Always cleave up your mess: targeting collagen degradation to treat tissue fibrosis

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PULMONARY FIBROSIS, characterized by replacement of the normal alveolar architecture with collagen-rich matrix (140, 178), is a common pathological response to lung injury. Fibrosis can occur because of aberrant remodeling during recovery from acute lung injury, in the setting of systemic autoimmune and inflammatory disease, or as an idiopathic process without identifiable antecedent cause (48, 59, 100). Once fibrosis is established there are no effective pharmacological interventions that can reverse the process, leaving patients with significant pulmonary compromise and in many cases progressive clinical deterioration ultimately leading to death (138). As with many fundamental biological processes, the production, deposition, and resorption of collagen, the main component of fibrotic scar, are dynamic processes with the balance between collagen production and removal determining tissue architecture (43, 91). For fibrosis to develop there has to be a disruption of this homeostasis favoring matrix production over degradation. From this perspective, the development of pulmonary fibrosis represents a failure of matrix degradation as much as matrix overproduction. The pathogenesis of pulmonary fibrosis is complex and has been recently reviewed (31, 46, 79, 165). The objective of this article is to review our understanding of the pathways that regulate collagen degradation and how they relate to the development and resolution of fibrosis.

The Clinical Impact of Pulmonary Fibrosis

Fibrosis is the final common pathway of a myriad of insults and injuries to the lung (29). Increased production of extracellular matrix (ECM) is a normal response to parenchymal damage that serves to preserve tissue integrity in anticipation of tissue repair and remodeling. Fibrosis occurs when there is a failure of compensatory remodeling and reestablishment of normal tissue architecture. Lung fibrosis can occur after lung infections, as part of the pathological response to cigarette smoke in chronic obstructive pulmonary disease, as a response to drug or radiation treatment, or as one of the interstitial lung diseases (ILD). Although many different etiologies can induce a fibrotic response in the lung, the persistence and progression of fibrosis only occur in certain diseases (Fig. 1). A single episode of lung infection rarely leads to significant fibrosis. In acute bacterial pneumonias, even when severe, residual areas
of fibrosis generally do not impact long-term patient quality of life and in many cases are resolved with tissue remodeling leaving near-normal lung architecture. Repeated bouts of infection, however, can lead to a significant fibrotic response as seen in cystic fibrosis (61). A subset of patients with acute respiratory distress syndrome (ARDS) develop a fibroproliferative phase characterized by accumulation of collagen-rich material within the air spaces and severe gas-exchange abnormalities (164). Interestingly, even these patients can have resolution of the fibrotic response over time (114).

Pulmonary fibrosis that occurs in the setting of ILD is a more insidious and problematic process that can be progressive. Of the several disease processes that collectively make up the ILD, usual interstitial pneumonitis (UIP), often referred to as idiopathic pulmonary fibrosis (IPF), carries the worst prognosis (139). Although most of the other disorders respond to varying degrees with treatment of the underlying etiology, UIP is uniformly progressive with a median life expectancy of approximately 3 years after diagnosis. For the purposes of this review, we will use the term IPF when discussing literature that did not use the term UIP in classifying their patient populations.

Production of Excess Collagen

An excess in collagen production is necessary for the development of pulmonary fibrosis. The cells responsible for collagen production are believed to be fibroblasts and myofibroblasts (47, 188). Myofibroblasts are differentiated fibroblasts that express α-smooth muscle actin and secrete significantly greater amounts of collagen and ECM components than their fibroblast counterparts (188). The source of fibroblasts that differentiate into myofibroblasts in fibrotic tissue is an area of contention, with studies supporting migration of local fibroblasts into the alveolar space (134), transformation of local epithelial cells into cells expressing mesenchymal markers through epithelial-to-mesenchymal transformation (EMT) (77), transformation of lung pericytes and pleural mesothelial cells into myofibroblasts (120, 123), and homing of bone marrow-derived cells of the monocyte lineage (fibrocytes) into areas of injury (134). Since local fibroblast populations are plentiful in the lung it may seem surprising that other cellular sources are required to produce myofibroblasts. Although the exact importance of different populations has yet to be elucidated, there is some evidence to suggest that the source of cells that differentiate into myofibroblasts has pathological relevance. For example, neutralizing antibodies to the chemokine CXCL12 prevents recruitment of fibrocytes into the lung after bleomycin injury and reduces the amount of collagen accumulation by greater than 50% (134). Similarly, prevention of EMT has been reported to reduce bleomycin-induced pulmonary fibrosis (78).

Collagen Synthesis, Processing, and Structure

With the appropriate stimulation and environment, fibroblasts and myofibroblasts increase their production of collagen mRNA transcripts. Translation results in collagen propeptides that undergo modifications including hydroxylation of proline and lysine residues, glycosylation of lysine residues, and disulfide bond formation, ultimately leading to the formation of a triple helix consisting of three propeptides in the endoplasmic reticulum. Following packaging in the Golgi and secretion from the cell, peptidases further process collagen resulting in the mature monomeric collagen molecule. In type I collagen, five monomeric collagen molecules make up a microfibril that is staggered by a multiple of 67 nm, giving a characteristic banding pattern termed a D-period (71, 131, 183). Each microfibril interdigitates with neighboring microfibrils, creating a collagen fibril. There are both inter- and intramicrofibrillar cross-links mediated by aldehyde groups on lysine and hydroxylysine residues created by lysyl oxidase (125). A bundle of collagen fibrils then creates a collagen fiber (Fig. 2). The combination of extensive cross-linking and a right-handed

Fig. 1. Many etiologies can induce a fibrotic response in the lung, but fibrosis only persists in certain types of disease. ECM, extracellular matrix; UIP, usual interstitial pneumonitis; ARDS, acute respiratory distress syndrome.
The composition of collagen is different in fibrotic tissue compared with normal tissue. The uninjured lung contains primarily type I and type III collagen with type I approximately twice as frequent as type III (62, 155). This relationship changes in lung fibrosis, favoring a predominance of type I collagen. A study examining the collagen composition of biopsy samples obtained from patients with a variety of fibrotic diseases found an increase in the proportion of type III collagen and an increase in total type I and type III collagen in early lung fibrosis. However, in more established areas of scar formation there was almost exclusively type I collagen (7). In studies conducted in the 1970s and 1980s of what was then called IPF (likely representing a mix of ILD pathologies with UIP predominating), the increase in collagen was found to be primarily an increase in type I collagen that represented ~80% of the total collagen (7, 101, 151, 155, 160). These studies indicate that in addition to an increase in total collagen in IPF (150, 151) there is also a shift toward the less elastic type I collagen, which contributes to pathophysiological abnormalities in fibrosis (51, 125, 145). A relative increase in type I collagen production (as well as an increase in total lung collagen content) has also been demonstrated in the lungs of patients with ARDS (87, 140) as well as in human fibrotic livers (85).

In addition to differences in the type of collagen that is expressed in scar tissue, there is an increase in production of other ECM molecules that intermingle with collagen (95). In liver fibrosis, for example, there is an increase in fibronectin, elastin, laminin, hyaluronan, chondroitin sulfate and heparin sulfate proteoglycans, and undulin in addition to increases in collagens (9, 60, 149). Similarly, in pulmonary fibrosis there is an increase in elastin, fibronectin, hyaluronan, and proteoglycans in addition to collagen (8, 39, 67, 163). This conglomeration of ECM molecules provides a unique ultrastructural challenge for matrix remodeling pathways and may prevent proteolytic enzymes from accessing their binding sites on collagen. Furthermore, some of these matrix molecules modulate the fibrotic process. Decorin, a small proteoglycan that has potentially opposing effects on fibrosis due to its dual roles in promoting fibril formation (184, 187) and suppressing the activity of the profibrotic cytokine TGF-β (80), is produced in excess by fibroblasts from patients with pulmonary fibrosis. And although TGF-β has been shown to reduce the production of decorin, long-standing connective tissue-associated fibrosis is associated with increased decorin production, which by promoting fibril formation may reduce collagen resorption.
The importance of proteoglycans in modulating fibrosis is evident in data showing that intranasal installation of decorin in mice by viral delivery can prevent fibrosis induced by TGF-β (81) or bleomycin (80). Preferential production of fibrosis-promoting isoforms of ECM molecules is another mechanism by which altered ECM can exacerbate the fibrotic response. For example, the splice variant of fibronectin containing the extra type III domains (EDA) is induced by TGF-β (14) and promotes TGF-β-dependent induction of myofibroblasts and fibrosis (154). EDA is found interspersed between fibroblast and collagen in tissue sections from patients with IPF (83) and is produced in excess by fibroblasts from patients with IPF (121). EDA-null mice are protected from bleomycin-induced pulmonary fibrosis (121).

Collagen cross-linking by lysyl oxidase is another important structural modification that can prevent degradation in fibrosis. Lysyl oxidase expression and the proportion of hydroxylated lysine residues are increased in fibrotic states (104). An increase in cross-linking and altered cross-linking patterns have been implicated in dermal fibrosis (17), in ARDS in the lung (86), in myocardial fibrosis (98), and in pulmonary fibrosis (56, 142). A recent study demonstrated that inhibition of lysyl oxidase-like-2 prevented the development of bleomycin-induced lung fibrosis and reversed established fibrosis (6). Finally, collagen orientation in areas of fibrosis can also be altered. In skin scar tissue, collagen has a more parallel orientation relative to the epithelial surface (176, 177) compared with collagen in normal skin. Taken together, these data suggest that the type, hydroxylysine content, orientation, and cross-linking pattern of collagens as well as the molecules that interact with collagen differ in areas of fibrosis.

Collagen Turnover in the Lung

As outlined above, the appropriate stimulus leads to an increase in collagen production in the lung. However, not all collagen that is produced gets incorporated into fibrillar collagen. Newly synthesized collagen can be degraded prior to being secreted by the cell or prior to being incorporated into fibrils in the ECM (10, 12, 13). There is some evidence to suggest that with aging there is an increase in degradation of newly synthesized collagen prior to secretion from the cell and a decrease in degradation of extracellular collagen (108). Collagen that does become part of the ECM likely undergoes constant remodeling. Human lung explant studies demonstrated that adult lungs continuously produce collagen and the adult rat lung has been shown to undergo continuous collagen turnover (5, 15, 109, 126, 135), although other groups have used assays measuring labeled hydroxylsine cross-links to contend that there is very little or no degradation of collagen once it has been cross-linked and firmly incorporated into the ECM (88). More recent studies evaluating the kinetics of collagen metabolism have measured (by mass spectrometry) the incorporation of deuterium-labeled amino acids into collagen after replacing the drinking water of rodents with deuterium-labeled water (20, 54). This technique provides detailed data regarding the relative rates of collagen production and degradation in the resting state and after injury. Whereas the proportion of new collagen (collagen labeled with deuterium) after 14 days of deuterated water intake is roughly 10% in the normal adult lung, the proportion increases to ~40% after challenge with a dose of bleomycin that produces a 20% increase in total collagen (4). These studies delineate the dynamic turnover of collagen both in the normal lung and the lung after injury. What is unclear from this technique is whether all collagen in the lung is degraded at similar rates or whether there are pools of collagen that are more or less accessible to degradation (55, 58, 90). It has been suggested that the increased ratio of more stable dihydroxylysinoonorleucine cross-links to less stable hydroxylysinoonorleucine cross-links found in fibrotic lung tissue may protect “fibrotic collagen” from enzymatic degradation or mechanical damage (56). Further studies examining whether collagen that is incorporated into fibrillar scar is less prone to degradation than collagen that is part of normal tissue architecture are warranted.

**Extracellular Pathway of Collagen Degradation**

The molecular pathways responsible for collagen degradation have been incompletely described. Collagen metabolism is a two-step process that involves extracellular protease-mediated cleavage followed by cellular uptake and intracellular lysosomal degradation (43, 102, 161) (Fig. 3). Extracellular collagen degradation is mediated by recognition of specific cleavage sites that have characteristic imino acid content by matrix metalloproteinase enzymes (89, 166). Intact fibrillar collagen can only be cleaved by a subset of matrix metalloproteinases (MMPs) including MMP-1, MMP-8, MMP-13, MMP-14 (also known as MT1-MMP), MMP-16 (also known as MT3-MMP), and MMP-18 (161). Most MMPs consist of a catalytic domain and hemopexin domain. The hemopexin domain is thought to bind and unwind collagen, allowing access of the catalytic domain to the appropriate cleavage site. In fact, incubation of collagen with just the catalytic domain is inadequate for cleavage to take place (27, 28). Fragments of cleaved fibrillar collagen rapidly denature (i.e., lose their triple-helical structure) at 37°C and may then be further degraded by

![Fig. 3. Collagen monomers are incorporated into fibrils, which aggregate to form fibers. Degradation of collagen fibers involves cleavage of fibrils by collagenolytic enzymes and uptake of collagen fragments by macrophages and fibroblasts or further cleavage by gelatinases. MMP, matrix metalloproteinase.](http://ajplung.physiology.org/)

**L712 COLLAGEN DEGRADATION AND FIBROSIS**

Review

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the gelatinases (MMP-2 and MMP-9). Collagen can also be cleaved by cathepsin K (18, 162), a lysosomal cysteine protease that is also active in the extracellular compartment. Cathepsin K is induced by inflammation (23) and cleaves type I and type II collagen both at the ends of the molecule and at multiple sites within the triple helix.

Several metalloproteinase enzymes, including MMP-1, 2, 3, 7, 9, and 14, are either highly expressed in the lungs of human patients with fibrosis (132) or have a defined role in experimental pulmonary fibrosis (132). The contribution of these enzymes to in vivo remodeling of fibrotic tissue, however, has been difficult to discern because of their multifunctional roles. For example, although the expression of MMP-1, the prototypical metalloproteinase for degradation of fibrillar collagens, is increased in lungs of patients with IPF (50, 132), expression is restricted to the alveolar compartment rather than the interstitium. Increased expression of MMP-1 in fibrotic lungs may be related to its role in alveolar reepithelialization rather than collagen degradation (42, 132). Furthermore, most experiments with MMP knockout mice show protection from bleomycin-induced fibrosis rather than increased collagen accumulation as might be expected from an in vivo defect in collagen degradation (Table 1). This is because, even though MMPs are proteolytic enzymes, their effects on targets other than collagens in vivo are greater determinants of their ultimate effects on the development of fibrosis. Although expression of both gelatinases (MMP-2 and MMP-9) is increased in pulmonary fibrosis, there is conflicting data regarding the consequences of gelatinase deficiency on the severity of experimentally induced fibrosis (11, 21, 129, 181) in the lung and other organs (64, 68, 107). A profibrotic role for these enzymes has been attributed to the fact that they mediate cleavage of basement membrane collagen during the acute phase of lung injury, allowing fibroblasts to migrate into the alveolar compartment and produce collagen leading to fibrosis (129, 167). MMP-3 expression is also increased in the lungs of patients with IPF, but it appears to promote fibrosis by promoting EMT (137, 185). MMP-3 overexpression in rats induces lung fibrosis and MMP-3 null mice are protected from bleomycin-induced pulmonary fibrosis. MMP-7 expression is also increased in IPF. As with MMP-3 and MMP-8 (53) knockout mice, MMP-7 knockout mice are protected from bleomycin-induced lung fibrosis (192).

Even when mice deficient in collagen-cleaving metalloproteinases accumulate interstitial collagens during development, they may be protected from fibrosis after injury. For example, MMP-13 knockout mice have defects in the development of bone and cartilage with accumulation of interstitial collagens (73). However, in a bile duct ligation model of liver fibrosis and in a lung model of radiation-induced fibrosis, MMP-13 knockouts are protected from developing fibrosis because of the effect of MMP-13 in regulating the initial inflammatory response (49, 173). In the carbon tetrachloride model of hepatic injury, MMP-13 knockouts developed the same initial degree of fibrosis but have a delay in resolution of fibrosis because of impaired collagen degradation (45). These conflicting results in different in vivo models of fibrosis emphasize the complex biological roles played by metalloproteinase enzymes.

The most convincing evidence for increased fibrosis resulting from a defect in extracellular collagen degradation comes from studies with MMP-14 and cathepsin K-deficient mice. MMP-14 knockout mice accumulate collagen extracellularly in the soft tissues (69, 92) and MMP-14 expression and activity is modulated by type I collagen (52, 191). Cathepsin K knockout mice develop more severe bleomycin-induced fibrosis and transgenic mice overexpressing cathepsin K have an abrogated response to bleomycin-induced fibrosis (18, 162). Additional support for an important role in ECM degradation comes from macrophage depletion studies. In carbon tetrachloride-induced liver fibrosis, the depletion of CD11B+ cells after fibrosis is established prevents remodeling and resorption of fibrotic tissue (36) by decreasing local concentrations of MMP-13 secretion by scar-associated macrophages (45).

Table 1. Experimental models of fibrosis in mice deficient in proteolytic enzymes

<table>
<thead>
<tr>
<th>Proteolytic Enzyme</th>
<th>Experimental Model</th>
<th>Results</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 KO</td>
<td>Cardiac pressure overload</td>
<td>Protection from fibrosis</td>
<td>107</td>
</tr>
<tr>
<td>MMP-2 KO</td>
<td>Myocardial infarction</td>
<td>Protection from fibrosis</td>
<td>64</td>
</tr>
<tr>
<td>MMP-2 KO</td>
<td>Diabetic nephropathy</td>
<td>Increased fibrosis</td>
<td>169</td>
</tr>
<tr>
<td>MMP-2 KO</td>
<td>CCl4 liver fibrosis</td>
<td>Increased fibrosis</td>
<td>136</td>
</tr>
<tr>
<td>MMP-3 KO</td>
<td>Bleomycin lung fibrosis</td>
<td>Protection from fibrosis</td>
<td>185</td>
</tr>
<tr>
<td>MMP-3 overexpression</td>
<td>Transient adenoviral expression in rat lung</td>
<td>Induction of pulmonary fibrosis</td>
<td>185</td>
</tr>
<tr>
<td>MMP-7 KO</td>
<td>Bleomycin lung fibrosis</td>
<td>Protection from fibrosis</td>
<td>192</td>
</tr>
<tr>
<td>MMP-8 KO</td>
<td>Bleomycin lung fibrosis</td>
<td>Protection from fibrosis</td>
<td>53</td>
</tr>
<tr>
<td>MMP-9 KO</td>
<td>Renal obstruction</td>
<td>Protection from fibrosis</td>
<td>181</td>
</tr>
<tr>
<td>MMP-9 overexpression in macrophages</td>
<td>Bleomycin lung fibrosis</td>
<td>Protection from fibrosis</td>
<td>21</td>
</tr>
<tr>
<td>MMP-9 KO</td>
<td>OVA sensitization</td>
<td>Protection from fibrosis</td>
<td>96</td>
</tr>
<tr>
<td>MMP-9 KO</td>
<td>Cardiac pressure overload</td>
<td>Protection from fibrosis</td>
<td>68</td>
</tr>
<tr>
<td>MMP-13 KO</td>
<td>Normal development</td>
<td>Interstitial collagen accumulation</td>
<td>73</td>
</tr>
<tr>
<td>MMP-13 KO</td>
<td>Bile duct ligation</td>
<td>Protection from fibrosis</td>
<td>173</td>
</tr>
<tr>
<td>MMP-13 KO</td>
<td>CCl4 liver fibrosis</td>
<td>Prolonged fibrosis</td>
<td>45</td>
</tr>
<tr>
<td>MMP-13 KO</td>
<td>Thoracic irradiation</td>
<td>Protection from fibrosis</td>
<td>49</td>
</tr>
<tr>
<td>MMP-13 KO</td>
<td>Lung hyperoxia</td>
<td>No change from WT</td>
<td>153</td>
</tr>
<tr>
<td>MMP-14 (MT1-MMP) KO</td>
<td>Normal development</td>
<td>Collagen accumulation in multiple tissues</td>
<td>69</td>
</tr>
<tr>
<td>Cathepsin K KO</td>
<td>Normal development</td>
<td>Increased fibrosis</td>
<td>186</td>
</tr>
<tr>
<td>Cathepsin K KO</td>
<td>Bleomycin lung fibrosis</td>
<td>Increased fibrosis</td>
<td>18</td>
</tr>
<tr>
<td>Cathepsin K overexpression</td>
<td>Bleomycin lung fibrosis</td>
<td>Protection from fibrosis</td>
<td>162</td>
</tr>
<tr>
<td>Neutrophil elastase KO</td>
<td>Bleomycin lung fibrosis</td>
<td>Protection from fibrosis</td>
<td>25</td>
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KO, knockout; WT, wild type; OVA, ovalbumin.
The plasminogen activator system has recently been implicated in the resolution of fibrosis, and this effect may be mediated by modulation of extracellular collagen degradation pathways. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of urokinase plasminogen activator (uPA), which cleave plasminogen into active plasmin. PAI-1 is present in increased levels in human IPF samples (82) and is induced by TGF-β in an experimental mouse model of lung fibrosis (174). The PAI-1 knockout mouse is protected from lung (40) and kidney (127) fibrosis. One of the proposed mechanisms by which PAI-1 regulates fibrogenesis is by modulating activity of the proteinases necessary for ECM breakdown. Plasmin can directly or indirectly activate several matrix proteases, including pro-MMP-2, MMP-3, and MMP-9 (65, 117, 122, 141), as well as degrade ECM components present in fibrotic plaques including fibronectin and laminins (116). Furthermore, tPA has been shown to cause increased MMP-9 activity independent of plasmin, either by inducing release from neutrophils (32), by increasing MMP-9 transcription (189), or by liberating and activating ECM-bound HGF (63, 106), a potent antifibrotic growth factor that can cause upregulation of MMP-9 (57). Studies in lung (70) and liver (180) fibrosis models have shown that the profibrotic effect of PAI-1 depends on its inhibition of tPA or uPA and support the notion that impairment of normal extracellular collagen breakdown pathways can lead to pathologic fibrosis. However, another recent study on PAI-1-/- mice that had been transgenically engineered to selectively express PAI-1 protein constructs including or lacking PAI-1’s antiprotease domain concluded that the major contribution of PAI-1 to fibrogenesis rests not on its antiproteolytic function but its ability to bind vitronectin (30). PAI-1 is clearly important in the development and resolution of fibrosis. However, like the metalloproteinases, its multiple functions make it difficult to precisely define its role in fibrosis.

**Intracellular Pathway of Collagen Degradation**

Compared with the pathways of extracellular collagen degradation, the pathways of intracellular collagen degradation, defined here as ingestion of collagen from the extracellular compartment, have been less extensively studied. Collagen phagocytosis by macrophages and fibroblasts was initially described by electron microscopy (35, 43, 99, 175). The identification of intracellular collagen fibrils in these studies argued against the possibility that intracellular staining represented newly synthesized collagen rather than collagen being internalized for degradation (41, 43). Studies of periodontal ligament fibroblasts indicated that internalized collagen is ultimately degraded in the lysosomal network (43) by cysteine proteinases (41, 42).

For collagen to be internalized it must first be recognized by specific cell surface receptors (Fig. 4). Collagen phagocytosis has been studied through the use of collagen-coated beads or native fibrillar collagen (156) and is dependent on the α2β1 integrin (3, 24, 93). Fibroblasts using this integrin receptor bind and internalize collagen through a Gelsolin- and Rac-dependent pathway (2). A receptor-mediated endocytosis pathway of collagen uptake and intracellular degradation has been described for the transmembrane mannose receptors uPARAP/Endo180 (38) and mannose receptor/CD206 (105), both members of the mannose receptor family (103). The in vivo importance of uPARAP/Endo180-mediated collagen uptake is apparent in the increased collagen accumulation around areas of malignancy (33) as well as increased pulmonary fibrosis after treatment with bleomycin (111) in uPARAP/Endo180 knockout mice. The extracellular glycoprotein Mfge8 also mediates collagen uptake by binding collagen fragments and targeting them for uptake and digestion by macrophages. Mice deficient in Mfge8 have impaired collagen uptake and develop increased fibrosis after bleomycin injury because of an in vivo defect in collagen degradation (4). Additionally, a macropinocytic pathway of uptake has been reported for dendritic cell and macrophage-mediated uptake of collagen in studies using pharmacological inhibitors (179).

Given the importance of inflammation in injury and repair, it is not surprising that, at least in vitro, the intracellular pathways of collagen degradation are modulated by cytokines. In in vitro assays, the role of the critical cytokines TNF-α, TGF-β, and IL-1α on collagen uptake have been examined. TGF-β, generally considered an anti-inflammatory cytokine, stimulates collagen phagocytosis by perioveal fibroblasts and inhibits collagenase release (175). TNF-α and IL-1α, both pro-inflammatory cytokines, inhibit collagen uptake and induce MMP release/activity (24, 44, 158, 175). Since uncleared collagen fragments are thought to be proinflammatory (143, 182), it is possible that one mechanism by which these cytokines regulate inflammation is through removal of collagen fragments from the extracellular compartment. The effect of inflammation on the intracellular collagen degradation pathway in vivo has not been evaluated experimentally.

**Consequences of Impaired Extra- or Intracellular Degradation Pathways on Fibrosis in Humans**

Though increased collagenase expression has been reported in both human and experimental murine models of pulmonary fibrosis (66, 75, 192), there is some evidence for impaired extracellular collagen degrading capacity in fibrotic tissue. In human patients with IPF there is an increase in the ratio of tissue inhibitors of metalloproteinases (TIMP) to collagenases
(118, 152) evaluated by immunohistochemistry of tissue sections and decreased collagenolytic activity in lung homogenates (151). Similarly, in a rat model of experimental lung silicosis, there is a relative increase in TIMP-1 and TIMP-2 relative to MMP expression by immunohistochemistry and in situ hybridization in late silicotic granulomas (133). A decrease in collagenolytic activity has also been reported in cirrhotic livers (119, 128) and in the skin of patients with scleroderma (16). Patients with acute lung injury are at risk of developing lung fibrosis have and been reported to have reduced lung collagenolytic activity. One study of patients with ARDS and acute lung injury measured the ratio of the COOH-terminal propeptide of type I collagen (a marker of collagen synthesis) to the carboxy terminal sequence of the 3/4 degradation fragment of type I collagen (a marker of collagen degradation). The authors found that patients with more severe lung injury had less total and relative quantities of degraded collagen fragments and higher levels of collagen propeptide, indicating an in vivo defect in collagen degradation (1). Taken together, these data suggest that in areas of fibrosis there is inhibition of the pathways that mediate extracellular collagen degradation. It is unclear how this occurs. One possibility is that even though there are a large number of fibroblasts and macrophages in fibrotic tissue, these cells have altered profiles of collagenase and TIMP expression due to cues provided by the fibrotic microenvironment surrounding them.

The first evidence that a defect in the intracellular degradation pathway can lead to an increase in extracellular collagen accumulation comes from studies of patients who develop gingival hyperplasia as an adverse reaction to drug therapy with medications such as cyclosporine, dilantin, and nifedipine (22, 110, 112). Cyclosporine induces accumulation of subepithelial collagen in part by inhibiting integrin-mediated release of intracellular calcium stores that are required for collagen binding prior to phagocytosis (22, 34). Human gingival fibroblasts isolated from fibrotic lesions have impaired collagen bead phagocytosis that can be replicated by incubation of normal fibroblasts in vitro with dilantin or nifedipine (111). A similar defect in fibroblast-mediated collagen phagocytosis is thought to occur in the chronic mucosal condition oral submucous fibrosis (OSF). OSF lesions are characterized by an increase in collagen deposition in the subepithelial layer of the oral mucosa. In vitro evidence suggests that fibroblasts cultured from patients with OSF have increased production and impaired uptake of collagen (170). Incubation of normal fibroblasts with areca nut alkaloids, the putative toxin that causes OSF, inhibits collagen phagocytosis, and this effect is reversed by treatment with corticosteroids (157, 172) (Table 2).

### Relationship Between Extracellular and Intracellular Degradation Pathways

One interesting area of active investigation is focused on understanding the relationship between the extracellular and intracellular degradation pathways in vivo. The logical assumption has been that these pathways function in a serial fashion with extracellular degradation of fibrillar collagen preceding cellular uptake and intracellular degradation. Since the collagen fibril is much larger than a single cell it would seem absolutely necessary for the fibril to be partially degraded prior to uptake. Although some studies do not support this model (24, 42, 44), there is compelling evidence that, at least in vitro, uptake of collagen proceeds much more efficiently after metalloproteinase-mediated cleavage (92, 102) and denaturation of the collagen fragments. The most satisfying model is one where cells that take up collagen for intracellular degradation express metalloproteinases in a geographically restricted manner at areas of contact with collagen (43, 84, 92). This allows for local proteolytic cleavage of fibrillar collagen while avoiding unrestricted exposure of normal tissue to increased collagenase levels. Cells can then take up collagen for further intracellular degradation by either phagocytosis, receptor-mediated endocytosis, or macropinocytosis. One might wonder why uptake of cleaved collagen is necessary since gelatinase enzymes can further degrade fragments in the extracellular space. However, the orderly removal of collagen fragments is important for preventing both acute and chronic inflammation. Cleaved collagen fragments can induce acute inflammation through neutrophil recruitment (143, 182) and can serve as antigens inducing autoimmunity (148, 171).

Assuming that this model is correct, a provocative question is why a defect in intracellular degradation would lead to increased extracellular accumulation of collagen. One attractive hypothesis is that the binding of collagen by cell surface receptors or subsequent internalization can regulate the production and secretion of extracellular proteolytic enzymes. Therefore, an increase in intracellular collagen or cell surface binding of collagen would signal to the cell to increase its production of collagenases and a subsequent decrease in intracellular collagen or cell surface binding of collagen would decrease collagenase production. In this model, the uptake of collagen for intracellular degradation could regulate extracel-

### Table 2. Evidence for impaired collagen degradation leading to fibrosis in vivo

<table>
<thead>
<tr>
<th>Experimental Population</th>
<th>Mechanism of Impaired Degradation</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with IPF/UIP</td>
<td>Increase ratio of TIMPs to collagens</td>
<td>118, 152</td>
</tr>
<tr>
<td>Patients with IPF/UIP</td>
<td>Decreased collagenolytic activity</td>
<td>151</td>
</tr>
<tr>
<td>Patients with IPF/UIP</td>
<td>Decreased collagenolytic activity</td>
<td>16</td>
</tr>
<tr>
<td>Patients with drug-induced gingival hyperplasia</td>
<td>Subepithelial collagen accumulation with associated impaired collagen binding/phagocytosis by fibroblasts</td>
<td>22, 113, 34, 111</td>
</tr>
<tr>
<td>Patients with oral submucous fibrosis</td>
<td>Subepithelial collagen accumulation with reduced collagen phagocytosis by fibroblasts</td>
<td>157, 172</td>
</tr>
<tr>
<td>Patients with ARDS/ALI</td>
<td>Decreased degradation of type I collagen in vivo with more severe disease</td>
<td>1</td>
</tr>
<tr>
<td>Exaggerated bleomycin-induced lung fibrosis in mice</td>
<td>Collagen accumulation due to impaired collagen binding/phagocytosis</td>
<td>4, 19</td>
</tr>
</tbody>
</table>

IPF, idiopathic pulmonary fibrosis; UIP, usual interstitial pneumonitis; ARDS, acute respiratory distress syndrome; ALI, acute lung injury; TIMPs, tissue inhibitors of metalloproteinases.
lular cleavage by regulating proteolytic enzyme production. Although there are no data supporting this hypothesis, there is some work to suggest the opposite. uPARAP/Endo180-mediated collagen uptake inhibits the cell surface activity level of MMP-14 uptake, therefore decreasing the collagenase activity and preventing MMP-14 induced cleavage and activation of the gelatinase MMP-2 (115). As discussed above, TNF-α, TGF-β, and IL-1α have reciprocal effects on collagen phagocytosis and collagenase activity (24).

To further complicate matters, some evidence suggests that a defect in extracellular cleavage of collagen can lead to an increase in the activity of collagen uptake and intracellular degradation. Deficiency of MMP-14 leads to an increase in extracellular collagen accumulation (147) but is also associated with a large increase in intracellular collagen accumulation (69), suggesting that a compensatory increase in intracellular degradation may occur with defective extracellular degradation. There is also some evidence that the extracellular or intracellular pathway may be more active under different experimental conditions. For example, in animal models of emphysema, the extracellular pathway of collagen degradation predominates when levels of collagenolytic elastases (26) are high, whereas the intracellular pathway predominates when the development of emphysema is not associated with a high elastase burden (99). It is unclear whether these differences are due to collagen cleavage by elastase or mediated by its interactions with MMPs and/or TIMPs.

Summary and Conclusions

Collagen is the most abundant protein in our bodies and its production and tissue deposition are exquisitely regulated to maintain tissue homeostasis. Collagen is continuously produced and degraded, and under homeostatic conditions the rate of production and degradation are perfectly matched to prevent the development of fibrosis with subsequent organ dysfunction. With tissue injury there is an appropriate increase in collagen production to form a provisional matrix and allow for tissue repair. Injury also induces an increase in collagen degradation pathways that remodel areas of fibrosis. These pathways in the lung can lead to the restoration of normal or near-normal tissue architecture with the ability to engage in efficient gas exchange. Despite the presence of these degradation pathways, tissue injury can lead to unresolved and sometimes progressive fibrosis. Whether the development of permanent fibrosis requires a failure of collagen degradation is unknown although there is some evidence of inhibition of degradation pathways in pulmonary fibrosis.

Although no therapies currently exist that effectively treat pulmonary fibrosis, the majority of scientific effort has been and continues to be directed toward understanding the pathways that lead to excess collagen production with the goal of inhibiting these pathways to prevent the development of fibrosis. In UIP, the most vexing disease from a treatment and prognosis perspective, most patients have a significant fibrotic burden at the time of initial diagnosis and would benefit greatly from therapies that increase collagen degradation. If we accept that there is a need for therapies that promote resolution of established fibrosis in addition to those that prevent collagen production, we must consider what the most promising avenues are for future investigation. The use of proteolytic enzymes to augment ECM degradation is fraught with potentially disastrous adverse effects due to uncontrolled parenchymal destruction of normal and abnormal tissue and is therefore not a realistic option. The use of inhibitors of collagen cross-linking is a much more promising approach in that it may allow endogenous proteolytic enzymes to access previously hidden degradation sites on collagen molecules. Additionally, these inhibitors may decrease the synthesis of highly cross-linked and stable collagen and thus allow more rapid cleavage of less cross-linked and less stable collagen. Ideally, this will lead to gradual and persistent degradation of fibrillar scar without having a significant impact on collagens that are part of the normal tissue architecture. The ability of an inhibitor of lysyl oxidase-like-2 to reverse established bleomycin-induced lung fibrosis in mice (6) highlights the importance of further treatments aimed at preventing the structural changes that make collagen resistant to degradation in fibrotic tissue. Another area that warrants further evaluation is examining whether therapies that augment intracellular pathways of collagen degradation will lead to enhanced extracellular remodeling of fibrotic tissue. There is now convincing evidence that defective intracellular collagen degradation leads to excess fibrosis in experimental mouse models (4, 19). These data show that inhibition of key pathways mediating collagen uptake by cells promote extracellular accumulation of fibrillar collagen. What remains to be established is whether there are cellular pathways that inhibit uptake of collagen for intracellular degradation. If so, antagonizing these inhibitory pathways may increase collagen uptake and degradation and optimally lead to enhanced resorption of extracellular fibrillar collagens in areas of fibrosis. Even if these therapies do not restore normal gas exchange, the reduction in scar tissue may improve lung mechanics and oppose the increase in parenchymal stiffness that can further activate collagen producing pathways (97, 190). Despite extensive research, there is still much to be learned about the structural modifications that prevent breakdown of scar-associated collagen and the interplay between the intracellular and extracellular pathways of collagen degradation. A better understanding of these processes may lead to novel therapies for the treatment of pulmonary fibrosis.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

W.M. and K.A. prepared figures; W.M., T.-H.L., and K.A. drafted manuscript; K.A. conception and design of research; K.A. approved final version of manuscript.

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