase production of TGF-β in pulmonary endothelial cells (259) and T cells (260).
- TGF-β has been reported to stimulate (261) and inhibit (262) IL-1β production in monocytes.
- TGF-β can also block the effects of IL-1 by stimulating production of IL-1ra (262, 263) and by downregulating IL-1 receptors (264).
- IL-1 can have both inhibitory and stimulatory effects on collagen gene expression. PGE inhibits collagen production; TGF-β stimulates transcription, and PGE counters this effect (176) and also inhibits transcription directly.
- IL-1 stimulates collagenase production in several different cell types. In chondrocytes TGF-β decreases this effect by downregulating the number of IL-1 receptors (265).
- TGF-β inhibits collagenase production (179).

Two notes of caution must be raised regarding this scheme: (i) it combines results from different types of cells; and (ii) the temporal dimension is missing and one cannot appreciate how interactions among the regulators and their effects on collagen and collagenase production might evolve.

As noted, some cytokines can exert opposite effects on collagen metabolism. Figure 4 illustrates how this might happen. Consider a cell that synthesizes a cytokine that has a direct inhibitory effect on collagen production in a target cell. The cytokine also stimulates production of a second cytokine in another cell, which, in turn, has a stimulatory effect on collagen production in the same target cell. Depending on whether interactions between the cytokines and their receptors are permitted or blocked, the net apparent effect of the first cytokine on collagen production might be enhancement, inhibition, or nothing. This situation might occur when IFN-γ, which inhibits collagen production, stimulates PDGF production in alveolar macrophages, which, in turn, can stimulate collagen production (266, 267).

Summary. Many *in vitro* studies of the effects of cytokines on collagen metabolism appear contradictory. It is likely that all the studies are correct in the sense that they illustrate the range of possible responses to cytokines. Perhaps the systems used, mainly cultured cells, are not appropriate and we do not always appreciate their limitations. For example, fibroblasts respond in different ways to cytokines such as IL-1, PDGF and TGF-β depending on whether they are grown on collagen gels or plastic (268). It can be fundamentally misleading to assume that studying the effects of a single cytokine, or even pairs of cytokines, on collagen metabolism in cultured cells can yield valid insights into control mechanisms. The most striking impression one gets from studying diagrams of the interactions of cytokines with cells is the complexity of the networks. It is probably not far off the mark to say that in charting the interactions, all connections

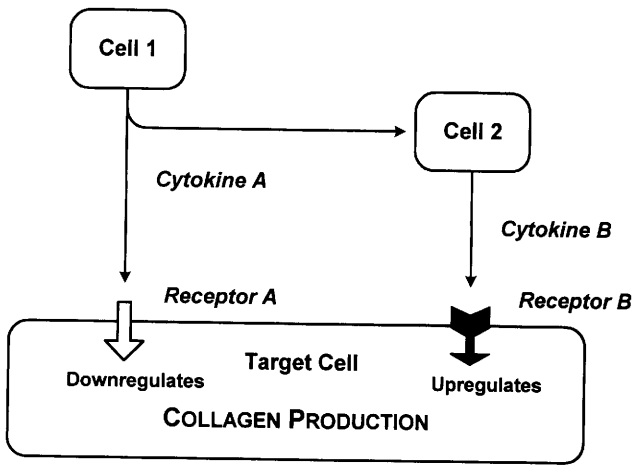

Figure 4. Schematic diagram illustrating how a single cytokine can elicit opposite effects on collagen production.

are possible, although some are more likely than others. Nevertheless, the following generalizations seem reasonable:

- TGF-β enhances collagen production at several levels (more on this later).
- IL-6 increases TIMP production, and so contributes to the net production of collagen.
- IFN-γ inhibits production of collagen and collagenase, and may function to moderate changes in net collagen production.
- IL-1α/β, TNF-α, FGF, and PDGF have variable effects on collagen production. At any given time their effects probably depend on the immediate cellular environment, and in particular on levels of other cytokines. In this sense, it may be appropriate to call these cytokines *coregulators* of collagen metabolism.

Overview: The Matrix Programs in Lung

The various states of the extracellular matrix in lung can be divided into two groups: stable and unstable. (Fig. 5). There is only one stable state, namely the normal adult lung in which net matrix production is zero—the amount of matrix produced is balanced by the amount degraded. However, we speak of balance in a global sense, and there may be regional inbalances. The unstable states can be further classified as centripetal and centrifugal. Development is centripetal because it is characterized by a steady accumulation of collagen, tending toward the adult steady state lung. When subjected to insult or injury, the extracellular matrix responds in one of two ways.

The first possible response is that of a centripetal repair leading back to the steady state. Usually, this response is associated with mild injury and may involve recapitulating certain developmental stages, such as regenerating alveolar epithelium and repairing or replacing some collagen fibrils. The compensatory growth of the contralateral lung following pneumonectomy is also a centripetal state because the net accumulation of collagen approaches a limiting value. The pneumonectomy response exhibits many features of normal development.

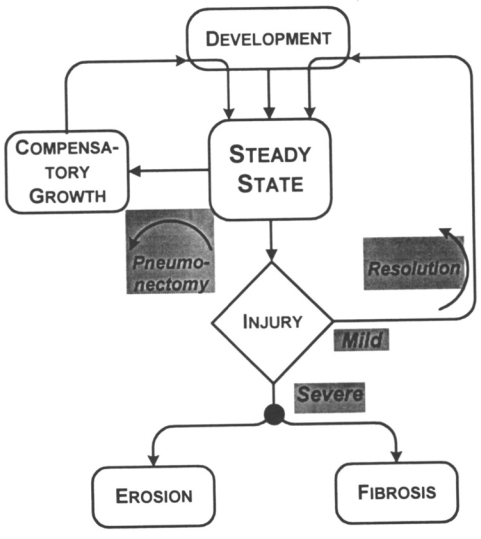

Figure 5. Extracellular matrix "programs" in lung.

The second possible response is centrifugal and leads away from the steady state. The better-known form of this response is fibrosis, defined here as uncontrolled and inappropriate production of extracellular matrix components, particularly collagen I. The other form of centrifugal response is erosion, due largely to uncontrolled proteolysis. Erosion of elastin is accepted in the commonly held picture of emphysema caused by cigarette smoke. We suggest that erosion is to be expected as an end state of collagen accumulation in lung, and we shall adduce below experimental evidence supporting this contention.

Associated with each state is a program that specifies how the component processes of collagen production—cell number, transcription, posttranslational modifications, deposition, and degradation—are modulated.

Normal Development. The current state of extracellular matrix in developing lung is reviewed in reference (269).

Collagen production. In a pioneering study, Bradley *et al.* showed that there was a burst of collagen synthesis during the period of alveolarization in rabbits (270). Message levels for collagen I peak during this period (271).

Collagen fibrils and branching morphogenesis. Collagen fibrils stabilize clefts during the branching period (272). Treating lung rudiments with collagenase or with TNF-α (which probably stimulates collagenase production) inhibits branching, whereas TIMP inhibits the effect of collagenase (174, 273). Branching morphogenesis is normal in the Mov13 mouse, which indicates that collagen I is not absolutely necessary for this process (13). It is possible that collagen III, or perhaps collagen IV arranged in fibrils (64, 65), stabilizes the clefts. It is noteworthy that collagen IV and laminin, which are major constituents of basement membranes, are expressed throughout the epithelium and mesenchyme of embryonic mouse lungs (274), and nidogen, another constituent of basement membranes, is expressed only in mesenchyme (274, 275).

Role of degradation during development. Remodeling—the replacement of one structure by another—is an integral part of many developmental processes. In mouse lungs, there is extensive degradation of interstitial and basement collagens (276), and basement membrane-degrading collagenase is expressed at high levels during the immediate postnatal period when alveolar structures form (277). Constituitive ex-

pression of interstitial collagenase during embryogenesis, due to the action of a transgene, leads to a pathology similar to emphysema (278). A problem in interpreting this work is that the original transgene was constructed with a haptoglobin promoter in order to be expressed in liver. It is not clear why it was expressed in lung.

Modulation of cell types during development. Lipid-laden interstitial cells (LIC) and myofibroblasts are observed in lung during the alveolarization period (279); they are rare in normal adult lung, but reappear during fibrotic response. As noted, LIC synthesize high levels of collagen and myofibroblasts may organize the new matrix.

Role of TGF-β during development. TGF-β is present in high levels in the developing lung (280, 281) and it colocalizes with collagens I and III during branching morphogenesis (282). In targeted gene disruption experiments, TGF-β null mice manifest widespread inflammation, especially in lungs, and die soon after birth (283). However, endogenously produced TGF-β is not absolutely necessary for relatively normal development. There are at least two reasons for this: (i) given the extensive homology among members of the TGF-β family, compensation among the isoforms is possible; and (ii) maternal TGF-β1 can cross the placenta and rescue a TGF-β1 null fetus (284).

Fibrosis. Fibrosis, the excessive accumulation of extracellular matrix, is a response to injury and exhibits many characteristics of wound healing. The major phases of wound healing, exemplified by simple dermal wounds, are hemostasis, granulation, and laying down new extracellular matrix. However, the details of the response depend on the nature of the insult. Skin wounds are usually limited in extent and time, but insults to the lung are often continual. Crystal and colleagues have emphasized that one of the cardinal features of lung fibrosis is an ongoing alveolitis (285). The most prominent features of the collagen matrix in fibrotic lung are an increase in total amount, an increase in the amount of collagen I relative to collagen III, and deposition in the interstitium and alveolar spaces (286, 287). Immunofluorescence and *in situ* hybridization studies suggest that fibroblasts in fibrotic lungs are much more active in synthesizing collagen than cells in normal lungs (288, 289). It is not clear what induces a region of lung to enter the fibrotic program rather than recover from an insult. Damage to the alveolar basement membrane sufficient to allow passage of mesenchymal cells into the alveolar space may be the trigger (175, 285, 290–294). For recent reviews of lung fibrosis, consult references (295–298).

Models for fibrosis. Pulmonary fibrosis can be induced in laboratory animals in various ways (296). Intratracheal administration of the chemotherapeutic agent bleomycin provides an excellent model system for studying fibrosis and has been used extensively (292). Bleomycin has several direct effects on lung cells. For example, it increases mRNA levels of TGF-β, collagen I and collagen III in rat lung fibroblasts (299). This study is very important because it demonstrates a "fibrotic response" that is independent of an inflammatory component. Bleomycin also increases production of TGF-β and IL-6 in lung endothelial cells (300, 301) and of FGF and IL-1β in alveolar macrophages (302, 303). Several strains of mice are resistant to the fibrogenic effects of bleomycin (304, 305) and continued study of these animals will surely provide additional insights into pathogenesis and potential treatment of the human disease.

Modulation of cell types in fibrosis. Cytokines released locally during the initial stages of injury can be powerful chemoattractants for lung fibroblasts. Interestingly, fibroblast migration in lungs does not seem to have been demonstrated *in vivo*. However, there is clear evidence of fibroblast replication in injured lungs (306). It has been postulated that in the development of fibrosis, clones of high collagen-producing fibroblasts are subject to activation, selective expansion, and differentiation under the influence of cytokines secreted by inflammatory cells, or by interaction with fibronectin or other components of the extracellular matrix (307, 308). The recent report that fibroblasts derived from fibrotic lung tissue can grow in soft agar (i.e., they are anchorage-independent) whereas cells derived from normal tissue cannot, supports the idea of selective expansion (309).

As noted previously, LIC appear during fibrotic response. LIC and hepatic lipocytes are very similar, and consideration of the liver cells can give insight into the nature of the LIC. Normal hepatic lipocytes synthesize mostly collagen III and some collagen I; in contrast, lipocytes from fibrotic tissue synthesize mainly collagen I. These cells proliferate in response to PDGF (310) and IGF-I (311); increase synthesis of collagen I without increasing mRNA when exposed to TNF-α (312); and down-regulate collagen I gene expression in response to IL-1α (313).

Both $Thy1^+$ and $Thy1^-$ cells can synthesize IL-6. TNF-α and IL-1 can induce the production of IL-6, which can then amplify the induction of TIMP, thus contributing to the fibrotic response. Although $Thy1^+$ cells express higher levels of IL-1 receptor, both populations respond to IL-1 by increasing collagen synthesis. In contrast, $Thy1^+$ cells respond to interleukin-4 by proliferating and increasing collagen production, whereas $Thy1^-$ cells do not (314). IFN-γ reduces collagen synthesis in these cells. C3H mice, which are resistant to radiation-induced fibrosis, have significantly fewer $Thy1^+$ cells than cells from radiation-

sensitive strains (315). There is evidence that $Thy1^+$ fibroblasts are selectively increased in radiation-induced fibrosis and these cells may correspond to the LIC observed in this condition (45). $Thy1^-$ cells synthesize and secrete IL-1α, and TNF-α can stimulate this process (44).

An increase in myofibroblasts has been observed in hyperoxia (40) and in fibrosis induced by bleomycin (41, 316, 317) and paraquat (287). Myofibroblasts from fibrotic lungs, which may be $Thy1^-$ cells, contain higher levels of TGF-β mRNA than cells from normal lungs (318).

Collagen degradation and fibrosis. Several lines of evidence indicate that collagen degradation is an integral part of the fibrotic process:

- Large numbers of activated macrophages, which secrete collagenase, are present in the initial stages of fibrosis (285).
- Bronchoalveolar lavage fluid from fibrosis patients contains high levels of active collagenase (319).
- Collagen fibrils in interstitium of monkeys exposed to paraquat are frayed and disorganized and appear to be partially digested (287).
- Exposure of lung tissue to 95% O_2 in an organ culture system leads to significant degradation of collagen (320).
- Intracellular collagen degradation is increased in lung fibroblasts exposed to bleomycin (321).
- Although much of the degradation is probably due to the action of collagenase secreted by alveolar macrophages and fibroblasts, there is also evidence, from nonlung systems, that phagocytosis of collagen fibrils is active during remodeling processes (89, 322, 323).

Role of cytokines in lung fibrosis: Potential therapeutic approaches. Cytokines are released from damaged cells and platelets immediately after injury. Inflammatory cells that migrate to the site of injury can secrete proteases capable of releasing growth factors bound to ECM components (324, 325). In addition, cytokines are presented to the tissue by inflammatory cells (e.g., macrophages) recruited to the site of injury. In turn, these cells secrete factors that induce resident cells to migrate to the injury, proliferate, and synthesize collagen and cytokines.

An important, but little understood, concept is "activation" of macrophages in inflamed and fibrotic lungs (326, 327). Cells that are activated in response to various challenges synthesize higher levels of PGE2 (328) and various cytokines than unstimulated cells, and many of the cytokines are secreted in activated, rather than latent, forms (329). Lipopolysaccharide is commonly used to activate cells *in vitro*. Recently, Laskin *et al.* showed that collagen and collagen fragments (such as might be released when inflammatory cells recruited to the site of an injury secrete proteases) activate alveolar macrophages *in vitro* (330). Activated macrophages lavaged from lungs of patients with interstitial disease (102, 331, 332) or cells from normal volunteers activated *in vitro* by radiation (333) secrete large amounts of IL-1α/β. TNF-α can be detected in macrophages in normal lungs; in fibrotic lungs, however, alveolar epithelial type 2 cells also show high level expression (334, 335). Macrophages lavaged from fibrotic lungs synthesize PDGF at rates comparable to cells lavaged from normal lungs and activated *in vitro* (218).

TNF-α levels rise in lungs of mice that are susceptible to bleomycin-induced fibrosis, but not in lungs of mice that are genetically resistant (304). Net destruction of tissue in the initial stages would be consistent with IL-1 and TNF-α having dominant effects in stimulating collagenase production. However, it is likely that during the later phase of response to injury these agents participate in exerting net positive effects on collagen production. Skin fibroblasts from scleroderma patients have much higher levels of IL-1 receptor than normal cells, suggesting that cells in fibrotic tissue are more sensitive to the cytokine (336).

Consistent with the results of studies showing that levels of certain cytokines rise before or during fibrotic response, additional studies have shown that interventions that increase cytokine levels in normal animals induce fibrosis. Conversely, inhibiting such increases in animals administered fibrogenic agents can ameliorate the fibrosis. Infusion of TNF-α increases collagen deposition in lung (337), whereas infusion of anti-TNF-α antibodies abrogates fibrotic response in mouse lungs exposed to either bleomycin (338) or silica (337), as well as in lungs of lupus-prone mice (339). Infusions of IL-1 receptor antagonist (IL-1ra) or TNF-α antagonist (recombinant soluble TNF-α receptor) also inhibit fibrosis in mice exposed to bleomycin or silica (340, 341). Administration of antibody against the IL-1 receptor diminishes fibrosis in lungs of rats challenged with bacillus Calmette-Guerin (342). Tyrosine kinase activity increases in lungs of rats exposed to paraquat and in fibroblasts isolated from paraquat-exposed lungs, probably as a result of induction by cytokines (343, 344). Tyrosine kinase inhibitors might prevent the fibrosis (345). Inhibiting the processing of TNF-α from a membrane-bound form to a soluble form protects mice from the lethal effects of endotoxin (346); this maneuver might prevent fibrosis in animals exposed to fibrogenic agents. Administration of anti-PDGF antibodies to rats with mesangial proliferative nephritis inhibited the accompanying fibrosis (347). A similar experiment might show inhibition of pulmonary fibrosis in rats administered bleomycin.

In vivo studies suggest that IL-6 has an antifibrotic effect. As noted, the major effect of IL-6 on collagen metabolism is to stimulate TIMP production. In a murine model of hypersensitivity pneumonitis, infusion of IL-6 lessened fibrosis and infusion of anti-IL-6 antibody increased fibrosis (348). A possible interpretation of this work is that inhibiting collagenase activity during an initial inflammatory stage is sufficient to inhibit the fibrotic sequelae. Initial studies with IFN-γ as an antifibrotic agent are promising. Administration of IFN-γ and IFN-γ inducers to rodents exposed to bleomycin reduced the amount of collagen in the lungs (157, 349, 350). Aerosols can be used to deliver recombinant IFN-γ to alveolar spaces (351).

TGF-β—The "master switch" for the fibrotic program? TGF-β has a central role in fibrosis (183, 352). In an experimental model of lung fibrosis, areas containing activated fibroblasts showed high levels of TGF-β (353). Immunohistochemical analysis of human fibrotic lung tissue revealed TGF-β concentrated in regenerating epithelium (354). Mice that are genetically resistant to bleomycin-induced fibrosis have much lower levels of TGF-β than susceptible mice (304, 305, 355). Zugmaier *et al.* injected large amounts of TGF-β into nude mice and found it was taken up by the lungs and other organs and caused a generalized interstitial fibrosis (356). Converse experiments have also been carried out: Antibodies against TGF-β effectively prevented fibrosis in lungs of mice exposed to bleomycin (357) and ameliorated the fibrotic response in mice challenged with bacilus Calmette-Guerin and diminished the amounts of IL-1β and TNF-α in the lungs (358).

In addition to activating and maintaining a generalized fibrotic response, TGF-β, secreted first by invading macrophages and then by resident epithelial and mesenchymal cells, might also inhibit repair of alveolar epithelium. It is known, for example, that low concentrations of TGF-β stimulate proliferation, and inhibit differentiation of alveolar epithelial cells (359). High concentrations of TGF-β may inhibit both proliferation and differentiation of epithelial cells (360). Furthermore, TGF-β stimulates production of basement-membrane-degrading collagenase (361, 362). A consequence of these processes would be continual access of interstitial cells to, and deposition of collagen within, alveolar spaces.

On the basis of this experimental evidence, we propose that TGF-β functions as a master switch that activates an entire "fibrotic program" (Fig. 6). (It should be noted that Sharma and Ziyadeh present a similar argument for the role of TGF-β in renal disease [363].) Although this scheme accounts for much of the experimental data, there are still large gaps in our knowledge of the role of TGF-β in fibrosis. One piece

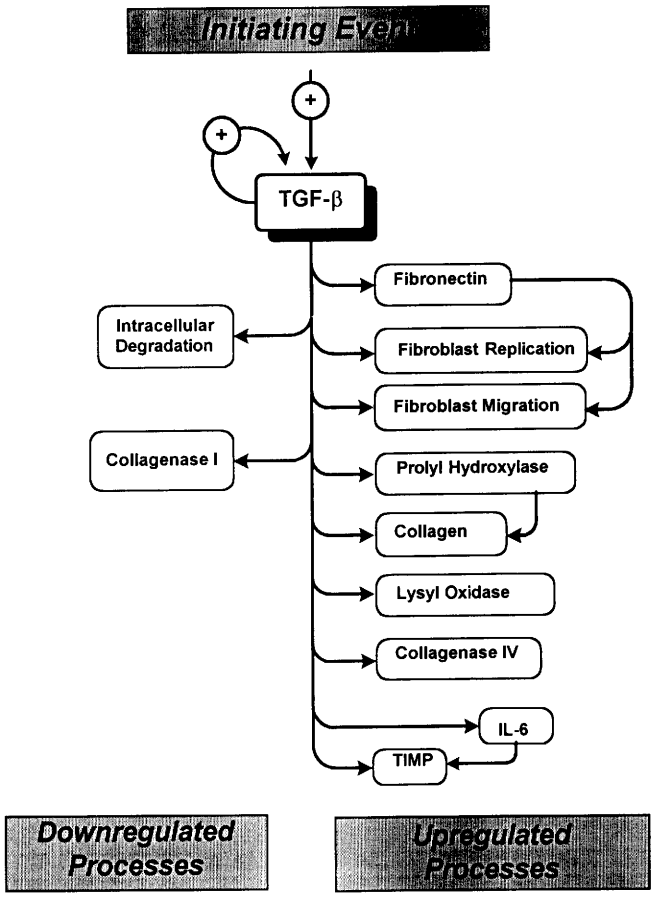

Figure 6. TGF-β, the "master switch" for the fibrotic program.

of important information that is missing from this picture is how TGF-β receptor levels are regulated during fibrosis. (Crouch presents a very interesting discussion of this point [293].) Information on changes in TGF-β receptors is scanty, and none relates directly to lung (364, 365). Also not known is whether the isoforms of TGF-β are modulated in fibrosis. The relative amounts of TGF-β isoforms can vary in a tissue, depending on its state (172). For example, TGF-β1 is observed in regenerating dermis after burn injury, but not in pressure wounds (366). It is also interesting to speculate whether TGF-β selectively stimulates expansion of fibroblast clones with constituitively high capacity for collagen synthesis and reduced sensitivity to PGE.

As noted previously, TGF-β is secreted in a latent form and must be activated before it can exert an effect. Alveolar macrophages from rats exposed to bleomycin secrete active TGF-β1, whereas cells from control animals secrete small quantities of the inactive form (367). Rodent macrophages exposed *in vitro* to bleomycin secrete inactive TGF-β (368). The situation appears different in humans. Shaw *et al.* reported that TGF-β mRNA levels were similar in alveolar macrophages from patients with interstitial lung disease and normal volunteers (267).

The scheme presented in Figure 6 does not take account of time. It is not clear that all the processes depicted can be regulated simultaneously in the manner depicted. Finally, it should be noted that TGF-β is synthesized by both inflammatory and interstitial cells, and Figure 6 does not show how the cell types might interact.

Fibril formation. Formation of new fibrils may be extremely difficult in the fibrotic environment because of the constant production of collagenase by inflammatory cells in the lungs (369). However, Last *et al.* found that there is very little turnover of mature (cross-linked) collagen in fibrotic lung (370). A possible explanation is that the collagenase acts on relatively "young" collagen that has not been incorporated into cross-linked structures. Recently, Pinnell and colleagues reported that minoxidil can inhibit formation of covalent crosslinks. This drug may be useful in treating fibrosis (371).

Erosion. Chronic inflammation of the lung leads to a generalized erosion of connective tissue (372). It is widely accepted that an important cause of emphysema is the imbalance between elastase production and inhibition in lung. However, Snider and colleagues have pointed out that an emphysema-like morphology can be induced experimentally without disrupting elastic fibers (373), and Laurent has raised the possibility that an emphysema-like condition can result from degradation of other matrix elements such as collagen (374). Moreover, as noted previously, chronic expression in lung of a transgene coding for collagenase leads to morphologic changes similar to those seen in emphysema (278).

Mice bearing the tight-skin mutation (*tsk*) exhibit a histologic picture resembling both scleroderma, with excessive accumulation of collagen in skin (375) and heart (376), and emphysema, with dilated air spaces and destruction of alveolar walls (377). There is disagreement about whether unmodulated activity of elastase is the cause of the pathology (378–381). Of particular note, Gardi *et al.* observed localized accumulation of collagen, as assessed histologically, but only a moderate increase as measured biochemically (382). They attributed this effect to a continuing process of collagen degradation in the lungs of the *tsk* mice.

An erosion program. It is much more difficult to define an erosion program than it was to define the fibrotic program. Nevertheless, experimental studies in lung and other systems suggest that IL-1, TNF-α, and PGE could mediate a generalized degradative response in which cell replication is inhibited, collagen synthesis and TIMP production are decreased, and collagenase production and intracellular degradation are decreased. Unchecked production of PGE may be the hallmark of this condition in a way similar to the central role of TGF-β in fibrosis.

Pneumonectomy. The changes in collagen and other extracellular matrix components following unilateral pneumonectomy have been described for rabbits and rodents (383–385). The pneumonectomy response recapitulates some features of normal development, in particular, the burst of collagen synthesis that accompanies the multiplication of alveoli in rabbits (384) and the expansion of alveoli in mice (385). A very interesting aspect of the compensatory growth process is the increase in inflammatory cells and levels of certain cytokines (notably IGF-I) in a manner similar, but not identical, to the response to other kinds of insult (386). Compensatory lung growth may be considered a response to injury, and from this point of view, one might expect increases in TGF-β and TNF-α, at least during the early stages of growth.

Steady State. In the steady state, the processes of collagen metabolism are generally quiescent. In the adult lung, collagen production is very low (270), and fibroblasts do not actively synthesize collagen (42). As noted previously, macrophages recovered from normal rat lungs secrete small amounts of inactive TGF-β (367). This is not to say that nothing is happening. Although Last *et al.* did not detect turnover of mature (cross-linked) collagen in adult lung (387), Laurent and colleagues demonstrated high levels of collagen turnover in lung from adult animals (388–390). A possible explanation for this apparent discrepancy is that the newly synthesized collagen is degraded rapidly, rather than incorporated into cross-linked fibrils.

As indicated earlier, TGF-β and PGE2 have a complex interaction. TGF-β stimulates PGE2 production in lung fibroblasts, whereas PGE2 abrogates the stimulatory effect of TGF-β upon collagen production (176). This interaction may be part of a feedback control system to regulate the level of collagen production at the steady state. In such a scheme, IFN-γ, which inhibits collagen synthesis and collagenase synthesis, may play the role of a "balance-seeking" regulator.

Suggestions for Future Studies

Several questions about control of collagen deposition in lung are unresolved and strike us as particularly interesting and meriting further investigation:

- How are fibrils formed in normal and fibrotic lung, and what is the role of the connective tissue cells in constructing the fibrils?
- How are the powerful effects of PGE, which seems to be ubiquitously produced and which inhibits collagen production, overcome in making the transition to a fibrotic state?

- What is the mechanism of action of PGE in controlling collagen production? Does it operate via cyclic AMP, or by another pathway that is independent of cyclic AMP?
- How are receptors for TGF-β regulated during fibrotic response?
- How do cytokine levels change during response to pneumonectomy?

Research in our laboratory has been supported by a Lung Research Award from the New York Lung Association and an Esther Hoffman Beller Research Grant from the New York Chapter of the Arthritis Foundation (R.S.B.), and a Graduate Fellowship from the Society of Cosmetic Chemists (M.E.G.).

1. McGowan SE. Extracellular matrix and the regulation of lung development and repair. FASEB J **6**:2895–2904, 1992.
2. Guzowski D, Blau H, Bienkowski RS. Extracellular matrix in developing lungs. In: Scarpelli EM, Ed. Pulmonary Physiology of the Fetus, Newborn, Child, and Adolescent. Philadelphia: Lea & Febiger, pp83–105, 1990.
3. Mosher DF, Sottile J, Wu C, McDonald JA. Assembly of extracellular matrix. Curr Opin Cell Biol **4**:810–818, 1992.
4. Roman J, Limper AH, McDonald JA. Lung extracellular matrix: Physiology and pathophysiology. Hosp Pract Off Ed **25**:125–128;131–135;139–140, 1990.
5. Davidson JM. Biochemistry and turnover of lung interstitium. Eur Respir J **3**:1048–1063, 1990.
6. van-der-Rest M, Garrone R. Collagen family of proteins. FASEB J **5**:2814–2823, 1991.
7. Bienkowski RS. Interstitial collagens. In: Crystal RG, West JB, Eds. The Lung: Scientific Foundations. New York: Raven Press, Vol **1**:pp381–388, 1991.
8. Sandell LJ, Boyd CD. Conserved and divergent sequence and functional elements within collagen genes. In: Sandell LJ, Boyd CD, Eds. Extracellular Matrix Genes. San Diego: Academic Press, pp1–56, 1990.
9. Fine A, Poliks CF, Donahue LP, Smith BD, Goldstein RH. The differential effect of prostaglandin E2 on transforming growth factor-beta and insulin-induced collagen formation in lung fibroblasts. J Biol Chem **264**:16988–16991, 1989.
10. Fine A, Poliks CF, Smith BD, Goldstein RH. The accumulation of type I collagen mRNAs in human embryonic lung fibroblasts stimulated by transforming growth factor-beta. Connect Tissue Res **24**:237–247, 1990.
11. Liska DJ, Robinson VR, Bornstein P. Elements in the first intron of the alpha 1(I) collagen gene interact with Sp 1 to regulate gene expression. Gene Exp **2**:379–389, 1992.
12. Barker DD, Wu H, Hartung S, Breindl M, Jaenisch R. Retrovirus-induced insertional mutagenesis: Mechanism of collagen mutation in Mov13 mice. Mol Cell Biol **11**:5154–5163, 1991.
13. Kratochwil K, Dziadek M, Lohler J, Harbers K, Jaenisch R. Normal epithelial branching morphogenesis in the absence of collagen I. Dev Biol **117**:596–606, 1986.
14. Kratochwil K, von-der-Mark K, Kollar EJ, et al. Retrovirus-induced insertional mutation in Mov13 mice affects collagen I expression in a tissue-specific manner. Cell **57**:807–816, 1989.
15. Schwarz M, Harbers K, Kratochwil K. Transcription of a mutant collagen I gene is a cell type and stage-specific marker for odontoblast and osteoblast differentiation. Development **108**:717–726, 1990.
16. Fouser L, Sage EH, Clark J, Bornstein P. Feedback regulation of collagen gene expression: A Trojan horse approach. Proc Natl Acad Sci USA **88**:10158–10162, 1991.
17. Katayama K, Armendariz-Borunda J, Raghow R, Kang AH, Seyer JM. A pentapeptide from type I procollagen promotes extracellular matrix production. J Biol Chem **268**:9941–9944, 1993.
18. Goldstein RH. Control of type I collagen formation in the lung. Am J Physiol **261**:L29–L40, 1991.
19. Adams SL. Collagen gene expression. Am J Respir Cell Mol Biol **1**:161–168, 1989.
20. Ramirez F, Di-Liberto M. Complex and diversified regulatory programs control the expression of vertebrate collagen genes. FASEB J **4**:1616–1623, 1990.
21. Slack JL, Liska DJ, Bornstein P. Regulation of expression of the type I collagen genes. Am J Med Genet **45**:140–151, 1993.
22. Oohashi T, Sugimoto M, Mattei MG, Ninomiya Y. Identification of a new collagen IV chain, alpha 6(IV), by cDNA isolation and assignment of the gene to chromosome Xq22, which is the same locus for COL4A5. J Biol Chem **269**:7520–7526, 1994.
23. Hudson BG, Reeders ST, Tryggvason K. Type IV collagen: Structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis. J Biol Chem **268**:26033–26036, 1993.
24. Crouch EC, Martin GR, Brody JS. Basement membranes. In: Crystal RG, West JB, Eds. The Lung: Scientific Foundations. New York: Raven Press, Vol **1**:pp421–437, 1991.
25. Bitterman PB, Rennard SI, Adelberg S, Crystal RG. Role of fibronectin as a growth factor for fibroblasts. J Cell Biol **97**:1925–1932, 1983.
26. Akiyama SK, Yamada SS, Chen WT, Yamada KM. Analysis of fibronectin receptor function with monoclonal antibodies: Roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. J Cell Biol **109**:863–875, 1989.
27. Postlethwaite AE, Keski-Oja J, Balian G, Kang AH. Induction of fibroblast chemotaxis by fibronectin. Localization of the chemotactic region to a 140,000-molecular weight non-gelatin-binding fragment. J Exp Med **153**:494–499, 1981.
28. Shoji S, Rickard KA, Ertl RF, Robbins RA, Linder J, Rennard SI. Bronchial epithelial cells produce lung fibroblast chemotactic factor: Fibronectin. Am J Respir Cell Mol Biol **1**:13–20, 1989.
29. Hance AJ, Bradley K, Crystal RG. Lung collagen heterogeneity. Synthesis of type I and type III collagen by rabbit and human lung cells in culture. J Clin Invest **57**:102–111, 1976.
30. Rennard SI, Jaurand MC, Bignon J, et al. Role of pleural mesothelial cells in the production of the submesothelial connective tissue matrix of lung. Am Rev Respir Dis **130**:267–274, 1984.
31. Macarak EJ. Collagen synthesis by cloned pulmonary artery endothelial cells. J Cell Physiol **119**:175–182, 1984.
32. Sage H, Farin FM, Striker GE, Fisher AB. Granular pneumocytes in primary culture secrete several major components of the extracellular matrix. Biochemistry **22**:2148–2155, 1983.
33. Simon RH, Scott MJ, Reza MM, Killen PD. Type IV collagen production by rat pulmonary alveolar epithelial cells. Am J Respir Cell Mol Biol **8**:640–646, 1993.
34. Alitalo K. Production of both interstitial and basement membrane procollagens by fibroblastic WI-38 cells from human embryonic lung. Biochem Biophys Res Commun **93**:873–880, 1980.
35. Phipps RP, Ed. Pulmonary Fibroblast Heterogeneity. Boca Raton: CRC Press, 1992.
36. Fries KM, Blieden T, Looney RJ, et al. Evidence of fibroblast heterogeneity and the role of fibroblast subpopulations in fibrosis. Clin Immunol Immunopathol **72**:283–292, 1994.
37. Kaplan NB, Grant MM, Brody JS. The lipid interstitial cell of the pulmonary alveolus. Age and species differences. Am Rev Respir Dis **132**:1307–1312, 1985.
38. Leslie KO, Mitchell JJ, Woodcock-Mitchell JL, Low RB. Alpha smooth muscle actin expression in developing and adult human lung. Differentiation **44**:143–149, 1990.
39. Vaccaro C, Brody JS. Ultrastructure of developing alveoli. I. The role of the interstitial fibroblast. Anat Rec **192**:467–479, 1978.
40. Durr RA, Dubaybo BA, Thet LA. Repair of chronic hyperoxic lung injury: Changes in lung ultrastructure and matrix. Exp Mol Pathol **47**:219–240, 1987.
41. Evans JN, Kelley J, Low RB, Adler KB. Increased contractility of isolated lung parenchyma in an animal model of pul-

monary fibrosis induced by bleomycin. Am Rev Respir Dis **125**:89–94, 1982.
42. Kuhn C, McDonald JA. The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. Am J Pathol **138**:1257–1265, 1991.
43. Mollenhauer J, Bayreuther K. Donor-age-related changes in the morphology, growth potential, and collagen biosynthesis in rat fibroblast subpopulations in vitro. Differentiation **32**:165–172, 1986.
44. Froncek MJ, Derdak S, Felch ME, Silvera MR, Watts HB, Phipps RP. Cellular and molecular characterization of Thy-1$^+$ and Thy-1$^-$ murine lung fibroblasts. In: Phipps RP, Ed. Pulmonary Fibroblast Heterogeneity. Boca Raton: CRC Press, pp135–198, 1992.
45. Penney DP, Keng PC, Derdak S, Phipps RP. Morphologic and functional characteristics of subpopulations of murine lung fibroblasts grown *in vitro*. Anat Rec **232**:432–443, 1992.
46. Silvera MR, Sempowski GD, Phipps RP. Expression of TGF-beta isoforms by Thy-1(+) and Thy-1(−) pulmonary fibroblast subsets evidence for TGF-beta as a regulator of il-1-dependent stimulation of IL-6. Lymph Cyto Res **15**:277–285, 1994.
47. Brody JS, Kaplan NB. Proliferation of alveolar interstitial cells during postnatal lung growth. Evidence for two distinct populations of pulmonary fibroblasts. Am Rev Respir Dis **127**:763–770, 1983.
48. Makela JK, Vuorio E. Type I collagen messenger RNA levels in experimental granulation tissue and silicosis in rats. Med Biol **64**:15–22, 1986.
49. Geesin JC, Darr D, Kaufman R, Murad S, Pinnell SR. Ascorbic acid specifically increases type I and type III procollagen messenger RNA levels in human skin fibroblast. J Invest Dermatol **90**:420–424, 1988.
50. Goldenberg R, Fine RE. Generalized inhibition of cell-free translation by the amino-terminal propeptide of chick type I procollagen. Biochim Biophys Acta **826**:101–107, 1985.
51. Kivirikko KI, Myllyla R, Pihlajaniemi T. Protein hydroxylation: Prolyl 4-hydroxylase, an enzyme with four cosubstrates and a multifunctional subunit. FASEB J **3**:1609–1617, 1989.
52. Wang QJ, Giri SN, Hyde DM, Nakashima JM, Javadi I. Niacin attenuates bleomycin-induced lung fibrosis in the hamster. J Biochem Toxicol **5**:13–22, 1990.
53. Keene DR, Sakai LY, Bachinger HP, Burgeson RE. Type III collagen can be present on banded collagen fibrils regardless of fibril diameter. J Cell Biol **105**:2393–2402, 1987.
54. Xu LX, Ohsaki Y, Nagata K, Kurisu K. Immunohistochemical studies on the distributions and age-related changes of types I and III collagen in the oral mucosa of mice. J Dent Res **72**:1336–1343, 1993.
55. Fleischmajer R, MacDonald ED, Perlish JS, Burgeson RE, Fisher LW. Dermal collagen fibrils are hybrids of type I and type III collagen molecules. J Struct Biol **105**:162–169, 1990.
56. Prockop DJ. Mutations that alter the primary structure of type I collagen. The perils of a system for generating large structures by the principle of nucleated growth. J Biol Chem **265**:15349–15352, 1990.
57. Birk DE, Trelstad RL. Extracellular compartments in matrix morphogenesis: Collagen fibril, bundle, and lamellar formation by corneal fibroblasts. J Cell Biol **99**:2024–2033, 1984.
58. Birk DE, Trelstad RL. Extracellular compartments in tendon morphogenesis: Collagen fibril, bundle, and macroaggregate formation. J Cell Biol **103**:231–240, 1986.
59. Ploetz C, Zycband EI, Birk DE. Collagen fibril assembly and deposition in the developing dermis: Segmental deposition in extracellular compartments. J Struct Biol **106**:73–81, 1991.
60. Contard P, Jacobs L II, Perlish JS, Fleischmajer R. Collagen fibrillogenesis in a three-dimensional fibroblast cell culture system. Cell Tissue Res **273**:571–575, 1993.
61. Fleischmajer R, Perlish JS, Olsen BR. The carboxylpropeptide of type I procollagen in skin fibrillogenesis. J Invest Dermatol **89**:212–215, 1987.
62. Last JA, Armstrong LG, Reiser KM. Biosynthesis of collagen crosslinks. Int J Biochem **22**:559–564, 1990.
63. Reiser K, McCormick RJ, Rucker RB. Enzymatic and nonenzymatic cross-linking of collagen and elastin. FASEB J **6**:2439–2449, 1992.
64. Chen JM, Little CD. Cells that emerge from embryonic explants produce fibers of type IV collagen. J Cell Biol **101**:1175–1181, 1985.
65. Chen JM, Little CD. Cellular events associated with lung branching morphogenesis including the deposition of collagen type IV. Dev Biol **120**:311–321, 1987.
66. Bienkowski RS. Intracellular degradation of newly synthesized collagen. Prog Clin Biol Res **180**:565–572, 1985.
67. Barile FA, Guzowski DE, Ripley C, Siddiqi ZA, Bienkowski RS. Ammonium chloride inhibits basal degradation of newly synthesized collagen in human fetal lung fibroblasts. Arch Biochem Biophys **276**:125–131, 1990.
68. Bienkowski RS, Baum BJ, Crystal RG. Fibroblasts degrade newly synthesised collagen within the cell before secretion. Nature **276**:413–416, 1978.
69. Barile FA, Ripley-Rouzier C, Siddiqi ZE, Bienkowski RS. Effects of prostaglandin E1 on collagen production and degradation in human fetal lung fibroblasts. Arch Biochem Biophys **265**:441–446, 1988.
70. Berg RA, Schwartz ML, Crystal RG. Regulation of the production of secretory proteins: Intracellular degradation of newly synthesized "defective" collagen. Proc Natl Acad Sci USA **77**:4746–4750, 1980.
71. Bienkowski RS, Ripley CR, Gitzelmann R, Steinmann B. Collagen degradation in I-cells is normal. Biochem Biophys Res Commun **168**:479–484, 1990.
72. Ripley CR, Fant J, Bienkowski RS. Brefeldin A inhibits degradation as well as production and secretion of collagen in human lung fibroblasts. J Biol Chem **268**:3677–3682, 1993.
73. Klausner RD, Sitia R. Protein degradation in the endoplasmic reticulum. Cell **62**:611–614, 1990.
74. Fra A, Sitia R. The endoplasmic reticulum as a site of protein degradation. Subcell Biochem **21**:143–168, 1993.
75. Horwitz AL, Hance AJ, Crystal RG. Granulocyte collagenase: Selective digestion of type I relative to type III collagen. Proc Natl Acad Sci USA **74**:897–901, 1977.
76. D'Ortho MP, Jarreau PH, Delacourt C, et al. Matrix metalloproteinase and elastase activities in LPS-induced acute lung injury in guinea pigs. Am J Physiol **266**:L209–L216, 1994.
77. Birkedal-Hansen H, Moore WG, Bodden MK, et al. Matrix metalloproteinases: A review. Crit Rev Oral Biol Med **4**:197–250, 1993.
78. Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet **6**:121–125, 1990.
79. Woessner JF Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J **5**:2145–2154, 1991.
80. Wilhelm SM, Collier IE, Marmer BL, Eisen AZ, Grant GA, Goldberg GI. SV40-transformed human lung fibroblasts secrete a 92-kDa type IV collagenase which is identical to that secreted by normal human macrophages (published erratum appears in J Biol Chem **265**[36]:22570, 1990). J Biol Chem **264**:17213–17221, 1989.
81. Pluznik DH, Fridman R, Reich R. Correlation in the expression of type IV collagenase and the invasive and chemotactic abilities of myelomonocytic cells during differentiation into macrophages. Exp Hematol **20**:57–63, 1992.
82. Albin RJ, Senior RM, Welgus HG, Connolly NL, Campbell EJ. Human alveolar macrophages secrete an inhibitor of metalloproteinase elastase. Am Rev Respir Dis **135**:1281–1285, 1987.
83. Shapiro SD, Kobayashi DK, Welgus HG. Identification of TIMP-2 in human alveolar macrophages. Regulation of biosynthesis is opposite to that of metalloproteinases and TIMP-1. J Biol Chem **267**:13890–13894, 1992.
84. Pardo A, Selman M, Ramirez R, et al. Production of collagenase and tissue inhibitor of metalloproteinases by fibroblasts derived from normal and fibrotic human lungs. Chest **102**:1085–1089, 1992.
85. Leblond CP. Synthesis and secretion of collagen by cells of connective tissue, bone, and dentin. Anat Rec **224**:123–138, 1989.
86. Sasaki T, Garant PR. Multinucleated fibroblastic cells in the

periodontal ligaments of aged rats. J Periodontal Res **28**:65–71, 1993.
87. Salonen J, Uitto VJ, Pan YM, Oda D. Proliferating oral epithelial cells in culture are capable of both extracellular and intracellular degradation of interstitial collagen. Matrix **11**:43–55, 1991.
88. Everts V, Hembry RM, Reynolds JJ, Beertsen W. Metalloproteinases are not involved in the phagocytosis of collagen fibrils by fibroblasts. Matrix **9**:266–276, 1989.
89. McCulloch CA, Knowles GC. Deficiencies in collagen phagocytosis by human fibroblasts *in vitro:* A mechanism for fibrosis? J Cell Physiol **155**:461–471, 1993.
90. Klien JH, Adamson IY. Fibroblast inhibitor and prostaglandin secretion by alveolar epithelial cells exposed to silica. Lab Invest **60**:808–813, 1989.
91. Goldstein RH, Polgar P. The effect and interaction of bradykinin and prostaglandins on protein and collagen production by lung fibroblasts. J Biol Chem **257**:8630–8633, 1982.
92. Varga J, Diaz-Perez A, Rosenbloom J, Jimenez SA. PGE2 causes a coordinate decrease in the steady state levels of fibronectin and types I and III procollagen mRNAs in normal human dermal fibroblasts. Biochem Biophys Res Commun **147**:1282–1288, 1987.
93. Baum BJ, Moss J, Breul SD, Berg RA, Crystal RG. Effect of cyclic AMP on the intracellular degradation of newly synthesized collagen. J Biol Chem **255**:2843–2847, 1980.
94. Berg RA, Moss J, Baum BJ, Crystal RG. Regulation of collagen production by the beta-adrenergic system. J Clin Invest **67**:1457–1462, 1981.
95. Bhatnagar R, Schade U, Rietschel ET, Decker K. Involvement of prostaglandin E and adenosine 3′,5′-monophosphate in lipopolysaccharide-stimulated collagenase release by rat Kupffer cells. Eur J Biochem **125**:125–130, 1982.
96. Fine A, Matsui R, Zhan X, Poliks CF, Smith BD, Goldstein RH. Discordant regulation of human type I collagen genes by prostaglandin E2. Biochim Biophys Acta **1135**:67–72, 1992.
97. Saltzman LE, Moss J, Berg RA, Hom B, Crystal RG. Modulation of collagen production by fibroblasts. Effects of chronic exposure to agonists that increase intracellular cyclic AMP. Biochem J **204**:25–30, 1982.
98. Raisz LG, Fall PM, Gabbitas BY, McCarthy TL, Kream BE, Canalis E. Effects of prostaglandin E2 on bone formation in cultured fetal rat calvariae: Role of insulin-line growth factor-I. Endocrinology **133**:1504–1510, 1993.
99. Kelley J. Cytokines of the lung. Am Rev Respir Dis **141**:765–788, 1990.
100. Goldring MB, Goldring SR. Cytokines and cell growth control. Crit Rev Eukaryot Gene Exp **1**:301–326, 1991.
101. Manson JC, Symons JA, Di-Giovine FS, Poole S, Duff GW. Autoregulation of interleukin 1 production. Eur J Immunol **19**:261–265, 1989.
102. Zhang Y, Lee TC, Guillemin B, Yu MC, Rom WN. Enhanced IL-1 beta and tumor necrosis factor-alpha release and messenger RNA expression in macrophages from idiopathic pulmonary fibrosis or after asbestos exposure. J Immunol **150**:4188–4196, 1993.
103. Xing Z, Jordana M, Gauldie J. IL-1 beta and IL-6 gene expression in alveolar macrophages: Modulation by extracellular matrices. Am J Physiol **262**:L600–L605, 1992.
104. Gauldie J, Jordana M, Cox G. Cytokines and pulmonary fibrosis. Thorax **48**:931–935, 1993.
105. Partanen J, Vainikka S, Korhonen J, Armstrong E, Alitalo K. Diverse receptors for fibroblast growth factors. Prog Growth Factor Res **4**:69–83, 1992.
106. Taga T, Kishimoto T. Cytokine receptors and signal transduction. FASEB J **6**:3387–3396, 1992.
107. Kishimoto T, Taga T, Akira S. Cytokine signal transduction. Cell **76**:253–262, 1994.
108. Sadowski HB, Shuai K, Darnell JE Jr., Gilman MZ. A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. Science **261**:1739–1744, 1993.
109. Darnell J JE, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science **264**:1415–1421, 1994.
110. Yan Z, Winawer S, Friedman E. Two different signal transduction pathways can be activated by transforming growth factor beta 1 in epithelial cells. J Biol Chem **269**:13231–13237, 1994.
111. Givol D, Yayon A. Complexity of FGF receptors: Genetic basis for structural diversity and functional specificity. FASEB J **6**:3362–3369, 1992.
112. Thornberry NA, Bull HG, Calaycay JR, et al. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature **356**:768–774, 1992.
113. Cerretti DP, Kozlosky CJ, Mosley B, et al. Molecular cloning of the interleukin-1 beta converting enzyme. Science **256**:97–100, 1992.
114. Harpel JG, Metz CN, Kojima S, Rifkin DB. Control of transforming growth factor-beta activity: Latency vs. activation. Prog Growth Factor Res **4**:321–335, 1992.
115. McGeehan GM, Becherer JD, Bast RC Jr., et al. Regulation of tumour necrosis factor-alpha processing by a metalloproteinase inhibitor. Nature **370**:558–561, 1994.
116. Gearing AJ, Beckett P, Christodoulou M, et al. Processing of tumour necrosis factor-alpha precursor by metalloproteinases. Nature **370**:555–557, 1994.
117. Hardingham TE, Fosang AJ. Proteoglycans: Many forms and many functions. FASEB J **6**:861–870, 1992.
118. Arend WP. Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. J Clin Invest **88**:1445–1451, 1991.
119. Martinet N, Charles T, Vaillant P, Vignaud JM, Lambert J, Martinet Y. Characterization of a tumor necrosis factor-alpha inhibitor activity in cancer patients. Am J Respir Cell Mol Biol **6**:510–515, 1992.
120. Clemmons DR. IGF binding proteins and their functions. Mol Reprod Dev **35**:368–374, 1993.
121. Jones JI, Gockerman A, Busby WH Jr., Camacho-Hubner C, Clemmons DR. Extracellular matrix contains insulin-like growth factor binding protein-5: Potentiation of the effects of IGF-I. J Cell Biol **121**:679–687, 1993.
122. Price WA, Moats-Staats BM, D'Ercole AJ, Stiles AD. Insulin-like growth factor binding protein production and regulation in fetal rat lung cells. Am J Respir Cell Mol Biol **8**:425–432, 1993.
123. Pollock RA, Richardson WD. The alternative-splice isoforms of the PDGF A-chain differ in their ability to associate with the extracellular matrix and to bind heparin *in vitro*. Growth Factors **7**:267–277, 1992.
124. McCaffrey TA, Falcone DJ, Du B. Transforming growth factor-beta 1 is a heparin-binding protein: Identification of putative heparin-binding regions and isolation of heparins with varying affinity for TGF-beta 1. J Cell Physiol **152**:430–440, 1992.
125. McCaffrey TA, Falcone DJ, Vicente D, Du B, Consigli S, Borth W. Protection of transforming growth factor-beta 1 activity by heparin and fucoidan. J Cell Physiol **159**:51–59, 1994.
126. Lopez-Casillas F, Payne HM, Andres JL, Massague J. Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: mapping of ligand binding and GAG attachment sites. J Cell Biol **124**:557–568, 1994.
127. LaMarre J, Wollenberg GK, Gonias SL, Hayes MA. Cytokine binding and clearance properties of proteinase-activated alpha 2-macroglobulins. Lab Invest **65**:3–14, 1991.
128. Kusaka Y, Cullen RT, Donaldson K. Immunomodulation in mineral dust-exposed lungs: Stimulatory effect and interleukin-1 release by neutrophils from quartz-elicited alveolitis. Clin Exp Immunol **80**:293–298, 1990.
129. Driscoll KE, Lindenschmidt RC, Maurer JK, Higgins JM, Ridder G. Pulmonary response to silica or titanium dioxide: Inflammatory cells, alveolar macrophage-derived cytokines, and histopathology. Am J Respir Cell Mol Biol **2**:381–390, 1990.
130. Driscoll KE, Maurer JK. Cytokine and growth factor release by alveolar macrophages: Potential biomarkers of pulmonary toxicity. Toxicol Pathol **19**:398–405, 1991.
131. Hori T, Yamanaka Y, Hayakawa M, et al. Prostaglandins antagonize fibroblast proliferation stimulated by tumor necrosis factor. Biochem Biophys Res Commun **174**:758–766, 1991.
132. Thornton SC, Por SB, Walsh BJ, Penny R, Breit SN. Interaction of immune and connective tissue cells: I. The effect of

lymphokines and monokines on fibroblast growth. J Leukoc Biol **47**:312–320, 1990.
133. Hunt JS, Atherton RA, Pace JL. Differential responses of rat trophoblast cells and embryonic fibroblasts to cytokines that regulate proliferation and class I MHC antigen expression. J Immunol **145**:184–189, 1990.
134. Postlethwaite AE, Seyer JM. Stimulation of fibroblast chemotaxis by human recombinant tumor necrosis factor alpha (TNF-alpha) and a synthetic TNF-alpha 31-68 peptide. J Exp Med **172**:1749–1756, 1990.
135. Schirren CG, Scharffetter K, Hein R, Braun-Falco O, Krieg T. Tumor necrosis factor alpha induces invasiveness of human skin fibroblasts *in vitro*. J Invest Dermatol **94**:706–710, 1990.
136. Elias JA, Freundlich B, Adams S, Rosenbloom J. Regulation of human lung fibroblast collagen production by recombinant interleukin-1, tumor necrosis factor, and interferon-gamma. Ann NY Acad Sci **580**:233–244, 1990.
137. Diaz A, Munoz E, Johnston R, Korn JH, Jimenez SA. Regulation of human lung fibroblast alpha 1(I) procollagen gene expression by tumor necrosis factor alpha, interleukin-1 beta, and prostaglandin E2. J Biol Chem **268**:10364–10371, 1993.
138. Mauviel A, Heino J, Kahari VM, et al. Comparative effects of interleukin-1 and tumor necrosis factor-alpha on collagen production and corresponding procollagen mRNA levels in human dermal fibroblasts. J Invest Dermatol **96**:243–249, 1991.
139. Owens MW, Grimes SR. Pleural mesothelial cell response to inflammation: Tumor necrosis factor-induced mitogenesis and collagen synthesis. Am J Physiol **265**:L382–L388, 1993.
140. Matsushima K, Bano M, Kidwell WR, Oppenheim JJ. Interleukin 1 increases collagen type IV production by murine mammary epithelial cells. J Immunol **134**:904–909, 1985.
141. Thornton SC, Robbins JM, Penny R, Breit SN. Fibroblast growth factors in connective tissue disease associated interstitial lung disease. Clin Exp Immunol **90**:447–452, 1992.
142. Richards CD, Shoyab M, Brown TJ, Gauldie J. Selective regulation of metalloproteinase inhibitor (TIMP-1) by oncostatin M in fibroblasts in culture. J Immunol **150**:5596–5603, 1993.
143. Meikle MC, Atkinson SJ, Ward RV, Murphy G, Reynolds JJ. Gingival fibroblasts degrade type I collagen films when stimulated with tumor necrosis factor and interleukin 1: Evidence that breakdown is mediated by metalloproteinases. J Periodontal Res **24**:207–213, 1989.
144. Lefebvre V, Peeters-Joris C, Vaes G. Modulation by interleukin 1 and tumor necrosis factor alpha of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochim Biophys Acta **1052**:366–378, 1990.
145. Unemori EN, Hibbs MS, Amento EP. Constitutive expression of a 92-kD gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. J Clin Invest **88**:1656–1662, 1991.
146. Galve-de-Rochemonteix B, Nicod LP, Chicheportiche R, Lacraz S, Baumberger C, Dayer JM. Regulation of interleukin-1ra, interleukin-1 alpha, and interleukin-1 beta production by human alveolar macrophages with phorbol myristate acetate, lipopolysaccharide, and interleukin-4. Am J Respir Cell Mol Biol **8**:160–168, 1993.
147. Galve-de-Rochemonteix B, Nicod LP, Junod AF, Dayer JM. Characterization of a specific 20- to 25-kD interleukin-1 inhibitor from cultured human lung macrophages. Am J Respir Cell Mol Biol **3**:355–361, 1990.
148. Okamura K, Sato Y, Matsuda T, et al. Endogenous basic fibroblast growth factor-dependent induction of collagenase and interleukin-6 in tumor necrosis factor-treated human microvascular endothelial cells. J Biol Chem **266**:19162–19165, 1991.
149. Eckes B, Hunzelmann N, Ziegler-Heitbrock HW, et al. Interleukin-6 expression by fibroblasts grown in three-dimensional gel cultures. FEBS Lett **298**:229–232, 1992.
150. Lacraz S, Nicod L, Galve-de-Rochemonteix B, Baumberger C, Dayer JM, Welgus HG. Suppression of metalloproteinase biosynthesis in human alveolar macrophages in interleukin-4. J Clin Invest **90**:382–388, 1992.
151. Sato T, Ito A, Mori Y. Interleukin 6 enhances the production of tissue inhibitor of metalloproteinases (TIMP) but not that of matrix metalloproteinases by human fibroblasts. Biochem Biophys Res Commun **170**:824–829, 1990.
152. Lotz M, Guerne PA. Interleukin-6 induces the synthesis of tissue inhibitor of metalloproteinases-1/erythroid potentiating activity (TIMP-1/EPA). J Biol Chem **266**:2017–2020, 1991.
153. Ito A, Itoh Y, Sasaguri Y, Morimatsu M, Mori Y. Effects of interleukin-6 on the metabolism of connective tissue components in rheumatoid synovial fibroblasts. Arthritis Rheum **35**:1197–1201, 1992.
154. Shingu M, Nagai Y, Isayama T, Naono T, Nobunaga M, Nagai Y. The effects of cytokines on metalloproteinase inhibitors (TIMP) and collagenase production by human chondrocytes and TIMP production by synovial cells and endothelial cells. Clin Exp Immunol **94**:145–149, 1993.
155. Emonard H, Munaut C, Melin M, Lortat-Jacob H, Grimaud JA. Interleukin-6 does not regulate interstitial collagenase, stromelysin and tissue inhibitor of metalloproteinases synthesis by cultured human fibroblasts. Matrix **12**:471–474, 1992.
156. Prior C, Haslam PL. *In vivo* levels and *in vitro* production of interferon-gamma in fibrosing interstitial lung diseases. Clin Exp Immunol **88**:280–287, 1992.
157. Okada T, Sugie I, Aisaka K. Effects of gamma-interferon on collagen and histamine content in bleomycin-induced lung fibrosis in rats. Lymphokine Cytokine Res **12**:87–91, 1993.
158. Narayanan AS, Whithey J, Souza A, Raghu G. Effect of gamma-interferon on collagen synthesis by normal and fibrotic human lung fibroblasts. Chest **101**:1326–1331, 1992.
159. Akamine A, Raghu G, Narayanan AS. Human lung fibroblast subpopulations with different C1q binding and functional properties. Am J Respir Cell Mol Biol **6**:382–389, 1992.
160. Clark JG, Dedon TF, Wayner EA, Carter WG. Effects of interferon-gamma on expression of cell surface receptors for collagen and deposition of newly synthesized collagen by cultured human lung fibroblasts. J Clin Invest **83**:1505–1511, 1989.
161. Adelmann-Grill BC, Hein R, Wach F, Krieg T. Inhibition of fibroblast chemotaxis by recombinant human interferon gamma and interferon alpha. J Cell Physiol **139**:270–275, 1987.
162. Wahl LM, Corcoran ML. Regulation of monocyte/macrophage metalloproteinase production by cytokines. J Periodontol **64**:467–473, 1993.
163. Shapiro SD, Campbell EJ, Kobayashi DK, Welgus HG. Immune modulation of metalloproteinase production in human macrophages. Selective pretranslational suppression of interstitial collagenase and stromelysin biosynthesis by interferon-gamma. J Clin Invest **86**:1204–1210, 1990.
164. Nakajima H, Hiyama Y, Tsukada W, Warabi H, Uchida S, Hirose S. Effects of interferon gamma on cultured synovial cells from patients with rheumatoid arthritis: inhibition of cell growth, prostaglandin E2, and collagenase release. Ann Rheum Dis **49**:512–516, 1990.
165. Duncan MR, Berman B. Differential regulation of glycosaminoglycan, fibronectin, and collagenase production in cultured human dermal fibroblasts by interferon-alpha, -beta, and -gamma. Arch Dermatol Res **281**:11–18, 1989.
166. Scharffetter K, Heckmann M, Hatamochi A, et al. Synergistic effect of tumor necrosis factor-alpha and interferon-gamma on collagen synthesis of human skin fibroblasts *in vitro*. Exp Cell Res **181**:409–419, 1989.
167. Elias JA, Gustilo K, Baeder W, Freundlich B. Synergistic stimulation of fibroblast prostaglandin production by recombinant interleukin 1 and tumor necrosis factor. J Immunol **138**:3812–3816, 1987.
168. Granstein RD, Flotte TJ, Amento EP. Interferons and collagen production. J Invest Dermatol **95**:75S–80S, 1990.
169. Kelley J, Shull S, Walsh JJ, Cutroneo KR, Absher M. Autoinduction of transforming growth factor-beta in human lung fibroblasts. Am J Respir Cell Mol Biol **8**:417–424, 1993.
170. Jordana M, Richards C, Irving LB, Gauldie J. Spontaneous *in vitro* release of alveolar-macrophage cytokines after the intratracheal instillation of bleomycin in rats. Characterization and kinetic studies. Am Rev Respir Dis **137**:1135–1140, 1988.
171. Kim SJ, Angel P, Lafyatis R, et al. Autoinduction of transforming growth factor beta 1 is mediated by the AP-1 complex. Mol Cell Biol **10**:1492–1497, 1990.

172. Roberts AB, Sporn MB. Differential expression of the TGF-beta isoforms in embryogenesis suggests specific roles in developing and adult tissues. Mol Reprod Dev **32**:91–98, 1992.
173. Massague J, Cheifetz S, Laiho M, Ralph DA, Weis FM, Zentella A. Transforming growth factor-beta. Cancer Surv **12**:81–103, 1992.
174. Matrisian LM, Ganser GL, Kerr LD, Pelton RW, Wood LD. Negative regulation of gene expression by TGF-beta. Mol Reprod Dev **32**:111–120, 1992.
175. Raghow R. Role of transforming growth factor-beta in repair and fibrosis. Chest **99**:61S–65S, 1991.
176. Diaz A, Varga J, Jimenez SA. Transforming growth factor-beta stimulation of lung fibroblast prostaglandin E2 production. J Biol Chem **264**:11554–11557, 1989.
177. Rossi P, Karsenty G, Roberts AB, Roche NS, Sporn MB, de-Crombrugghe B. A nuclear factor 1 binding site mediates the transcriptional activation of a type I collagen promoter by transforming growth factor-beta. Cell **52**:405–414, 1988.
178. Ritzenthaler JD, Goldstein RH, Fine A, Smith BD. Regulation of the alpha 1(I) collagen promoter via a transforming growth factor-beta activation element. J Biol Chem **268**:13625–13631, 1993.
179. Kerr LD, Miller DB, Matrisian LM. TGF-beta 1 inhibition of transin/stromelysin gene expression is mediated through a Fos binding sequence. Cell **61**:267–278, 1990.
180. Wright JK, Cawston TE, Hazleman BL. Transforming growth factor beta stimulates the production of the tissue inhibitor of metalloproteinases (TIMP) by human synovial and skin fibroblasts. Biochim Biophys Acta **1094**:207–210, 1991.
181. Overall CM, Wrana JL, Sodek J. Independent regulation of collagenase, 72-kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-beta. J Biol Chem **264**:1860–1869, 1989.
182. McAnulty RJ, Campa JS, Cambrey AD, Laurent GJ. The effect of transforming growth factor beta on rates of procollagen synthesis and degradation *in vitro*. Biochim Biophys Acta **1091**:231–235, 1991.
183. Raghow B, Irish P, Kang AH. Coordinate regulation of transforming growth factor beta gene expression and cell proliferation in hamster lungs undergoing bleomycin-induced pulmonary fibrosis. J Clin Invest **84**:1836–1842, 1989.
184. Schreier T, Degen E, Baschong W. Fibroblast migration and proliferation during *in vitro* wound healing. A quantitative comparison between various growth factors and a low molecular weight blood dialysate used in the clinic to normalize impaired wound healing. Res Exp Med Berl **193**:195–205, 1993.
185. Heino J, Ignotz RA, Hemler ME, Crouse C, Massague J. Regulation of cell adhesion receptors by transforming growth factor-beta. Concomitant regulation of integrins that share a common beta 1 subunit. J Biol Chem **264**:380–388, 1989.
186. Kawaguchi Y, Kitani A, Hara M, et al. Cytokine regulation of prolyl 4-hydroxylase production in skin fibroblast cultures from patients with systemic sclerosis: contribution to collagen synthesis and fibrosis. J Rheumatol **19**:1195–1201, 1992.
187. Schultz G, Chegini N, Grant M, Khaw P, MacKay S. Effects of growth factors on corneal wound healing. Acta Ophthalmol (Suppl) **202**:60–66, 1992.
188. Roberts AB, Sporn MB. Regulation of endothelial cell growth, architecture, and matrix synthesis by TGF-beta. Am Rev Respir Dis **140**:1126–1128, 1989.
189. Elias JA, Lentz V, Cummings PJ. Transforming growth factor-beta regulation of IL-6 production by unstimulated and IL-1-stimulated human fibroblasts (published erratum appears in J Immunol **147**[4]:1460, 1991). J Immunol **146**:3437–3443, 1991.
190. Turner M, Chantry D, Feldmann M. Transforming growth factor beta induces the production of interleukin 6 by human peripheral blood mononuclear cells. Cytokine **2**:211–216, 1990.
191. Grant MB, Khaw PT, Schultz GS, Adams JL, Shimizu RW. Effects of epidermal growth factor, fibroblast growth factor, and transforming growth factor-beta on corneal cell chemotaxis. Invest Ophthalmol Vis Sci **33**:3292–3301, 1992.
192. Pierce GF, Mustoe TA, Lingelbach J, et al. Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. J Cell Biol **109**:429–440, 1989.
193. Lucas PA, Caplan AI. Chemotactic response of embryonic limb bud mesenchymal cells and muscle-derived fibroblasts to transforming growth factor-beta. Connect Tissue Res **18**:1–7, 1988.
194. Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. J Exp Med **165**:251–256, 1987.
195. Osornio-Vargas AR, Kalter VG, Badgett A, Hernandez-Rodriguez N, Aguilar-Delfin I, Brody AR. Early-passage rat lung fibroblasts do not migrate in vitro to transforming growth factor-beta. Am J Respir Cell Mol Biol **8**:468–471, 1993.
196. Chen RH, Ebner R, Derynck R. Inactivation of the type II receptor reveals two receptor pathways for the diverse TGF-beta activities. Science **260**:1335–1338, 1993.
197. Bassing CH, Howe DJ, Segarini PR, Donahoe PK, Wang X-F. A single heteromeric receptor complex is sufficient to mediate biological effects of transforming growth factor-beta ligands. J Biol Chem **269**:14861–14864, 1994.
198. Partanen J, Vainikka S, Alitalo K. Structural and functional specificity of FGF receptors. Philos Trans R Soc Lond Biol **249**:297–303, 1993.
199. Basilico C, Moscatelli D. The FGF family of growth factors and oncogenes. Adv Cancer Res **59**:115–165, 1992.
200. Kondo H, Matsuda R, Yonezawa Y. Autonomous migration of human fetal skin fibroblasts into a denuded area in a cell monolayer is mediated by basic fibroblast growth factor and collagen. *In Vitro* Cell Dev Biol Anim **29A**:929–935, 1993.
201. Adamson IY, Bowden DH. Pulmonary reaction to long and short asbestos fibers is independent of fibroblast growth factor production by alveolar macrophages. Am J Pathol **137**:523–529, 1990.
202. Panos RJ, Rubin JS, Aaronson SA, Mason RJ. Keratinocyte growth factor and hepatocyte growth factor/scatter factor are heparin-binding growth factors for alveolar type II cells in fibroblast-conditioned medium. J Clin Invest **92**:969–977, 1993.
203. Yamashita Y, Nakagomi K, Takeda T, Hasegawa S, Mitsui Y. Effect of heparin on pulmonary fibroblasts and vascular cells. Thorax **47**:634–639, 1992.
204. Gessin JC, Brown LJ, Gordon JS, Berg RA. Regulation of collagen synthesis in human dermal fibroblasts in contracted collagen gels by ascorbic acid, growth factors, and inhibitors of lipid peroxidation. Exp Cell Res **206**:283–290, 1993.
205. Tan EM, Rouda S, Greenbaum SS, Moore JH Jr., Fox JW IV, Sollberg S. Acidic and basic fibroblast growth factors down-regulate collagen gene expression in keloid fibroblasts. Am J Pathol **142**:463–470, 1993.
206. Majors A, Ehrhart LA. Basic fibroblast growth factor in the extracellular matrix suppresses collagen synthesis and type III procollagen mRNA levels in arterial smooth muscle cell cultures. Arterioscler Thromb **13**:680–686, 1993.
207. Kay EP, Gu X, Ninomiya Y, Smith RE. Corneal endothelial modulation: A factor released by leukocytes induces basic fibroblast growth factor that modulates cell shape and collagen. Invest Ophthalmol Vis Sci **34**:663–672, 1993.
208. Sasaki T. The effects of basic fibroblast growth factor and doxorubicin on cultured human skin fibroblasts: Relevance to wound healing. J Dermatol **19**:664–666, 1992.
209. Chua CC, Chua BH, Zhao ZY, Krebs C, Diglio C, Perrin E. Effect of growth factors on collagen metabolism in cultured human heart fibroblasts. Connect Tissue Res **26**:271–281, 1991.
210. Rifkin DB, Moscatelli D, Bizik J, et al. Growth factor control of extracellular proteolysis. Cell Differ Dev **32**:313–318, 1990.
211. Chandrasekhar S, Harvey AK. Differential regulation of metalloprotease steady-state mRNA levels by IL-1 and FGF in rabbit articular chondrocytes. FEBS Lett **296**:195–200, 1992.
212. Khachigian LM, Chesterman CN. Platelet-derived growth factor and alternative splicing: A review. Pathology **24**:280–290, 1992.
213. Westermark B, Heldin CH. Platelet-derived growth factor. Structure, function and implications in normal and malignant cell growth. Acta Oncol **32**:101–105, 1993.
214. Seppa H, Grotendorst G, Seppa S, Schiffmann E, Martin GR.

Platelet-derived growth factor in chemotactic for fibroblasts. J Cell Biol **92**:584–588, 1982.
215. Haynes AR, Shaw RJ. Dexamethasone-induced increase in platelet-derived growth factor (B) mRNA in human alveolar macrophages and myelomonocytic HL60 macrophage-like cells. Am J Respir Cell Mol Biol **7**:198–206, 1992.
216. Antoniades HN, Galanopoulos T, Neville-Golden J, O'Hara CJ. Malignant epithelial cells in primary human lung carcinomas coexpress *in vivo* platelet-derived growth factor (PDGF) and PDGF receptor mRNAs and their protein products. Proc Natl Acad Sci USA **89**:3942–3946, 1992.
217. Brody AR, Bonner JC, Overby LH, et al. Interstitial pulmonary macrophages produce platelet-derived growth factor that stimulates rat lung fibroblast proliferation *in vitro*. J Leukoc Biol **51**:640–648, 1992.
218. Nagaoka I, Trapnell BC, Crystal RG. Upregulation of platelet-derived growth factor-A and -B gene expression in alveolar macrophages of individuals with idiopathic pulmonary fibrosis. J Clin Invest **85**:2023–2027, 1990.
219. Martinet Y, Rom WN, Grotendorst GR, Martin GR, Crystal RG. Exaggerated spontaneous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. N Engl J Med **317**:202–209, 1987.
220. Geesin JC, Hendricks LJ, Gordon JS, Berg RA. Modulation of collagen synthesis by growth factors: the role of ascorbate-stimulated lipid peroxidation. Arch Biochem Biophys **289**:6–11, 1991.
221. Amento EP, Ehsani N, Palmer H, Libby P. Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. Arterioscler Thromb **11**:1223–1230, 1991.
222. Matsuda N, Lin WL, Kumar NM, Cho MI, Genco RJ. Mitogenic, chemotactic, and synthetic responses of rat periodontal ligament fibroblastic cells to polypeptide growth factors *in vitro*. J Periodontol **63**:515–525, 1992.
223. Pierce GF, Tarpley JE, Yanagihara D, Mustoe TA, Fox GM, Thomason A. Platelet-derived growth factor (BB homodimer), transforming growth factor-beta 1, and basic fibroblast growth factor in dermal wound healing. Neovessel and matrix formation and cessation of repair. Am J Pathol **140**:1375–1388, 1992.
224. Okada Y, Katsuda S, Watanabe H, Nakanishi I. Collagen synthesis of human arterial smooth muscle cells: effects of platelet-derived growth factor, transforming growth factor-beta 1 and interleukin-1. Acta Pathol Jpn **43**:160–167, 1993.
225. Circolo A, Welgus HG, Pierce GF, Kramer J, Strunk RC. Differential regulation of the expression of proteinases/antiproteinases in fibroblasts. Effects of interleukin-1 and platelet-derived growth factor. J Biol Chem **266**:12283–12288, 1991.
226. Hiraoka K, Sasaguri Y, Komiya S, Inoue A, Morimatsu M. Cell proliferation-related production of matrix metalloproteinases 1 (tissue collagenase) and 3 (stromelysin) by cultured human rheumatoid synovial fibroblasts. Biochem Int **27**:1083–1091, 1992.
227. Kato S, Sasaguri Y, Morimatsu M. Down-regulation in the production of matrix metalloproteinase 1 by human aortic intimal smooth muscle cells. Biochem Mol Biol Int **31**:239–248, 1993.
228. Griffin M, Bhandari R, Hamilton G, Chan YC, Powell JT. Alveolar type II cell-fibroblast interactions, synthesis and secretion of surfactant and type I collagen. J Cell Sci **105**:423–432, 1993.
229. Rom WN, Paakko P. Activated alveolar macrophages express the insulin-like growth factor-I receptor. Am J Respir Cell Mol Biol **4**:432–439, 1991.
230. Stiles AD, Moats-Staats BM. Production and action of insulin-like growth factor I/somatomedin C in primary cultures of fetal lung fibroblasts. Am J Respir Cell Mol Biol **1**:21–26, 1989.
231. Goldstein RH, Poliks CF, Pilch PF, Smith BD, Fine A. Stimulation of collagen formation by insulin and insulin-like growth factor I in cultures of human lung fibroblasts. Endocrinology **124**:964–970, 1989.
232. Gillery P, Leperre A, Maquart FX, Borel JP. Insulin-like growth factor-I (IGF-I) stimulates protein synthesis and collagen gene expression in monolayer and lattice cultures of fibroblasts. J Cell Physiol **152**:389–396, 1992.
233. Granot I, Halevy O, Hurwitz S, Pines M. Growth hormone and insulin-like growth factor I regulate collagen gene expression and extracellular collagen in cultures of avian skin fibroblasts. Mol Cell Endocrinol **80**:1–9, 1991.
234. Klein-Soyer C, Archipoff G, Beretz A, Cazenave JP. Opposing effects of heparin with TGF-beta or aFGF during repair of a mechanical wound of human endothelium. Influence of cAMP on cell migration. Biol Cell **75**:155–162, 1992.
235. Ellis I, Grey AM, Schor AM, Schor SL. Antagonistic effects of TGF-beta 1 and MSF on fibroblast migration and hyaluronic acid synthesis. Possible implications for dermal wound healing. J Cell Sci **102**:447–456, 1992.
236. Soma Y, Takehara K, Ishibashi Y. Alteration of the chemotactic response of human skin fibroblasts to PDGF by growth factors. Exp Cell Res **212**:274–277, 1994.
237. Adelmann-Grill BC, Wach F, Cully Z, Hein R, Krieg T. Chemotactic migration of normal dermal fibroblasts towards epidermal growth factor and its modulation by platelet-derived growth factor and transforming growth factor-beta. Eur J Cell Biol **51**:322–326, 1990.
238. Meyer FA, Yaron I, Yaron M. Synergistic, additive, and antagonistic effects of interleukin-1 beta, tumor necrosis factor alpha, and gamma-interferon on prostaglandin E, hyaluronic acid, and collagenase production by cultured synovial fibroblasts. Arthritis Rheum **33**:1518–1525, 1990.
239. Kumkumian GK, Lafyatis R, Remmers EF, Case JP, Kim SJ, Wilder RL. Platelet-derived growth factor and IL-1 interactions in rheumatoid arthritis. Regulation of synoviocyte proliferation, prostaglandin production, and collagenase transcription. J Immunol **143**:833–837, 1989.
240. Seitz M, Loetscher P, Dewald B, Towbin H, Ceska M, Baggiolini M. Production of interleukin-1 receptor antagonist, inflammatory chemotactic proteins, and prostaglandin E by rheumatoid and osteoarthritic synoviocytes—Regulation by IFN-gamma and IL-4. J Immunol **152**:2060–2065, 1994.
241. Elias JA, Gustilo K, Freundlich B. Human alveolar macrophage and blood monocyte inhibition of fibroblast proliferation. Evidence for synergy between interleukin-1 and tumor necrosis factor. Am Rev Respir Dis **138**:1595–1603, 1988.
242. Jackson BA, Goldstein RH, Roy R, Cozzani M, Taylor L, Polgar P. Effects of transforming growth factor beta and interleukin-1 beta on expression of cyclooxygenase 1 and 2 and phospholipase A2 mRNA in lung fibroblasts and endothelial cells in culture. Biochem Biophys Res Commun **197**:1465–1474, 1993.
243. Barr RM, Symonds PH, Akpan AS, Greaves MW. Culture of human dermal fibroblasts in collagen gels: modulation of interleukin 1-induced prostaglandin E2 synthesis by an extracellular matrix. Exp Cell Res **198**:321–327, 1992.
244. Bry K, Lappalainen U, Hallman M. Interleukin-1 binding and prostaglandin E2 synthesis by amnion cells in culture: Regulation by tumor necrosis factor-alpha, transforming growth factor-beta, and interleukin-1 receptor antagonist. Biochim Biophys Acta **1181**:31–36, 1993.
245. Haynes DR, Whitehouse MW, Vernon-Roberts B. The prostaglandin E1 analogue, misoprostol, regulates inflammatory cytokines and immune functions *in vitro* like the natural prostaglandins E1, E2 and E3. Immunology **76**:251–257, 1992.
246. Chensue SW, Remick DG, Higashi GI, Boros DL, Kunkel SL. Modulation of murine schistosomiasis by exogenously administered prostaglandins. Am J Pathol **125**:28–34, 1986.
247. Cahill J, Hopper KE. Immunoregulation by macrophages. III. Prostaglandin E suppresses lymphocyte activation but not macrophage effector function during Salmonella enteritidis infection. Int J Immunopharmacol **6**:9–17, 1984.
248. Utsunomiya I, Nagai S, Oh-ishi S. Differential effects of indomethacin and dexamethasone on cytokine production in carrageenin-induced rat pleurisy. Eur J Pharmacol **252**:213–218, 1994.
249. Viherluoto J, Palkama T, Silvennoinen O, Hurme M. Cyclic adenosine monophosphate decreases the secretion, but not the cell-associated levels, of interleukin-1 beta in lipopolysaccha-

ride-activated human monocytes. Scand J Immunol **34**:121–125, 1991.
250. Bailly S, Ferrua B, Fay M, Gougerot-Pocidalo MA. Differential regulation of IL 6, IL 1 A, IL 1 beta and TNF alpha production in LPS-stimulated human monocytes: Role of cyclic AMP. Cytokine **2**:205–210, 1990.
251. Takii T, Akahoshi T, Kato K, Hayashi H, Marunouchi T, Onozaki K. Interleukin-1 up-regulates transcription of its own receptor in a human fibroblast cell line TIG-1: Role of endogenous PGE2 and cAMP. Eur J Immunol **22**:1221–1227, 1992.
252. Schalkwijk CG, de-Vet E, Pfeilschifter J, van-den-Bosch H. Interleukin-1 beta and transforming growth factor-beta 2 enhance cytosolic high-molecular-mass phospholipase A2 activity and induce prostaglandin E2 formation in rat mesangial cells. Eur J Biochem **210**:169–176, 1992.
253. Pfeilschifter J, Pignat W, Leighton J, Marki F, Vosbeck K, Alkan S. Transforming growth factor beta 2 differentially modulates interleukin-1 beta- and tumour-necrosis-factor-alpha-stimulated phospholipase A2 and prostaglandin E2 synthesis in rat renal mesangial cells. Biochem J **270**:269–271, 1990.
254. Gilbert RS, Reddy ST, Kujubu DA, Xie W, Luner S, Herschman HR. Transforming growth factor beta 1 augments mitogen-induced prostaglandin synthesis and expression of the TIS 10/prostaglandin synthase 2 gene both in Swiss 3T3 cells and in murine embryo fibroblasts. J Cell Physiol **159**: 67–75, 1994.
255. Miller-Graziano CL, Szabo G, Griffey K, Mehta B, Kodys K, Catalano D. Role of elevated monocyte transforming growth factor beta (TGF beta) production in posttrauma immunosuppression. J Clin Immunol **11**:95–102, 1991.
265. Reddy ST, Gilbert RS, Xie W, Luner S, Herschman HR. TGF-beta 1 inhibits both endotoxin-induced prostaglandin synthesis and expression of the TIS 10/prostaglandin synthase 2 gene in murine macrophages. J Leukoc Biol **55**:192–200, 1994.
257. Taylor DJ, Feldmann M, Evanson JM, Woolley DE. Comparative and combined effects of transforming growth factors alpha and beta, interleukin-1 and interferon-gamma on rheumatoid synovial cell proliferation, glycolysis and prostaglandin E production. Rheumatol Int **9**:65–70, 1989.
258. Hamilton JA, Piccoli DS, Leizer T, Butler DM, Croatto M, Royston AK. Transforming growth factor beta stimulates urokinase-type plasminogen activator and DNA synthesis, but not prostaglandin E2 production, in human synovial fibroblasts. Proc Natl Acad Sci USA **88**:7180–7184, 1991.
259. Phan SH, Gharaee-Kermani M, McGarry B, Kunkel SL, Wolber FW. Regulation of rat pulmonary artery endothelial cell transforming growth factor-beta production by IL-1 beta and tumor necrosis factor-alpha. J Immunol **149**:103–106, 1992.
260. Bristol LA, Ruscetti FW, Brody DT, Durum SK. IL-1 alpha induces expression of active transforming growth factor-beta in nonproliferating T cells via a post-transcriptional mechanism. J Immunol **145**:4108–4114, 1990.
261. McCartney-Francis N, Mizel D, Wong H, Wahl L, Wahl S. TGF-beta regulates production of growth factors and TGF-beta by human peripheral blood monocytes. Growth Factors **4**:27–35, 1990.
262. Turner M, Chantry D, Katsikis P, Berger A, Brennan FM, Feldmann M. Induction of the interleukin 1 receptor antagonist protein by transforming growth factor-beta. Eur J Immunol **21**:1635–1639, 1991.
263. Wahl SM, Costa GL, Corcoran M, Wahl LM, Berger AE. Transforming growth factor-beta mediates IL-1-dependent induction of IL-1 receptor antagonist. J Immunol **150**:3553–3560, 1993.
264. Dubois CM, Ruscetti FW, Palaszynski EW, Falk LA, Oppenheim JJ, Keller JR. Transforming growth factor beta is a potent inhibitor of interleukin 1 (IL-1) receptor expression: Proposed mechanism of inhibition of IL-1 action. J Exp Med **172**:737–744, 1990.
265. Harvey AK, Hrubey PS, Chandrasekhar S. Transforming growth factor-beta inhibition of interleukin-1 activity involves down-regulation of interleukin-1 receptors on chondrocytes. Exp Cell Res **195**:376–385, 1991.
266. Wangoo A, Taylor IK, Haynes AR, Shaw RJ. Up-regulation of alveolar macrophage platelet-derived growth factor-B (PDGF-B) mRNA by interferon-gamma from Mycobacterium tuberculosis antigen (PPD)-stimulated lymphocytes. Clin Exp Immunol **94**:43–50, 1993.
267. Shaw RJ, Benedict SH, Clark RA, King TE Jr. Pathogenesis of pulmonary fibrosis in interstitial lung disease. Alveolar macrophage PDGF(B) gene activation and up-regulation by interferon gamma. Am Rev Respir Dis **143**:167–173, 1991.
268. Irwin CR, Schor SL, Ferguson MWJ. Effects of cytokines on gingival fibroblasts *in vitro* are modulated by the extracellular matrix. J Periodontal Res **29**:309–317, 1994.
269. Warburton D, Lee M, Berberich MA, Bernfield M. Molecular embryology and the study of lung development. Am J Respir Cell Mol Biol **9**:5–9, 1993.
270. Bradley KH, McConnell SD, Crystal RG. Lung collagen composition and synthesis. Characterization and changes with age. J Biol Chem **249**:2674–2683, 1974.
271. Larson JE. Developmental control of collagen gene expression in the rat lung: Confirmation of early observations of lung growth. Pediatr Pulmonol **15**:205–208, 1993.
272. Wessells NK, Cohen JH. Effects of collagenase on developing epithelia *in vitro:* Lung, uretic bud, and pancreas. Dev Biol **18**:294–309, 1968.
273. Ganser GL, Stricklin GP, Matrisian LM. EGF and TGF alpha influence *in vitro* lung development by the induction of matrix-degrading metalloproteinases. Int J Dev Biol **35**:453–461, 1991.
274. Thomas T, Dziadek M. Expression of collagen alpha 1(IV), laminin and nidogen genes in the embryonic mouse lung: Implications for branching morphogenesis. Mech Dev **45**:193–201, 1994.
275. Mayer U, Timpl R. Role of mesenchymal nidogen for epithelial morphogenesis *in vitro*. Development **120**:2003–2014, 1994.
276. Arden MG, Adamson IY. Collagen degradation during postnatal lung growth in rats. Pediatr Pulmonol **14**:95–101, 1992.
277. Reponen P, Sahlberg C, Huhtala P, Hurskainen T, Thesleff I, Tryggvason K. Molecular cloning of murine 72-kDa type IV collagenase and its expression during mouse development. J Biol Chem **267**:7856–7862, 1992.
278. D'Armiento J, Dalal SS, Okada Y, Berg RA, Chada K. Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema. Cell **71**:955–961, 1992.
279. Berk JL, Fine A, Goldstein RH. Pulmonary lipid interstitial cells. In: Phipps RP, Ed. Pulmonary Fibroblast Heterogeneity. Boca Raton: CRC Press, pp55–77, 1992.
280. Miller DA, Lee A, Pelton RW, Chen EY, Moses HL, Derynck R. Murine transforming growth factor-beta 2 cDNA sequence and expression in adult tissues and embryos. Mol Endocrinol **3**:1108–1114, 1989.
281. Jakowlew SB, Ciment G, Tuan RS, Sporn MB, Roberts AB. Pattern of expression of transforming growth factor-beta 4 mRNA and protein in the developing chicken embryo. Dev Dyn **195**:276–289, 1992.
282. Heine UI, Munoz EF, Flanders KC, Roberts AB, Sporn MB. Colocalization of TGF-beta 1 and collagen I and III, fibronectin and glycosaminoglycans during lung branching morphogenesis. Development **109**:29–36, 1990.
283. Kulkarni AB, Huh CG, Becker D, et al. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA **90**:770–774, 1993.
284. Letterio JJ, Geiser AG, Kulkarni AB, Roche NS, Sporn MB, Roberts AS. Maternal rescue of transforming growth factor-beta 1 null mice. Science **264**:1936–1938, 1994.
285. Crystal RG, Ferrans VJ, Basset F. Biologic basis of pulmonary fibrosis. In: Crystal RG, West JB, Eds. The Lung: Scientific Foundations. New York: Raven Press, Vol 2:pp2031–2046, 1991.
286. Seyer JM, Hutcheson ET, Kang AH. Collagen polymorphism in idiopathic chronic pulmonary fibrosis. J Clin Invest **57**:1498–1507, 1976.
287. Fukuda Y, Ferrans VJ, Schoenberger CI, Rennard SI, Crystal RG. Patterns of pulmonary structural remodeling after experimental paraquat toxicity. The morphogenesis of intraalveolar fibrosis. Am J Pathol **118**:452–475, 1985.

288. McDonald JA, Broekelmann TJ, Matheke ML, Crouch E, Koo M, Kuhn C III. A monoclonal antibody to the carboxyterminal domain of procollagen type I visualizes collagen-synthesizing fibroblasts. Detection of an altered fibroblast phenotype in lungs of patients with pulmonary fibrosis. J Clin Invest **78**:1237–1244, 1986.
289. Zhang K, Gharaee-Kermani M, McGarry B, Phan SH. *In situ* hybridization analysis of rat lung alpha 1(I) and alpha 2(I) collagen gene expression in pulmonary fibrosis induced by endotracheal bleomycin injection. Lab Invest **70**:192–202, 1994.
290. Vracko R. Significance of basal lamina for regeneration of injured lung. Virchows Arch A Pathol Anat **355**:264–274, 1972.
291. Vracko R. Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure. Am J Pathol **77**:314–346, 1974.
292. Bowden DH. Unraveling pulmonary fibrosis: The bleomycin model. (editorial) Lab Invest **50**:487–488, 1984.
293. Crouch E. Pathobiology of pulmonary fibrosis. Am J Physiol **259**:L159–L184, 1990.
294. Kuhn C. Patterns of lung repair. A morphologist's view. Chest **99**:11S–14S, 1991.
295. du-Bois RM. Idiopathic pulmonary fibrosis. Annu Rev Med **44**:441–450, 1993.
296. Fine A, Goldstein RH, Snider GL. Animal models of pulmonary fibrosis. In: Crystal RG, West JB, Eds. The Lung: Scientific Foundations. New York: Raven Press, Vol **2**:pp2073–2081, 1991.
297. Sheppard MN, Harrison NK. New perspectives on basic mechanisms in lung disease. 1. Lung injury, inflammatory mediators, and fibroblast activation in fibrosing alveolitis. Thorax **47**:1064–1074, 1992.
298. Cherniack RM, Crystal RG, Kalica AR. NHLBI Workshop summary. Current concepts in idiopathic pulmonary fibrosis: A road map for the future. Am Rev Respir Dis **143**:680–683, 1991.
299. Breen E, Shull S, Burne S, et al. Bleomycin regulation of transforming growth factor-beta mRNA in rat lung fibroblasts. Am J Respir Cell Mol Biol **6**:146–152, 1992.
300. Phan SH, Gharaee-Kermani M, Wolber F, Ryan US. Stimulation of rat endothelial cell transforming growth factor-beta production by bleomycin. J Clin Invest **87**:148–154, 1991.
301. Karmiol S, Remick DG, Kunkel SL, Phan SH. Regulation of rat pulmonary endothelial cell interleukin-6 production by bleomycin: effects of cellular fatty acid composition. Am J Respir Cell Mol Biol **9**:628–636, 1993.
302. Denholm EM, Phan SH. The effects of bleomycin on alveolar macrophage growth factor secretion. Am J Pathol **134**:355–363, 1989.
303. Scheule RK, Perkins RC, Hamilton R, Holian A. Bleomycin stimulation of cytokine secretion by the human alveolar macrophage. Am J Physiol **262**:L386–L391, 1992.
304. Phan SH, Kunkel SL. Lung cytokine production in bleomycin-induced pulmonary fibrosis. Exp Lung Res **18**:29–43, 1992.
305. Harrison JH Jr., Hoyt DG, Lazo JS. Acute pulmonary toxicity of bleomycin: DNA scission and matrix protein mRNA levels in bleomycin-sensitive and -resistant strains of mice. Mol Pharmacol **36**:231–238, 1989.
306. Adamson IY, Bakowska J, Bowden DH. Mesothelial cell proliferation: A nonspecific response to lung injury associated with fibrosis. Am J Respir Cell Mol Biol **10**:253–258, 1994.
307. Phipps RP, Baecher C, Frelinger JG, Penney DP, Keng P, Brown D. Differential expression of interleukin 1 alpha by Thy-1$^+$ and Thy-1$^-$ lung fibroblast subpopulations: Enhancement of interleukin 1 alpha production by tumor necrosis factor-alpha. Eur J Immunol **20**:1723–1727, 1990.
308. Korn JH, Thrall RS, Wilbur DC, Kream BE, Piela-Smith TH. Fibroblast heterogeneity: Clonal selection of fibroblasts as a model for fibrotic disease. In: Phipps RP, Ed. Pulmonary Fibroblast Heterogeneity. Boca Raton: CRC Press, pp119–133, 1992.
309. Torry DJ, Richards CD, Podor TJ, Gauldie J. Anchorage-independent colony growth of pulmonary fibroblasts derived from fibrotic human lung tissue. J Clin Invest **93**:1525–1532, 1994.
310. Friedman SL, Arthur MJ. Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. J Clin Invest **84**:1780–1785, 1989.
311. Pinzani M, Abboud HE, Aron DC. Secretion of insulin-like growth factor-I and binding proteins by rat liver fat-storing cells: Regulatory role of platelet-derived growth factor. Endocrinology **127**:2343–2349, 1990.
312. Weiner FR, Giambrone MA, Czaja MJ, et al. Ito-cell gene expression and collagen regulation. Hepatology **11**:111–117, 1990.
313. Matsuoka M, Pham NT, Tsukamoto H. Differential effects of interleukin-1 alpha, tumor necrosis factor alpha, and transforming growth factor beta 1 on cell proliferation and collagen formation by cultured fat-storing cells. Liver **9**:71–78, 1989.
314. Sempowski GD, Beckmann MP, Derdak S, Phipps RP. Subsets of murine lung fibroblasts express membrane-bound and soluble IL-4 receptors. Role of IL-4 in enhancing fibroblast proliferation and collagen synthesis. J Immunol **152**:3606–3614, 1994.
315. Phipps RP, Penney DP, Keng P, Silvera M, Harkins S, Derdak S. Immune functions of subpopulations of lung fibroblasts. Immunol Res **9**:275–286, 1990.
316. Zhang K, Rekhter MD, Gordon D, Phan SH. Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. A combined immunohistochemical and *in situ* hybridization study. Am J Pathol **145**:114–125, 1994.
317. Vyalov SL, Gabbiani G, Kapanci Y. Rat alveolar myofibroblasts acquire alpha-smooth muscle actin expression during bleomycin-induced pulmonary fibrosis. Am J Pathol **143**:1754–1765, 1993.
318. Breen E, Falco VM, Absher M, Cutroneo KR. Subpopulations of rat lung fibroblasts with different amounts of type I and type III collagen mRNAs. J Biol Chem **265**:6286–6290, 1990.
319. Gadek JE, Kelman JA, Fells G, et al. Collagenase in the lower respiratory tract of patients with idiopathic pulmonary fibrosis. N Engl J Med **301**:737–742, 1979.
320. Kerr JS, Chae CU, Nagase H, Berg RA, Riley DJ. Degradation of collagen in lung tissue slices exposed to hyperoxia. Am Rev Respir Dis **135**:1334–1339, 1987.
321. Sterling KM Jr., DiPetrillo TA, Kotch JP, Cutroneo KR. Bleomycin-induced increase of collagen turnover in IMR-90 fibroblasts: An *in vitro* model of connective tissue restructuring during lung fibrosis. Cancer Res **42**:3502–3506, 1982.
322. Sakai J, Hung J, Zhu G, Katakami C, Boyce S, Kao WW. Collagen metabolism during healing of lacerated rabbit corneas. Exp Eye Res **52**:237–244, 1991.
323. McGaw WT. The effect of tension on collagen remodelling by fibroblasts: A stereological ultrastructural study. Connect Tissue Res **14**:229–235, 1986.
324. Falcone DJ, McCaffrey TA, Haimovitz-Friedman A, Vergilio JA, Nicholson AC. Macrophage and foam cell release of matrix-bound growth factors. Role of plasminogen activation. J Biol Chem **268**:11951–11958, 1993.
325. Falcone DJ, McCaffrey TA, Haimovitz-Friedman A, Garcia M. Transforming growth factor-beta 1 stimulates macrophage urokinase expression and release of matrix-bound basic fibroblast growth factor. J Cell Physiol **155**:595–605, 1993.
326. Crystal RG. Alveolar macrophages. In: Crystal RG, West JB, Eds. The Lung: Scientific Foundations. New York: Raven Press, Vol **1**:pp527–538, 1991.
327. Rom WN, Travis WD, Brody AR. Cellular and molecular basis of the asbestos-related diseases. Am Rev Respir Dis **143**:408–422, 1991.
328. Elias JA, Ferro TJ, Rossman MD, et al. Differential prostaglandin production by unfractionated and density-fractionated human monocytes and alveolar macrophages. J Leukoc Biol **42**:114–121, 1987.
329. Friedlander MA, Hilbert CM, Wu YC, Finegan CK, Rich EA. Disparate cytochemical characteristics and production of cytokines and prostaglandin E2 by human mononuclear phagocytes from the blood, lung, and peritoneal cavity. J Lab Clin Med **123**:574–584, 1994.

330. Laskin DL, Soltys RA, Berg RA, Riley DJ. Activation of alveolar macrophages by native and synthetic collagen-like polypeptides. Am J Respir Cell Mol Biol **10**:58–64, 1994.
331. Kline JN, Schwartz DA, Monick MM, Floerchinger CS, Hunninghake GW. Relative release of interleukin-1 beta and interleukin-1 receptor antagonist by alveolar macrophages. A study in asbestos-induced lung disease, sarcoidosis, and idiopathic pulmonary fibrosis. Chest **104**:47–53, 1993.
332. Pueringer RJ, Schwartz DA, Dayton CS, Gilbert SR, Hunninghake GW. The relationship between alveolar macrophage TNF, IL-1, and PGE2 release, alveolitis, and disease severity in sarcoidosis. Chest **103**:832–838, 1993.
333. O'Brien-Ladner A, Nelson ME, Kimler BF, Wesselius LJ. Release of interleukin-1 by human alveolar macrophages after *in vitro* irradiation. Radiat Res **136**:37–41, 1993.
334. Piguet PF, Ribaux C, Karpuz V, Grau GE, Kapanci Y. Expression and localization of tumor necrosis factor-alpha and its mRNA in idiopathic pulmonary fibrosis. Am J Pathol **143**:651–655, 1993.
335. Nash JR, McLaughlin PJ, Butcher D, Corrin B. Expression of tumour necrosis factor-alpha in cryptogenic fibrosing alveolitis. Histopathology **22**:343–347, 1993.
336. Kawaguchi Y, Harigai M, Suzuki K, et al. Interleukin 1 receptor on fibroblasts from systemic sclerosis patients induces excessive functional responses to interleukin 1 beta. Biochem Biophys Res Commun **190**:154–161, 1993.
337. Piguet PF, Collart MA, Grau GE, Sappino AP, Vassalli P. Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. Nature **344**:245–247, 1990.
338. Piguet PF, Collart MA, Grau GE, Kapanci Y, Vassalli P. Tumor necrosis factor/cachectin plays a key role in bleomycin-induced pneumopathy and fibrosis. J Exp Med **170**:655–663, 1989.
339. Deguchi Y, Kishimoto S. Tumour necrosis factor/cachectin plays a key role in autoimmune pulmonary inflammation in lupus-prone mice. Clin Exp Immunol **85**:392–395, 1991.
340. Piguet PF, Vesin C, Grau GE, Thompson RC. Interleukin 1 receptor antagonist (IL-1ra) prevents or cures pulmonary fibrosis elicited in mice by bleomycin or silica. Cytokine **5**:57–61, 1993.
341. Piguet PF, Vesin C. Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. Eur Respir J **7**:515–518, 1994.
342. Denis M. Interleukin-1 (IL-1) is an important cytokine in granulomatous alveolitis. Cell Immunol **157**:70–80, 1994.
343. Dubaybo BA, Marwah GS, Fligiel SE, Hatfield JS, Majumdar AP. Tyrosine kinase activation during lung injury, fibrosis, and compensatory lung growth. Exp Lung Res **16**:257–266, 1990.
344. Dubaybo BA, Rubeiz GJ, Fligiel SE. Dynamic changes in the functional characteristics of the interstitial fibroblast during lung repair. Exp Lung Res **18**:461–477, 1992.
345. Novogrodsky A, Vanichkin A, Patya M, Gazit A, Osherov N, Levitzki A. Prevention of lipopolysaccharide-induced lethal toxicity by tyrosine kinase inhibitors. Science **264**:1319–1322, 1994.
346. Mohler KM, Sleath PR, Fitzner JN, et al. Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. Nature **370**:218–220, 1994.
347. Johnson RJ, Raines EW, Floege J, et al. Inhibition of mesangial cell proliferation and matrix expansion in glomerulonephritis in the rat by antibody to platelet-derived growth factor. J Exp Med **175**:1413–1416, 1992.
348. Denis M. Interleukin-6 in mouse hypersensitivity pneumonitis: changes in lung free cells following depletion of endogenous IL-6 or direct administration of IL-6. J Leukoc Biol **52**:197–201, 1992.
349. Zia S, Hyde DM, Giri SN. Effects of an interferon inducer bropirimine on bleomycin-induced lung fibrosis in hamster. Pharmacol Toxicol **71**:11–18, 1992.
350. Giri SN, Hyde DM. Ameliorating effect of an interferon polyinosinic-polycytidylic acid on bleomycin-induced lung fibrosis in hamsters. Morphologic and biochemical evidence. Am J Pathol **133**:525–536, 1988.
351. Jaffe HA, Buhl R, Mastrangeli A, et al. Organ specific cytokine therapy. Local activation of mononuclear phagocytes by delivery of an aerosol of recombinant interferon-gamma to the human lung. J Clin Invest **88**:297–302, 1991.
352. Pelton RW, Moses HL. The beta-type transforming growth factor. Mediators of cell regulation in the lung. Am Rev Respir Dis **142**:S31–S35, 1990.
353. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci USA **88**:6642–6646, 1991.
354. Khalil N, Greenberg AH. The role of TGF-beta in pulmonary fibrosis. Ciba Found Symp **157**:194–207, 1991.
355. Baecher-Allan CM, Barth RK. PCR analysis of cytokine induction profiles associated with mouse strain variation in susceptibility to pulmonary fibrosis. Reg Immunol **5**:207–217, 1993.
356. Zugmaier G, Paik S, Wilding G, et al. Transforming growth factor beta 1 induces cachexia and systemic fibrosis without an antitumor effect in nude mice. Cancer Res **51**:3590–3594, 1991.
357. Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor beta on bleomycin induced accumulation of lung collagen in mice. Thorax **48**:959–966, 1993.
358. Denis M. Neutralization of transforming growth factor-beta(1) in a mouse model of immune-induced lung fibrosis. Immunology **82**:584–590, 1994.
359. Caniggia I, Tseu I, Han RN, Smith BT, Tanswell K, Post M. Spatial and temporal differences in fibroblast behavior in fetal rat lung. Am J Physiol **261**:L424–L433, 1991.
360. Howe PH, Cunningham MR, Leof EB. Inhibition of mink lung epithelial cell proliferation by transforming growth factor-beta is coupled through a pertussis-toxin-sensitive substrate. Biochem J **266**:537–543, 1990.
361. Overall CM, Wrana JL, Sodek J. Transcriptional and post-transcriptional regulation of 72-kDa gelatinase/type IV collagenase by transforming growth factor-beta 1 in human fibroblasts. Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gene expression. J Biol Chem **266**:14064–14071, 1991.
362. Wahl SM, Allen JB, Weeks BS, Wong HL, Klotman PE. Transforming growth factor beta enhances integrin expression and type IV collagenase secretion in human monocytes. Proc Natl Acad Sci USA **90**:4577–4581, 1993.
363. Sharma K, Ziyadeh FN. The emerging role of transforming growth factor-beta in kidney diseases. (editorial) Am J Physiol **266**:F829–F842, 1994.
364. Sakata M, Kurachi H, Ikegami H, et al. Autocrine growth mechanism by transforming growth factor (TGF)-beta 1 and TGF-beta 1-receptor regulation by epidermal growth factor in a human endometrial cancer cell line IK-90. Int J Cancer **54**:862–867, 1993.
365. Franzen P, Ichijo H, Miyazono K. Different signals mediate transforming growth factor-beta 1-induced growth inhibition and extracellular matrix production in prostatic carcinoma cells. Exp Cell Res **207**:1–7, 1993.
366. Schmid P, Cox D, Bilbe G, et al. TGF-beta s and TGF-beta type II receptor in human epidermis: Differential expression in acute and chronic skin wounds. J Pathol **171**:191–197, 1993.
367. Khalil N, Whitman C, Zuo L, Danielpour D, Greenberg A. Regulation of alveolar macrophage transforming growth factor-beta secretion by corticosteroids in bleomycin-induced pulmonary inflammation in the rat. J Clin Invest **92**:1812–1818, 1993.
368. Denholm EM, Rollins SM. Expression and secretion of transforming growth factor-beta by bleomycin-stimulated rat alveolar macrophages. Am J Physiol **264**:L36–L42, 1993.
369. Weiland JE, Garcia JG, Davis WB, Gadek JE. Neutrophil collagenase in rheumatoid interstitial lung disease. J Appl Physiol **62**:628–633, 1987.
370. Last JA, Reiser KM. Biosynthesis of collagen crosslinks. III. *In vivo* labeling and stability of lung collagen in rats with bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol **1**:111–117, 1989.
371. Murad S, Walker LC, Tajima S, Pinnell SR. Minimum structural requirements for minoxidil inhibition of lysyl hydroxylase

in cultured fibroblasts. Arch Biochem Biophys **308**:42–47, 1994.
372. Gadek JE, Fells GA, Zimmerman RL, Crystal RG. Role of connective tissue proteases in the pathogenesis of chronic inflammatory lung disease. Environ Health Perspect **55**:297–306, 1984.
373. Snider GL, Ciccolella DE, Morris SM, Stone PJ, Lucey EC. Putative role of neutrophil elastase in the pathogenesis of emphysema. Ann NY Acad Sci **624**:45–59, 1991.
374. Laurent GJ. Lung collagen: More than scaffolding. Thorax **41**:418–428, 1986.
375. Osborn TG, Bauer NE, Ross SC, Moore TL, Zuckner J. The tight-skin mouse: Physical and biochemical properties of the skin. J Rheumatol **10**:793–796, 1983.
376. Osborn TG, Bashey RI, Moore TL, Fischer VW. Collagenous abnormalities in the heart of the tight-skin mouse. J Mol Cell Cardiol **19**:581–587, 1987.
377. Rossi GA, Hunninghake GW, Gadek JE, et al. Hereditary emphysema in the tight-skin mouse. Evaluation of pathogenesis. Am Rev Respir Dis **129**:850–855, 1984.
378. de-Santi MM, Gardi C, Martorana PA, van-Even P, Lungarella G. Immunoelectron-microscopic demonstration of elastase in emphysematous lungs of tight-skin mice. Exp Mol Pathol **51**:18–30, 1989.
379. Gardi C, Martorana PA, van-Even P, de-Santi MM, Lungarella G. Serum antielastase deficiency in tight-skin mice with genetic emphysema. Exp Mol Pathol **52**:46–53, 1990.
380. Gardi C, Martorana PA, de-Santi MM, van-Even P, Lungarella G. A biochemical and morphological investigation of the early development of genetic emphysema in tight-skin mice. Exp Mol Pathol **50**:398–410, 1989.
381. Starcher B, James H. Evidence that genetic emphysema in tight-skin mice is not caused by neutrophil elastase. Am Rev Respir Dis **143**:1365–1368, 1991.
382. Gardi C, Martorana PA, Calzoni P, et al. Lung collagen synthesis and deposition in tight-skin mice with genetic emphysema. Exp Mol Pathol **56**:163–172, 1992.
383. Watkins CA, Burkhart LR, Rannels DE. Lung growth in response to unilateral pneumonectomy in rapidly growing rats. Am J Physiol **248**:E162–E169, 1985.
384. Cowan MJ, Crystal RG. Lung growth after unilateral pneumonectomy: Quantitation of collagen synthesis and content. Am Rev Respir Dis **111**:267–277, 1975.
385. McAnulty RJ, Staple LH, Guerreiro D, Laurent GJ. Extensive changes in collagen synthesis and degradation during compensatory lung growth. Am J Physiol **255**:C754–C759, 1988.
386. McAnulty RJ, Guerreiro D, Cambrey AD, Laurent GJ. Growth factor activity in the lung during compensatory growth after pneumonectomy: Evidence of a role for IGF-1. Eur Respir J **5**:739–747, 1992.
387. Last JA, Summers P, Reiser KM. Biosynthesis of collagen crosslinks. II. *In vivo* labelling and stability of lung collagen in rats. Biochim Biophys Acta **990**:182–189, 1989.
388. Mays PK, McAnulty RJ, Laurent GJ. Age-related changes in lung collagen metabolism. A role for degradation in regulating lung collagen production. Am Rev Respir Dis **140**:410–416, 1989.
389. Mays PK, McAnulty RJ, Campa JS, Cambrey AD, Laurent GJ. Similar age-related alterations in collagen metabolism in rat tissues *in vivo* and fibroblasts *in vitro*. Biochem Soc Trans **18**:957, 1990.
390. Mays PK, McAnulty RJ, Campa JS, Laurent GJ. Age-related changes in collagen synthesis and degradation in rat tissues. Importance of degradation of newly synthesized collagen in regulating collagen production. Biochem J **276**:307–313, 1991.