invited review

Metalloproteinase and growth factor interactions:
do they play a role in pulmonary fibrosis?

MARGARET K. WINKLER1 AND JOHN L. FOWLKES2
1Department of Pediatrics, University of Alabama at Birmingham and Children’s Hospital of Alabama, Birmingham, Alabama 35233; and 2Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children’s Hospital, Little Rock, Arkansas 72202

Winkler, Margaret K., and John L. Fowlkes. Metalloproteinase and growth factor interactions: do they play a role in pulmonary fibrosis? Am J Physiol Lung Cell Mol Physiol 283: L1–L11, 2002; 10.1152/ajplung.00489.2001.—Chronic lung disease due to interstitial fibrosis can be a consequence of acute lung injury and inflammation. The inflammatory response is mediated through the migration of inflammatory cells, actions of proinflammatory cytokines, and the secretion of matrix-degrading proteinases. After the initial inflammatory insult, successful healing of the lung may occur, or alternatively, dysregulated tissue repair can result in scarring and fibrosis. On the basis of recent insights into the mechanisms underlying acute lung injury and its long-term consequences, data suggest that proteinases, such as the matrix metalloproteinases (MMPs), may not only be involved in the breakdown and remodeling that occurs during the injury but may also cause the release of growth factors and cytokines known to influence growth and differentiation of target cells within the lung. Through the release of and activation of fibrosis-promoting cytokines and growth factors such as transforming growth factor-\(\beta_1\), tumor necrosis factor-\(\alpha\), and insulin-like growth factors by MMPs, we propose that these metalloproteinases may be integral to the initiation and progression of pulmonary fibrosis.

acute respiratory distress syndrome; bronchopulmonary dysplasia; lung fibrosis; cytokines; emphysema

PULMONARY FIBROSIS CAN BE an all too common consequence of an acute inflammatory response of the lung to a host of inciting events. Chronic lung injury due to fibrotic changes can result from an identifiable inflammatory event, or an insidious, unknown event (i.e., idiopathic pulmonary fibrosis) may precipitate the fibroproliferative reaction (5, 94, 113). Although inflammation may be evident in the early stages of disease, fibrosis and interstitial scarring are generally considered late and ominous events in lung injury. The inflammatory process can include infiltration of various inflammatory cell types, such as neutrophils and macrophages, the release of inflammatory cytokines and chemokines, and the secretion of matrix remodeling proteinases, principally the matrix metalloproteinases (MMPs). The progression from the initial inflammatory reaction to the subsequent fibroproliferative manifestations is poorly understood. However, a large body of literature now points to several growth factors and cytokines as key modulators in the initiation and progression of fibrotic events in the lung (61, 69). It is through multiple attempts to heal itself that the injured lung ultimately fails to reepithelialize denuded surfaces, demonstrates dysregulated and inadequate repair of alveoli, undergoes impaired extracellular matrix (ECM) remodeling, experiences excessive fibroblast migration and proliferation, and shows an exaggerated response to fibrogenic cytokines (61, 69, 94). It is now known that the seminal event in the initiation of fibrosis occurs at primary sites of ongoing injury and repair that have been identified as regions or nests of fibroblastic proliferation, so-called fibroblast foci (90). These sites represent focal points for the abundant deposition of many constituents of the ECM and expression of MMP activity. Further-
more, within these aggregates, myofibroblasts and fibroblasts actively proliferate, resulting in microscopic sites of ongoing alveolar epithelial injury associated with progressive fibrosis (90).

In many acute and chronic inflammatory events, followed by a period of healing, MMPs have been shown to be upregulated, and their activities have been shown to be important for alveolar repair. Several studies demonstrate that type II pneumocytes are responsible for carrying out alveolar reepithelialization. They are capable of producing MMP-1 (collagenase-1), and the addition of MMPs to wound models promotes pneumocyte migration (77, 81). These same features are similarly observed in the repair and reepithelialization of the skin after injury (80). Therefore, MMPs likely are instrumental in the normal reepithelialization process of the alveolar surface that occurs after an acute inflammatory event and during the regenerative process. Indeed, several animal models of pulmonary fibrosis have shown that MMPs are important in the reepithelialization of the damaged lung (12, 60). Histological examination of normal lungs and lungs from patients with various degrees of interstitial disease also points to MMPs and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), as having a mechanistic role in the development of fibrosis (32, 42).

Interestingly, while MMPs appear to be involved in the initiation and progression of fibrosis, this would seem paradoxical since MMPs have classically been described as proteases involved in the destruction of ECM, whereas the process of fibrosis involves the building up and excess production of ECM molecules by hyperproliferating mesenchymal cells. Thus it appears that MMPs may also play ancillary roles in fibrosis that are not linked to ECM degradation. Recent data suggest that beyond the effects of MMPs to enhance ECM turnover and promote tissue remodeling, they may also have profound effects on the release of growth factors and cytokines known to affect fibrosis such as insulin-like growth factors (IGFs), transforming growth factor-β (TGF-β), and tumor necrosis factor-α (TNF-α) (14, 101, 112, 116). From an extensive survey of the literature, it is now well established that a large part of alveolar macrophage action is mediated by production of a large number of growth factors (22).

In the context of the lung, we review direct and indirect evidence suggesting there is a potentially important link between MMP activity and profibrotic growth factor bioavailability, thus creating an environment of potentiating fibrosis in the lung.

**MMPs**

MMPs currently comprise a family of zinc-dependent, matrix-degrading proteinases that are highly homologous and number over 20 family members (for a recent review, see Ref. 74). Their activities are highly regulated by TIMPs, of which four members have been described (TIMPs 1–4) (15). Classically, MMPs have been subclassified into functional groups based on their substrate specificity (Table 1): collagenases that are active against fibrillar forms of collagen; gelatinases that have high activity against denatured collagens (type IV collagen); stromelysins that exhibit activity against a wide array of noncollagen components of the ECM; and the recently described membrane type MMPs (MT-MMPs) that are transmembrane MMPs that have activity against some ECM molecules as well as activating other MMP family members (74, 101).

With the exception of MT-MMPs, MMPs are secreted as inactive proenzymes, requiring the cleavage of a propeptide for activation (74). Once activated, their proteolytic activity within tissues is primarily regulated through a balance between the production and secretion of TIMPs and MMPs. TIMPs 1–4 can form stable complexes with MMPs, usually in a 1:1 molar fashion (3, 13); thus a highly coordinated and intricate balance among production, activation, and inhibition is necessary to prevent untoward effects of these proteinases on tissue morphology and homeostasis.

**MMPs AND THE LUNG**

Under normal circumstances, MMPs are likely involved in the normal development of the lung. They have been implicated in promoting branching morphogenesis and the development of airway glands (55, 79). For instance, during the pseudoglandular stage of lung development, epithelial cells must invade the submucosa, which requires remodeling of the basal lamina. Animal studies suggest that expression of MMP-2 is important in this process (55). Review of the literature also suggests a role for MMPs in the regulation of lung

<table>
<thead>
<tr>
<th>MMP nomenclature</th>
<th>Alternative Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal domain</td>
<td></td>
</tr>
<tr>
<td>MMP-7</td>
<td>Matrilysin</td>
</tr>
<tr>
<td>MMP-26</td>
<td>Matrilysin-2/endometase</td>
</tr>
<tr>
<td>Collagenases</td>
<td></td>
</tr>
<tr>
<td>MMP-1</td>
<td>Collagenase-1</td>
</tr>
<tr>
<td>MMP-8</td>
<td>Collagenase-2</td>
</tr>
<tr>
<td>MMP-13</td>
<td>Collagenase-3</td>
</tr>
<tr>
<td>Stromelysins</td>
<td></td>
</tr>
<tr>
<td>MMP-3</td>
<td>Stromelysin-1</td>
</tr>
<tr>
<td>MMP-10</td>
<td>Stromelysin-2</td>
</tr>
<tr>
<td>MMP-11</td>
<td>Stromelysin-3</td>
</tr>
<tr>
<td>Gelatinases</td>
<td></td>
</tr>
<tr>
<td>MMP-2</td>
<td>Gelatinase A</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Gelatinase B</td>
</tr>
<tr>
<td>Membrane associated</td>
<td></td>
</tr>
<tr>
<td>MMP-14</td>
<td>MT1-MMP</td>
</tr>
<tr>
<td>MMP-15</td>
<td>MT2-MMP</td>
</tr>
<tr>
<td>MMP-16</td>
<td>MT3-MMP</td>
</tr>
<tr>
<td>MMP-17</td>
<td>MT4-MMP</td>
</tr>
<tr>
<td>MMP-24</td>
<td>MT5-MMP</td>
</tr>
<tr>
<td>MMP-25</td>
<td>MT6-MMP</td>
</tr>
<tr>
<td>MMP-23</td>
<td></td>
</tr>
<tr>
<td>Other MMPs</td>
<td></td>
</tr>
<tr>
<td>MMP-12</td>
<td>Metalloelastase</td>
</tr>
<tr>
<td>MMP-19</td>
<td>RASI</td>
</tr>
<tr>
<td>MMP-20</td>
<td>Enamelysin</td>
</tr>
<tr>
<td>MMP-28</td>
<td>Epilysin</td>
</tr>
</tbody>
</table>

MMP, matrix metalloproteinases; MT, membrane type; RASI, rheumatoid arthritis synovial inflammation.
matrix turnover, promoting angiogenesis, and also in the immunoprotection of the lung through allowing migration of inflammatory cells into infected or damaged lung tissues (79).

Although several groups of proteinases have been implicated in the lung damage observed in both acute and chronic lung injury, including proteinases such as neutrophil elastase, cathepsin G, and proteinase 3 (103), it is the MMPs that have recently received attention because of their capacity to cause severe damage to the lung when overexpressed in the lung parenchyma. Attempts to identify the source of these proteinases within the human lung show that several types of cells, including neutrophils, alveolar macrophages, and even airway epithelial cells, produce several different MMPs and TIMPs (96, 99). MMPs are produced by lung epithelial cells in sheets of airway lining cells, lung epithelial cell cultures from human lung explants, and in normal and neoplastic human lung tissues (17, 51, 117). Because inflammatory cell types such as neutrophils and macrophages produce several different forms of MMPs and their induction can be markedly enhanced under the influence of proinflammatory cytokines, these cells have been viewed as likely sources of MMPs in lung inflammation and injury (96, 99).

MMP-1, when transgenically overexpressed, results in emphysematous lung disease, whereas deletion of the MMP-12 gene in mice results in protection against cigarette smoke-induced emphysema (29, 31, 47). Similarly, lungs from mice made null for TIMP-3 show increased alveolar septation and histological evidence of emphysema, suggesting that unchecked proteinase activity can result in degradation of basement membranes within the lung (62). Thus on the basis of transgenic modeling, MMPs appear to be critical in the development and maintenance of lung architecture and function, with dysregulation in their activities resulting in lung damage. Furthermore, studies on humans now support a role for MMPs and an imbalance in favor of MMPs leading to degradation of ECM components.

MMPs in Lung Diseases

Acute lung injury and acute respiratory distress syndrome. Acute lung injury (ALI) can be a devastating disease, which not uncommonly can progress to ARDS. Although in recent years progress has been made in the definition and understanding of the natural history of ALI and ARDS, our understanding of the pathophysiology underlying these disorders remains incomplete. Although the mainstay of therapy in these patients remains primarily supportive in nature, even with the use of aggressive supportive care the current overall mortality rate in all age groups continues to be >40% (11, 49, 50, 114). Although the lungs from some individuals with ALI may heal normally, in others, progression to fibrosing alveolitis with persistent hypoxemia, increased alveolar dead space, and a persistent decrease in pulmonary compliance is seen (7, 83). Histologically, lungs from these individuals may show fibrosis associated with inflammatory cell infiltrates, while the alveolar space may be filled with mesenchymal cells and profibrotic factors (43). The initiation of fibrosing alveolitis likely begins early in the course of ARDS, during the time that the lung may be exposed to other inflammatory mediators, such as MMPs (114). The events leading to fibrosis in this disorder are critical to preventing mortality, because fibrosing alveolitis on histological analysis correlates with an increased risk of death (67).

In ARDS, MMPs and TIMPs can be easily detected by sampling fluids obtained from bronchoalveolar lavage (BAL). In adult patients with active ARDS, an increase in MMP-2 (72-kDa gelatinase or gelatinase A) and MMP-9 (92-kDa gelatinase or gelatinase B) as well as in TIMP-1 has been shown in BAL fluid (25). In addition, inflammatory cells, which are increased in the respiratory tract of patients with ARDS, appear to be involved in releasing MMPs into the alveolar and pericellular space (51). In vitro studies have shown that stimulation of human alveolar macrophages with lipopolysaccharides (LPS), to simulate ARDS, results in an increase in the release of MMPs, primarily MMP-9, as well as TIMPs (98). Neutrophils have also been shown to make and store MMPs, and in the presence of inflammatory mediators, to secrete preformed MMPs from storage vesicles (20). Furthermore, LPS increases the release of MMP-9 and MMP-2 by human bronchial epithelial cells obtained from biopsies but does not modify TIMP-1 release, suggesting an imbalance in favor of MMPs leading to degradation of ECM components.

In vivo models of acute lung injury have shown an important role for increased MMP activity in mediating pulmonary damage induced by immune complexes, hyperoxia, cardiopulmonary bypass, ozone, or LPS. LPS exposure leads to an increase of MMP-2 and MMP-9 in BAL fluid from several different animal models (27, 33, 34, 111), and a synthetic MMP inhibitor prevents the pathological changes typical of acute lung injury after cardiopulmonary bypass in an animal model of lung injury (18). This is corroborated in an endotoxin-induced lung injury model showing that a modified tetracycline (COL-3), a potent inhibitor of MMPs, prevents the development of ARDS. MMP-2 and MMP-9 levels were significantly increased in this ARDS model, but pretreatment with COL-3 ameliorated the rise in MMP-2 and MMP-9 levels (19). Recently, Gibbs and colleagues (45) have confirmed a specific role for MMPs in vivo in alveolar macrophage-mediated acute lung injury associated with both immune complexes and LPS by showing that TIMP-2 can diminish the lung damage seen in both models. Mechanical ventilation has also been associated with...
acute lung injury and has been thought to possibly contribute to and/or worsen the clinical course of individuals suffering from ARDS. In a rat model examining high-volume ventilation, upregulation of MMP-2, MMP-9, and MMP-14 was demonstrated, and pretreatment with the MMP inhibitor Prinomastat lessened the lung injury (36).

To directly examine the role of MMPs in acute lung injury, studies have now been performed in genetically modified animals made null for different MMPs. In mice made null for MMP-3 and MMP-9, acute lung injury from exposure to immunoglobulin G immune complexes results in less severe lung damage than in their genetically normal littermates, confirming a role for these proteinases in the destruction of lung tissue after an acute lung injury (115). Together, these data support that MMPs are elevated in adult humans with ARDS as well as in animal modeling of ARDS, suggesting that their inhibition may be a useful means to control the acute effects of these proteinases on the dissolution of lung tissue. However, the data does not address the issue of how enhanced MMP activity may in the long run contribute to the fibrotic and scarred lung seen in this disorder.

**Neonatal RDS.** RDS in infants is characterized by surfactant depletion and often leads to chronic lung disease, known as bronchopulmonary dysplasia (BPD). After the acute phase of the disease, lung regeneration begins to occur by reepithelialization of damaged alveoli. In the late and chronic stages of the disease, alveoli are lined primarily with type II pneumocytes, and within areas of fibrosis, increased numbers of fibroblasts are observed (4). Recent evidence suggests that the inflammation in RDS may contribute to lung damage by increasing the release of proteolytic enzymes. A recent study demonstrated that in preterm infants, an imbalance between MMP-8 and TIMP-2 exists (21). Tracheal aspirate samples were collected from preterm neonates during their first five postnatal days, and MMP-8 levels were found to be higher in tracheal fluid from the babies who subsequently developed BPD compared with children who did not go on to develop chronic lung disease. In addition, TIMP-2 levels were lower in the infants who required prolonged mechanical ventilation (21). Another recent study supported that MMP-8 can be found in BAL fluid from preterm babies, with higher levels being seen in the children who later develop BDP (106). Finally, immunohistochemistry localization of MMP-1, TIMP-1, and TIMP-2 has been investigated in postmortem lung tissue from infants who died during different phases of BDP development (32). These studies show that type II pneumocytes produce immunoreactive MMP-1 and both TIMPs. Furthermore, fibroblasts located within fibrotic foci express MMP-1, TIMP-1, and TIMP-2, supporting the hypothesis that MMPs contribute to the development of RDS, and that in the case of MMP-8, the degree of expression correlates with the long-term fibrotic picture seen in the lungs of these children.

**Asthma.** Asthma is a chronic inflammatory condition of the airways and lung parenchyma. Bronchial biopsies from patients with asthma demonstrate increased numbers of T helper lymphocytes and eosinophils, with inconsistently observed increases in mast cell numbers (56). New data support the concept that asthma may represent an aberrant repair response of the respiratory epithelium to injury (85). This results in a persistent proinflammatory milieu of epithelium-derived cytokines and growth factors that drive the chronic inflammatory response and remodeling activities seen in the subepithelial compartments, which include subepithelial fibrosis, activation of adjacent fibroblasts/myofibroblasts, increased smooth muscle cell mass, goblet cell hyperplasia, and submucosal gland hypertrophy (reviewed in Ref. 56).

Because airways in asthma display chronic inflammation, it is probable that MMPs may be oversecreted in this condition. Indeed, studies in asthmatics show that compared with normal subjects, MMP-9 is increased in BAL fluid and sputum of asthmatic subjects (58, 109, 119). Other studies reveal that MMP-9 immunoreactivity is increased in bronchial biopsies of asthmatics (54). In asthmatic patients with increased levels of MMP-9, MMP-1 (collagenase-1), MMP-2, and MMP-3 (stromelysin-1) levels were generally 8–30 times less than MMP-9 levels. However, other forms of MMPs were not measured (119). Thus it appears that an increase in MMP-9 production and/or secretion by cells lining the bronchial and alveolar surfaces, such as alveolar macrophages, may contribute to the pathogenesis of asthma and possibly other reactive airway disease, such as chronic obstructive pulmonary disease (COPD).

**Emphysema/COPD.** COPD is characterized by loss of lung parenchyma and enlargement of the air spaces with loss of functioning alveoli. In COPD, bronchoscopic evaluation reveals inflammation of bronchiolar epithelium and increased release of proinflammatory cytokines (108). There is infiltration of the wall of small airways by T suppressor/cytotoxic cells, and there is an increase in the number of macrophages in the airways and alveolar spaces (91). This inflammatory pattern appears to be associated with chronic changes referred to as “respiratory bronchiolitis-associated interstitial lung disease,” which includes septal thickening of alveolar walls and patchy alveolar wall fibrosis with a peribronchial distribution (56, 72).

There is significant evidence that an excess of proteolytic activity over the inhibitory capacity of the lung is associated with parenchymal destruction in COPD and emphysema. Recently, there has been considerable speculation on the potential involvement of MMPs in the matrix degradation in emphysematous lung disease. Finlay et al. (35) in 1997 were the first to report that MMPs were involved in the development of emphysema. In patients with emphysema, both MMP-1 and MMP-9 levels are increased in BAL fluid compared with control smoking patients without emphysema. In addition, increased activity of MMP-9 and MMP-2 in the lung parenchyma of patients with emphysema has been reported (9). Animal models also support the role of MMPs in the development of emphysema. Mice...
made null for MMP-12 are significantly protected from smoke-induced emphysema compared with wild-type animals (48, 96). Clearly, MMPs appear to play a role in the ECM destruction seen in COPD and possibly in the concomitant fibrosis. They may also be potentially critical to the continued distortion of the lung parenchyma seen with emphysematous changes. Although their role in the development of fibrosis and hyperplasia in these disorders is not as clear, cytokines and chemokines are elevated in the sputum of patients with COPD (57).

**Interstitial pulmonary fibrosis.** Interstitial pulmonary fibrosis (IPF) is a chronic fibrotic lung disorder of unknown origin characterized by a progressive interstitial fibrosis, which ultimately leads to respiratory failure. IPF is characterized by progressive dyspnea, dry cough, crackles, decreased lung volumes, and diffuse reticulonodular opacities on chest X-ray. Fibroblast proliferation and abnormal accumulation of ECM occurs in the damaged alveoli, leading to subsequent abnormal lung remodeling within fibroblast foci (90). The abnormal ECM remodeling observed in the lungs of patients with IPF is due, at least in part, to an imbalance between some MMPs and TIMPs (42, 48, 95). Normal lung fibroblasts do not make MMP-9 in vitro, whereas fibroblasts from IPF lungs strongly express MMP-9. In addition, fibroblasts from patients with IPF express increased levels of all TIMPs (90). In this setting, TIMPs may play a role in apoptosis in some cell populations or increased proliferation of other cell populations. Interestingly, TIMP-2 is almost exclusively expressed in the fibroblast foci, which is considered the site where most ongoing lung injury and fibrosis occur (90). In vitro studies of alveolar macrophages obtained from untreated patients with idiopathic pulmonary fibrosis showed marked increases in MMP-9 secretion compared with macrophages collected from normal individuals (64). In animal models of bleomycin-induced pulmonary fibrosis, MMPs have been shown to be elevated in BAL fluid. Indeed, a synthetic inhibitor of MMP, Batimastat, has been shown to significantly reduce bleomycin-induced lung fibrosis, again pointing to the importance of MMPs in the development of this fibrotic disease of the lung (26).

**GROWTH FACTOR-MMP INTERACTIONS**

Data clearly support a prominent role for MMPs in the pathogenesis of several well-recognized disorders of the lung as summarized above. However, it is unclear what molecules, beyond ECM, might be targets for these proteinases. Indeed, because MMP production precedes or parallels the development of fibroproliferative events in the lung, it seems plausible that MMPs may play an expanded role in profibrotic events that occur in lung pathology. Several mechanisms have been invoked to explain how cellular activities may be linked to MMP action (reviewed in Ref. 102). First, cell-matrix and cell-cell interactions may be modified. For example, MMP-mediated cleavage of laminin-5 generates a fragment (γ2-chain fragment) that can enhance cell motility (40). In addition, MMPs have been shown to cleave cell surface molecules involved in cell-cell interactions such as E-cadherin (75). Second, MMPs, such as MMP-7, have been shown to modify cell surface shedding of proteins such as Fas ligand. Therefore, MMP actions may be important in regulating Fas-mediated apoptosis (84). Third, MMPs may function to modulate the migration of cells into a given location, as has been shown for MMP-mediated cleavage of α1-proteinase inhibitor, which results in the release of a bioactive chemoattractant for neutrophils (8). Finally, a number of studies have shown that the actions of MMPs can result in the release of growth factors and cytokines, which may have a myriad of effects on cellular growth and proliferation (41, 112, 116). It is this final pathway of how MMPs may interface with growth factor release and activation in the lung that we will explore.

The roles of several well-described growth factors and cytokines have been implicated in the pathogenesis of lung fibrosis (2, 61, 69). However, a number of profibrotic growth factors require proteolytic processing for their activation or release from ECM or carrier proteins before they can exert their mitogenic and metabolic effects (reviewed in Ref. 107). Few studies have examined how sequestered or inactivated profibrotic growth factors are released during the pathogenesis of pulmonary fibrosis. Recent studies show that the proteolytic processing of several key growth factors involved in fibrosis occurs through the actions of MMPs, thereby activating or releasing them from inhibitory protein-protein interactions. Included among this group are several growth factors that have been shown to be involved in the fibrotic process, including IGFs, TGF-β, and TNF-α. We next review recent data suggesting that MMPs are critical proteinases in the release and/or activation of these three profibrotic growth factors and highlight how this interplay may be involved in the fibroproliferative process within the lung.

**IGFs.** Recent reports suggest a role for IGFs in the process of lung repair after acute lung injury (70). However, while IGFs may play important roles in the normal growth and restoration of lung tissue, they also have been implicated in fibrotic events taking place within several tissues, including the lung. Indeed, in IPF, studies have revealed that epithelial cells can express several cytokines and growth factors that can both promote fibroblast migration and proliferation as well as enhance the formation and accumulation of ECM (61). Among the growth factors produced by primary human airway epithelial cells, it is IGF-I secreted by these cells that accounts for the majority of the growth-promoting activity directed at lung fibroblasts (16). IGF-I has also been shown to be increased in early-stage IPF with minimal fibrosis and has been colocalized with several cell types, including alveolar macrophages and type II pneumocytes. However, it appears that as IPF progresses, IGF-I is expressed primarily by alveolar macrophages (53).
While IGFs are expected to be involved in the fibrotic process, IGFs in vivo are sequestered by six high-affinity IGF-binding proteins (IGFBPs 1–6), preventing their ability to interact with IGF receptors (23). Studies examining adults and children with IPF and interstitial lung disease show that IGFBP-3 and IGFBP-2 levels are increased in IPF BAL fluid (1, 22). Furthermore, increased IGF levels have been documented in idiopathic pulmonary fibrosis in adults, suggesting that both IGFs and their carrier proteins are overexpressed in interstitial lung disease (6).

MMPs have recently been shown to regulate the cleavage of IGFBPs, thereby liberating the complexed ligand to affect IGF actions in target cells. Because IGFBPs bind IGFs with equal or higher affinity than IGF receptors, proteolysis of IGFBPs is thought to play a major role in the regulation of IGF activity (38, 40). Specifically, IGFBP-3 can be cleaved by MMP-1, MMP-2, and MMP-3. In addition, IGFBP-5 is cleaved by MMP-1 and MMP-2 (37, 39, 110). The hypothesis that MMPs may affect IGF bioavailability in vivo has been corroborated by the finding that mice genetically susceptible to developing liver tumors are protected from tumor development through the overexpression of TIMP-1. This phenomenon is associated with increased levels of intact IGFBP-3 and decreased IGF signaling, demonstrating that MMPs are involved in IGF bioavailability and IGF action at the cellular level through modulating the levels of IGFBPs in the tissue compartment (68). The impact of MMP activity on IGF action in the lung is largely unknown. However, recent data from our laboratories have shown that normal human lung secretions contain several MMPs as well as several IGFBPs (Winkler, Folds, Ferguson, and Fowlkes, unpublished data). Furthermore, IGFBP-3 was found in both its intact form as well as multiple fragments, suggesting that IGFBP-3 may be degraded by MMPs in the lung (unpublished data).

The interaction of MMPs and IGF action has been demonstrated in airway smooth muscle cells. In this reactive airway disease model, the asthma-associated proinflammatory eicosanoid leukotriene D4 was shown to enhance IGFBP-2 degradation, and the proteinase implicated in the degradation was identified as MMP-1. Furthermore, TIMP-1 and the synthetic inhibitor of MMPs, Batimastat, inhibited the proteolysis of IGFBP-2 (89). In situ studies have also supported that MMPs may be involved in IGFBP proteolysis in the asthmatic lung. MMP-1 has been demonstrated in human airway tissue sections from nonasthmatic and asthmatic subjects with the immunostaining for MMP-1 being 12-fold higher in asthmatics in both the bronchial and tracheal smooth muscle cells compared with normal lung sections. Furthermore, levels of IGFBP-2 and IGFBP-3 were found to be extensively proteolyzed by extracts from asthmatic airway tissues. Interestingly, the IGFBP-degrading proteinase activity in the extracts could be specifically reduced using immunodepletion of MMP-1, suggesting strongly that MMPs are involved in IGFBP processing in the asthmatic lung (88). Indirect evidence also suggests that similar mechanisms may be operative in other conditions associated with pulmonary fibrosis, such as sarcoidosis, in which IGFBP-3 in BAL fluid has been shown to be extensively degraded (1). Although the interactions of MMPs on IGF action in vivo within the lung appear likely, more studies will be necessary to establish such an association. However, findings to date suggest that MMPs may be important regulators of IGF bioavailability, and this level of control may be important in modulating these mitogens, which can increase fibroblast proliferation and collagen production in the pathogenesis of lung fibrosis.

TGF-β. TGF-β is expressed as three different isoforms, and knockout studies examining each isoform have resulted in mice with significantly abnormal lungs, strongly suggesting that TGF-β is important in normal lung growth and development (92). However, despite its necessary developmental affects, TGF-β1 has been strongly associated with pulmonary fibrosis and has been demonstrated to be upregulated in the fibrotic lung at sites of fibrotic foci (24, 61). Furthermore, its administration via gene transfer into animal models results in severe parenchymal and airway fibrosis (100). However, normally, TGF-β is secreted in an inactive form due to its incorporation into a large latent complex with TGF-β latency-associated protein (LAP) and latent TGF-β-binding proteins, which can tether the latent complex in the extracellular matrix (ECM) (107). Under homeostatic conditions, very little free, active TGF-β is available compared with the LAP-bound latent form, thereby preventing signal transduction and uncontrolled fibrosis. In order for growth factor action to take place, TGF-β must first be liberated from these inhibitory proteins, a process generally believed to occur through proteolytic protein processing (14, 107). The precise mechanism by which TGF-β activation occurs in vivo is unclear; however, several molecules, such as plasmin, thrombospondin 1, and integrin may be involved (30, 66, 73), either by proteolytic cleavage of the latent TGF-β complex, or, as is the case with plasmin, through conformational changes induced through protein-protein interactions.

Recent observations by Yu and Stamenkovic (118) show that the gelatinases, MMP-9 and MMP-2, may be involved in proteolytic activation of latent TGF-β complexes. They have revealed an unexpected and potentially important relationship among the cell surface hyaluronan receptor CD44, the metalloproteinase MMP-9, and the cytokine TGF-β (118). CD44, through docking proteolytically active MMP-9 on the surface of cells, facilitates MMP-9 cleavage and activation of latent TGF-β. Although not working through precisely the same mechanism, they have also demonstrated that MMP-2 can perform a similar function in regard to latent TGF-β cleavage, yet MMP-2 displayed a different specificity than MMP-9 when all three isoforms of TGF-β were examined as substrates. To determine whether a relationship among CD44, MMP-9, and TGF-β activation exists in vivo in the lung, Lee et al. (63) have examined BAL fluid from interleukin (IL)-13 transgene (+), IL-13+/MMP-9−, and IL-13+/CD44−/− mice for bioactive TGF-β1. These studies demonstrated several aspects of how IL-13 may cause lung fibrosis.
First, MMP-9 appears to play a fundamental role in IL-13-induced TGF-β activation because bioactive TGF-β1 was significantly decreased in BAL fluid from IL-13 transgenics made null for MMP-9. In contrast, similar TGF-β1 bioactivity was present in BAL fluid from IL-13 transgene (+) mice irrespective of whether or not they expressed CD44 (63). These in vivo data demonstrate that MMP-9 does not require CD44 to activate TGF-β1 in the lung and raises the possibility that other surface molecules may be able to facilitate localizing MMP-9 to the surface to activate TGF-β. Although MMP-mediated activation of latent TGF-β may have various effects on lung pathology, it is likely that its release from matrix stores could promote fibrosis by affecting fibroblast proliferation and increasing the production of collagen as well as inhibiting the proliferation of epithelial cells (61).

Other indirect studies suggest a connection between MMP activity and the release of TGF-β. For instance, tranilast, an antifibrotic agent, inhibits MMP-2 expression and activity, suggesting strongly that one of the key mechanisms by which this compound inhibits fibrosis is through inhibiting MMP-mediated release of the profibrotic growth factors TGF-β1 and TGF-β2 (82). In other studies, the MMP inhibitor Batimastat has been examined for its capability to inhibit the release of both TNF-α and TGF-β into BAL fluid after mice have received aerosol administration of the inflammatory mediator, LPS (28). Batimastat reduced MMP-9 activity in BAL fluid, which was associated with decreases in TNF-α and TGF-β, signifying a possible connection between MMP action and release of these two growth factors in a model of acute lung injury (28).

TNF-α. Other growth factors, such as TNF-α, may be expressed as a membrane-bound protein, requiring proteolytic cleavage to release soluble and active ligand (65). TNF-α is an inflammatory cytokine that was originally described as being shed from cell surfaces by a cation-dependent proteinase (59). An important role for TNF-α in interstitial fibrosis of the lung has been established using transgenic mice, which in combination with its fibrinolytic and TNF-α-converting activity suggests a role in inflammation and its ramifications (41). In vivo, MMP-7 has been associated with the release of TNF-α and the subsequent upregulation of MMP-3, resulting in degradation of ECM in a model of herniated intervertebral disks (46). Because MMP-7 is secreted in airway and peribronchial epithelial cells and is upregulated in models of airway injury, it is possible that this MMP is also involved in the release of this profibrotic cytokine in the lung (78). This is supported by recent studies showing that the MMP inhibitor Batimastat decreases TNF-α levels in BAL fluid in an LPS model of acute lung injury (78). These studies suggest that by modulating MMP activity, the effects of proinflammatory and profibrotic cytokines, such as TNF-α, may be curtailed by inhibiting their shedding from cell surfaces.

Conclusions. The consequences of acute, persistent, or recurrent lung injury and inflammation can lead to various degrees of pulmonary fibrosis, alveolar scarring, and chronic lung dysfunction. Although there are likely many interactions among profibrotic events within the lung after injury such as ECM destruction and remodeling, cellular death and proliferation, and ultimately either adequate repair or dysregulated reconstruction, we have presented herein support for the idea that proteinases, primarily of the MMP family, may be critical enzymes not only in the breakdown and remodeling of lung tissues but also in the release and/or activation of profibrotic growth factors such as IGFs, TGF-β, and TNF-α. Indeed, there may even be more interplay among MMPs and growth factors and cytokines than we have detailed because MMPs appear to be capable also of releasing or activating such varied mitogens as interleukins, fibroblast growth factor, and heparin-binding epidermal growth factor-like growth factor (HB-EGF) (Table 2) (10, 41, membrane type 4-MMP (MT4-MMP) expressed in COS-7 cells has been shown to localize to the cell surface, but not activate pro-MMP-2, as do other MT-MMPs. However, MT4-MMP is able to cleave a peptide consisting of the pro-TNF-α cleavage site and is able to shed pro-TNF-α when cotransfected in COS-7 cells. MT4-MMP has been detected in monocyte/macrophage cell lines, which in combination with its fibrinolytic and TNF-α-converting activity suggests a role in inflammation and its ramifications (41). In vivo, MMP-7 is involved in the release of this profibrotic cytokine in the lung (78). This is supported by recent studies showing that the MMP inhibitor Batimastat decreases TNF-α levels in BAL fluid in an LPS model of acute lung injury (78). These studies suggest that by modulating MMP activity, the effects of proinflammatory and profibrotic cytokines, such as TNF-α, may be curtailed by inhibiting their shedding from cell surfaces.

Table 2. MMPs and cytokine/growth factor interactions

<table>
<thead>
<tr>
<th>MMP</th>
<th>Cytokine/Growth Factor Targeted or Released</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>FGF, IGF, TNF-α</td>
</tr>
<tr>
<td>MMP-2</td>
<td>TGF-β, TGF-β₂, IL1-β, IGF, TNF-α, FGF-R1/ectodomain</td>
</tr>
<tr>
<td>MMP-7</td>
<td>TGF-β, TNF-α</td>
</tr>
<tr>
<td>MMP-9</td>
<td>VEGF, TGF-β₂, IL1-β, IGF, TNF-α</td>
</tr>
<tr>
<td>MMP-11</td>
<td>IGF</td>
</tr>
<tr>
<td>MMP-14</td>
<td>IGF*, FGF</td>
</tr>
<tr>
<td>MMP-15</td>
<td>IGF*</td>
</tr>
<tr>
<td>MMP-16</td>
<td>IGF*</td>
</tr>
<tr>
<td>MMP-17</td>
<td>IGF*, TNF-α</td>
</tr>
</tbody>
</table>

Compiled from Refs. 41 and 71; *Fowlkes and Ferguson, unpublished data. FGF, fibroblast growth factor; IGF, insulin-like growth factor; TNF-α, tumor necrosis factor-α; TGF, transforming growth factor; IL, interleukin; VEGF, vascular endothelial growth factor.

Table 2. MMPs and cytokine/growth factor interactions
REFERENCES

26. Corbel M, Caulta-Maugendre S, Germain N, Molet S, Lagente V, and Boichot E. Inhibition of bleomycin-induced pul-


73. Munger JS, Huang X, Kawakatsu H, Grif


