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SERIES "MATRIX METALLOPROTEINASES IN LUNG HEALTH AND DISEASE"
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Metalloproteinases in idiopathic pulmonary fibrosis

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ABSTRACT: **In this article, we outline the current state of knowledge about the balance between collagen production and degradation in idiopathic pulmonary fibrosis (IPF). The dysregulated action of metalloproteinases implicated in IPF may play a central role in IPF pathogenesis. Inhibiting metalloproteinases in IPF may, therefore, have therapeutic potential, but our knowledge of their pathophysiological role is held back by limited animal models and the lack of specific inhibitors.**

KEYWORDS: **Idiopathic pulmonary fibrosis, matrix metalloproteinase**

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PULMONARY FIBROSIS: VARYING MECHANISMS WITH A FIBROTIC END-POINT

Diffuse interstitial lung disease (ILD) is characterised by varying degrees of inflammation and fibrosis resulting in derangement of the gas-exchange units of the lung. A hallmark of these diseases is the abnormal deposition of collagen. Many ILDs are of known aetiology, *i.e.* exposure to organic (*e.g.* farmer's lung) or inorganic (*e.g.* asbestosis) particles, induced by drugs (*e.g.* amiodarone), or associated with rheumatological disease, such as systemic sclerosis and rheumatoid arthritis. Around half of ILDs are of unknown aetiology and are classified as idiopathic interstitial pneumonias [1]. By far the most common is idiopathic pulmonary fibrosis (IPF), which has a prognosis that is worse than that of many cancers.

Current evidence suggests that IPF results from an abnormal response to a currently unidentified alveolar epithelial injury. Theories speculate that IPF results from abnormal wound healing in response to multiple microscopic sites of alveolar epithelial cell injury and activation (fig. 1). This is thought to result in a persistently abnormal epithelial repair, which promotes fibroblast proliferation, generating a reticulum of activated fibroblasts and collagen which progressively restructures the lung architecture [2]. In addition to this, there is increased epithelial cell apoptosis and cell loss, especially adjacent to the fibroblast foci. In IPF, aberrantly activated alveolar epithelial cells synthesise almost all, if not all, of the mediators that provoke and sustain the fibrotic reaction, probably through a bidirectional aberrant communication between epithelial and mesenchymal cells [3]. Fibroblast-type cells arise also by recruitment of fibrocytes from the circulation and, possibly, by the process of epithelial–mesenchymal transformation [4].

In addition to myofibroblast focus formation and epithelial cell injury, there is variable evidence of inflammation, as evidenced by increased macrophage and neutrophil counts [5], intra-alveolar coagulopathy [6] and the formation of new blood vessels in the IPF lung [7]. Abnormal angiogenesis has furthermore been linked to the development of fibrotic disorders of the lung [7]. Together, these changes result in an increase in the permeability of the alveolar capillary barrier, which can be detected clinically by increased diethylene triamine penta-acetic acid clearance [8]. Increased alveolar capillary barrier permeability may also be associated with early mortality in IPF [9].

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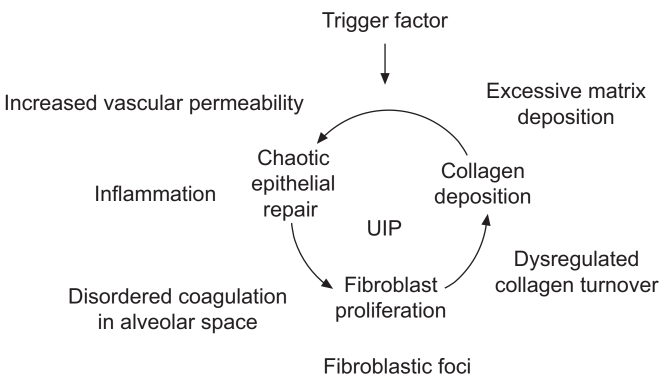

FIGURE 1. Mechanisms of interstitial pulmonary fibrosis (IPF). The aetiology of IPF is undetermined. It is postulated that whatever triggers IPF results in epithelial damage and that consequent epithelial activation leads to the core features of usual interstitial pneumonia (UIP), namely chaotic epithelial repair, fibroblast proliferation and collagen deposition, which become self-perpetuating.

Therefore, at least two different cellular routes exist – an inflammatory pathway, represented by most ILDs, and an epithelial pathway, as seen in IPF – that lead to the development of lung fibrosis [10]. In this article, we will focus on the importance of metalloproteinases in the pathophysiology of IPF.

COLLAGEN AND THE PATHOPHYSIOLOGY OF IPF

There are many different species of collagen, but types I and III predominate within both healthy and fibrotic lungs [11]. Fibrillar collagens are secreted as soluble precursors (bearing large extension propeptides at both their amino and carboxyl termini) that self associate to form an insoluble triple helix fibril. The triple helical conformation of collagen fibrils renders the molecule resistant to proteolytic attack by most enzymes except the metalloproteinases, the biology of which has been outlined in the first article of this Series [12].

Considerable evidence exists that both type I and III collagen production is increased in IPF. Most studies looking at type I or III production have looked at the procollagen carboxy-terminal propeptides (PICP and PIIICP, respectively). These have been used as surrogate markers of increased collagen production, since collagen itself is insoluble and cannot therefore be sampled directly without invasive biopsy.

In IPF, both PICP and PIIICP have been found to be elevated in bronchoalveolar lavage fluid (BALF), but not serum, of patients. PICP levels in the BALF and epithelial lining fluid had a significant negative correlation with diffusing capacity of the lung for carbon monoxide per unit of alveolar volume [13]. In immunohistochemical or *in situ* mRNA studies on lung tissue, type III collagen is predominant in the thickened alveolar septa and interstitium, whereas type I collagen appears to be the principal collagen at later stages in the disease course [14]. Type I procollagen is mostly present as intracellular spots in newly formed fibrosis in usual interstitial pneumonia (UIP) while type III procollagen is expressed extracellularly underneath metaplastic alveolar epithelium [15]. Increases in other constituents of the extracellular matrix, including type V, VI, and VII collagens, fibronectin, elastin, and proteoglycans are also present [10].

THE DEGRADATIVE ENVIRONMENT IN ILD

Elevated levels of procollagen production do not necessarily equate with increased collagen deposition, since collagen degradation is a dynamic process regulated by the matrix metalloproteinases (MMPs) and their inhibitors. In order to assess whether net collagen is deposited in the lung, some assessment of collagen degradation is also needed.

Several early lines of study pointed to the abnormalities of collagen degradation within the fibrotic lung. In 1979, using zymographic methods, GADEK *et al.* [16] demonstrated elevated collagenase activity in lung homogenates from 15 out of 21 IPF patients, but in none from normal controls or sarcoidosis patients. Conversely, there is evidence that collagenolysis is reduced in hypersensitivity pneumonitis, experimental silicosis and bleomycin-induced pulmonary fibrosis in animals [17]. Furthermore, immunohistochemical studies demonstrated high levels of expression of the tissue inhibitors of metalloproteinases (TIMPs) within the IPF lung. TIMP-1 was found in interstitial macrophages and TIMP-2 in fibroblast foci. TIMP-3 revealed an intense staining mainly decorating the elastic lamina in vessels. TIMP-4 was expressed in IPF lungs by epithelial and plasma cells [18]. Additionally MONTANO *et al*. [19] found that collagenase inhibitory activity was much higher in biopsy samples from patients with IPF and hypersensitivity pneumonitis than in those from control subjects. Given that IPF lung tissue-derived fibroblasts express a profibrotic secretory phenotype (reduced collagenase and elevated TIMP expression), these early studies suggested that a nondegrading fibrillar collagen microenvironment might prevail in ILD [18–20].

While initially, a defect in collagenolysis was suspected to lead to an excess of extracellular matrix deposition in pulmonary fibrosis, this view now seems overly simplistic. Several studies have suggested that there is an increase in MMPs, rather than a loss of MMPs, in IPF (fig. 2) [9]. Elevated levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8 and MMP-9 have been reported. MMP-12 and MMP-13 have also been implicated in experimental fibrosis. Their roles and potential significance are discussed here.

MMP-1

MMP-1 has been shown to be elevated in some, but not all, BALF studies of patients with IPF, with one study suggesting increased plasma levels as well [9, 21]. Microarray data suggest that MMP-1 mRNA is significantly upregulated in whole lung tissue from IPF patients compared with hypersensitivity pneumonitis [22] as well as normal control lung [23]. MMP-1 expression is also higher in patients with familial compared with sporadic IPF [24]. Interestingly, a polymorphism in the MMP-1 gene promoter is more common in smokers with IPF, revealing a putative gene–environment interaction in this disease [25].

The observation that MMP-1 is upregulated in IPF, a condition associated with accumulation of both type I and III collagen, is at first glance a paradox, especially as it has also been implicated in the pathogenesis of chronic obstructive pulmonary disease, where loss of elastic tissue is a feature. One potential explanation is that the expression of MMP-1 is primarily in the reactive epithelium, not in the interstitial compartment where collagen is accumulating [10]. Alternatively, the activity of MMP-1 may be counterbalanced by tissue inhibitors, resulting in only weak

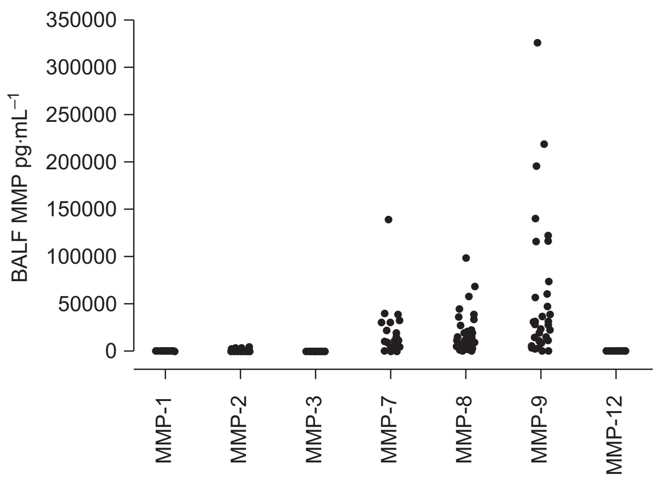

FIGURE 2. Bronchoalveolar lavage fluid (BALF) matrix metalloproteinase (MMP) levels expressed as a scatter plot. BALF data were combined from baseline and follow-up bronchoscopy (n=28). The scatter plot demonstrates that the majority of BALF MMP protein is MMP-7, MMP-8 and MMP-9. BALF levels of MMP-2, MMP-3, MMP-7, MMP-8 and MMP-9 were significantly elevated compared with normal controls. Reproduced from [9].

activity. However, the biological roles for MMP-1, in addition to collagen degradation, include processing of cytokines, such as pro-tumour necrosis factor (TNF)-α, regulation of cell migration, and potentially cell growth [26]. These multiple biological functions of MMP-1, along with the clinical data, suggest an important role in IPF pathogenesis.

MMP-2

MMP-2 (gelatinase A) has been reported to be widely expressed in fibrotic lungs, especially in areas of hyperplastic epithelial cells covering intra-alveolar fibrosis, as well as by mesenchymal cells in the fibroblast foci [18]. In BALF, MMP-2 has been reported to be elevated, but in another study, western blots for active MMP-2 suggested only weak activity (especially compared with bronchiolitis obliterans organising pneumonia (BOOP)) [9, 27]. MMP-2 degrades a wide range of matrix and nonmatrix substrates, particularly type IV collagen and other basement membrane proteins. In addition, MMP-2 is usually upregulated in experimental models of lung fibrosis and its overexpression, as well as that of MMP-9, has been suspected to be implicated in basement membrane disruption [28]. This may be important, because the structural integrity of the alveolar wall depends on the basement membrane and it is recognised that destruction of the subepithelial basement membrane may precede the development of alveolar fibrosis. A discontinuity of the basement membrane potentially allows greater access for exudative factors and interstitial cells to the alveolar space, promoting further tissue destruction and progressive fibrosis [9, 29].

MMP-3

MMP-3 (stromelysin 1) levels are elevated in the BALF of patients with IPF and were observed to be higher in those who died within 3 yrs of diagnosis [9]. MMP-3 may be important as a driver of fibrosis, since MMP-3 expression in epithelial cells of transgenic mice stimulates development of fibrosis and subsequent tumour formation. Further exposure of mammary epithelial cells to MMP-3 induces epithelial–mesenchymal transition, in which the cells acquire myofibroblast-like characteristics, and this process is dependent upon the generation of cellular reactive oxygen species. Data from culture models in which MMPs are inducibly expressed in human lung cell lines and transgenic mouse models in which MMPs are inducibly expressed in lung alveolar epithelial cells suggest that similar processes probably occur in the lung [30, 31]. MMP-3 has also been implicated in the release of antiangiogenic collagen degradation products, such as endostatin, that can promote alveolar epithelial apoptosis, which is believed to be an important driver of ongoing fibrosis [32, 33].

MMP-7

MMP-7, also known as matrilysin, has been reported to be one of the genes most consistently elevated in fibrotic lungs. MMP-7 expression does not differ between familial and sporadic IPF [34, 35]. In IPF lungs, the increased immunoreactive protein is expressed primarily by the abnormal alveolar epithelium and active protein has been demonstrated by tissue zymography in IPF lungs [34]. In BALF, MMP-7 levels relate to the severity of lung function impairment in IPF [9]. Recently, it has also been shown that elevated levels of MMP-7 can also be found in nonspecific interstitial pneumonia and sarcoidosis [36], suggesting that increased MMP-7 expression is not specific to IPF.

MMP-7 has been described as a profibrotic metalloproteinase [10]. Several lines of research suggest that MMP-7 may promote a fibrotic response *via* regulatory effects on epithelial repair and release of latent transforming growth factor (TGF)-β. MMP-7 null mice are relatively protected from bleomycin-induced fibrosis, suggesting that this MMP is a central driver of the tissue response in fibrosis. MMP-7 has a broad substrate affinity for extracellular matrix components, including type IV collagen, laminin, fibronectin, gelatine, elastin and osteopontin. MMP-7 also has the ability to process numerous bioactive substrates, such as Fas ligand (FasL), β_4-integrin, E-cadherin, pro-TNF-α, pro-α-defensin, endostatin, syndecan and α_2-macroglobulin. MMP-7 can also activate proteases including itself and pro-MMP-1, pro-MMP-2 and pro-MMP-9. Release of pre-formed TGF-β from the extracellular matrix by MMP-7 is the main regulator of TGF bioactivity, which could promote fibroblast growth, survival and collagen synthesis. Thus, the role of MMP-7 in pulmonary fibrosis is probably pleiotropic, due to its diverse biological roles, being implicated in apoptosis, inflammation, fibroproliferation and innate immunity [10].

MMP-8

MMP-8 (collagenase-2 or neutrophil collagenase) is derived from neutrophils and, to a lesser extent, from fibroblasts and endothelial, epithelial and plasma cells. MMP-8 levels are elevated in BALF from IPF patients and correlate with the collagenolytic capacity of the BALF [37]. MMP-8 levels in the BALF of IPF patients are highest in those with rapidly progressive disease and poor survival, and alveolar levels do not reduce with combination therapy (prednisolone, azathioprine and *N*-acetylcysteine (NAC)) [9]. Thus, elevated MMP-8 levels appear to be associated with adverse outcome in IPF.

Neutrophilic alveolitis is a feature of patients with IPF and the degree of neutrophilia in BALF has been related to mortality in one large series [5], so it is interesting to speculate that the neutrophilia drives matrix turnover *via* MMP-8, at least in some patients. Alternatively, MMP-8 has been implicated in the migration and homing of fibrocytes. Fibrocytes are unique bone marrow-derived mesenchymal progenitor cells that are defined by their growth characteristics and surface phenotype, as they express markers compatible with leukocytes, haematopoietic progenitor cells and fibroblasts. Fibrocytes have been found within the lungs of patients with IPF and circulating fibrocyte levels are a marker of poor prognosis in IPF [38, 39]. Attenuation of fibrocyte trafficking in mouse models also directly correlates with a reduction in pulmonary fibrosis. Clearly, the potentially important role that MMP-8 may play in this apparently important process needs further study.

MMP-9

MMP-9 (gelatinase B) has been widely studied in patients with IPF where it is predominantly expressed by alveolar macrophages, neutrophils and epithelial cells. In the normal lung, MMP-9 is not produced by resident cells, but under various forms of stimulation, bronchial epithelial cells, Clara cells, alveolar type II cells, fibroblasts, smooth muscle cells and endothelial cells can produce MMP-9 [40]. MMP-9 gene expression and protein have been found to be elevated in both human and experimental lung fibrosis [41, 42]. Fibroblasts and alveolar macrophages extracted from IPF patient lung produced elevated MMP-9 compared with normal cells [19, 41].

Levels of MMP-9 in BALF and MMP-9 activity are greatest in samples from rapidly progressive IPF cases [9, 43]. Whether elevations of MMP-9 are a marker of activated neutrophils or involved in the alveolar damage in this subset of patients is unknown. However, the elevations of MMP-9 in the BALF of patients with BOOP exceed those seen in IPF [44], suggesting an association with neutrophils rather than lung histology.

Animal studies using MMP-9 knockout mice display some conflicting results about the role of this metalloproteinase. After bleomycin installation, MMP-9-null mice develop fibrosis that is similar to that developed by wild-type animals, although the lungs of MMP-9-deficient mice show minimal alveolar bronchiolisation, suggesting that that MMP-9 facilitates migration of Clara cells and other bronchiolar cells into the regions of alveolar injury [45]. Alternatively, overexpression of MMP-9 in macrophages has been shown to attenuate bleomycin-induced fibrosis [46]. The reduction of profibrotic mediators, such as TIMP-1 and insulin-like growth factor-binding protein (IGFBP)-3, in MMP-9 transgenic mice was identified as a potential mechanism of the diminished fibrotic response. It is difficult to reconcile these findings, but they suggest that MMP-9 may promote or reduce the fibrotic response. It seems likely that the overall response depends upon which cell produces the MMP-9, the local tissue inhibitor levels and the available target molecules/substrates.

MMP-12

MMP-12 (macrophage elastase) has been implicated in the fibrotic response in animal studies of fibrosis using FasL. Mice treated with a Fas-activating antibody had increased caspase-3 activation in alveolar wall cells and increased total lung collagen on day 21 after exposure. Gene expression profiling showed sequential activation of co-regulated profibrotic genes, including marked upregulation of MMP-12. Targeted deletion of MMP-12 protected mice from Fas-induced pulmonary fibrosis, even though the inflammatory responses in the lungs were similar to those of wild-type mice [47].

There are few data on MMP-12 levels or expression in patients with IPF. In one small study, MMP-12 was only detectable in the BALF of three out of 18 patients with IPF and represented only 0.022% of total IPF BALF MMP levels, as measured by Luminex array [48]. It is important to recognise that BALF levels of MMP-12 do not necessarily clearly reflect tissue levels due to the complex regulation of MMPs by local inhibitors. Thus, current evidence does not support a clear role for MMP-12 in human lung fibrosis associated with IPF.

MMP-13

MMP-13 has been implicated in the severity of inflammation and fibrosis in experimental asbestos-induced lung injury, along with MMP-2, MMP-9 and MMP-12. Use of a general MMP inhibitor, GM6001, attenuated both the inflammation and the degree of fibrotic reaction [49]. MMP-13 knockout mice also have reduced acute inflammation and fibrosis when exposed to radiation [50].

In contrast, in pulmonary fibrosis induced in rats with paraquat and hyperoxia, RUIZ *et al*. [28] demonstrated reduced levels of collagenases MMP-8 and MMP-13 with an increase in TIMP-1 and TGF-β. Thus, similar to the results of the MMP-9 animal models described earlier, results for MMP-13 are conflicting. Little is known about the role of MMP-13 in human IPF, although MMP-13 is undetectable in IPF BALF (D.R. Thickett, unpublished observations). This data is backed up by immunohistochemistry and RT-PCR of IPF lungs [18].

MEMBRANE-ASSOCIATED AND OTHER METALLOPROTEINASES IN IPF

A subset of MMPs, the membrane-type (MT)-MMPs, participate in the activation of pro-MMP-2 to form MMP-2. These MT-MMPs have been shown to be present in IPF lung tissue. MT1-MMP and MT2-MMP were found in alveolar epithelial cells, MT3-MMP in fibroblasts from fibroblastic foci and alveolar epithelial cells, and MT5-MMP in basal bronchiolar epithelial cells and in areas of squamous metaplasia. In lung fibroblasts, TGF-β1 induced a strong upregulation of MT3-MMP, both at the gene and protein level [51].

The increasing diversity of known MMP biology means that many potentially important MMPs remain poorly studied in IPF. For example, microarray studies suggest elevated MMP-10 and MMP-28 in IPF tissue, but their cellular sources, substrates and function are poorly characterised. MMP-28 may be of particular interest, since it has been proposed to have a role in epithelial mesenchymal transformation and proteolytic cleavage of TGF-β [52]. Further work to clarify the importance of these novel MMPs is ongoing.

EFFECT OF CURRENT TREATMENT FOR IPF ON MMP EXPRESSION IN THE LUNG

Current treatment for IPF remains unsatisfactory and there is little evidence that the fibrosis seen in the UIP pattern on lung biopsy ever regresses with treatment. A recent trial has, however, suggested that treatment with prednisolone, azathioprine and NAC slows progression [25], but whether this treatment reduces aberrant collagen turnover is unclear. The only study to address MMP levels pre- and post-treatment in the same individual demonstrated that combination drug therapy does not have any suppressive activity upon BALF MMP immunoreactivity [9]. This is despite the fact that a previous study suggested that steroid treatment reduces MMP-9 production in IPF patients [26]; however, in that study, patients were not individually studied consecutively pre- and post-treatment.

Pirfenidone is a novel antifibrotic agent that has been shown to decrease collagen deposition in a variety of animal models *in vivo*. Trials in IPF patients [53] show that it reduces the rate of decline of lung function, which has led to the recent recommendation by the European Medicines Agency that pirfenidone be licensed for use in IPF in Europe. Although the mechanism of action of pirfenidone remains unclear, a few published nonclinical studies suggest that modulation of MMP activity may be involved. In a hepatic fibrosis model, the antifibrotic effect of pirfenidone was mainly due to the reduced expression of procollagen and TIMP-1, most likely through the downregulation of TGF-β1 mRNA, and of MMP-2 [54]. In mice given intratracheal lipopolysaccharide, pirfenidone reduces MMP-9 due to reduced neutrophil recruitment [55], whereas low-dose pirfenidone suppresses TGF-β1 and TIMP-1, and protects rats from lung fibrosis induced by bleomycin, but has no effect on the expression of MMP-13 [56].

METALLOPROTEINASES AS BIOMARKERS IN IPF

The findings of increased levels of metalloproteinases in IPF lungs and reported relationships to severity of lung function decline or progressive disease on BALF has led to interest in whether there is potential for MMPs as a biomarker panel for use in IPF. A BALF biomarker would have limited use given the logistics of bronchoalveolar lavage, variable dilution effects and wide biological variability in individual MMP levels.

Recently, a panel of 49 plasma proteins was measured in the plasma of IPF patients to define a five-protein signature that distinguished patients from controls. MMP-7 and MMP-1, the two plasma proteins whose levels were most increased in patients with IPF compared with controls, were key components of this signature (MMP-7, MMP-1, MMP-8, IGFBP1 and TNF receptor superfamily member 1A). The panel was sufficient to distinguish patients from controls with a sensitivity of 98.6%. These results were further verified in an independent validation cohort of patients with IPF, familial pulmonary fibrosis and subclinical ILD, and control individuals [57]. Given that these plasma proteins can be measured in a single Luminex assay, this panel of markers has some potential, especially as levels were elevated in patients with subclinical disease identified by high-resolution computed tomography. However, the relationship between this panel and disease progression was not reported. What we need in clinical practice is more a biomarker of disease activity than a diagnostic panel.

METALLOPROTEINASES AS THERAPEUTIC TARGETS IN IPF

The upregulation of MMPs in cancer and inflammatory diseases has made them attractive targets for drug development over the last 20 years. Can we expect MMP inhibitors to be effective in patients with IPF where excessive collagen deposition is a characteristic pathological finding?

The increasing recognition of the complexity of the biological functions of metalloproteinases in terms of wound repair, angiogenesis, and their effects on cytokine, chemokine and growth factor release suggest that there is potential for inhibition to modulate the aberrant alveolar remodelling seen in IPF. Bleomycin-induced pulmonary fibrosis is attenuated by the nonspecific MMP inhibitor actions of the antibiotic doxycycline [58]. This action is associated with reduced pulmonary inflammation and decreased MMP activity in BALF [59]. Furthermore, a small, open-label study of doxycycline therapy in seven IPF patients did not document any fall in forced vital capacity despite >17 months of daily doxycycline usage. Such a lack of progression would be unusual in a typical IPF patient cohort, but the quality of the study does not allow any conclusions to be drawn about the efficacy of doxycycline therapy in IPF patients [60].

Enthusiasm for metalloproteinase inhibition must also be tempered by the failure of early trials using broad-spectrum inhibitors in cancer, as well as concerns over the potential adverse effects that therapeutic reduction of collagen degradation may have in a fibrosing disease. Ideally, MMP inhibitors for use in IPF would have specificity for individual MMPs. The challenge for researchers in this area is, therefore, to identify whether any individual MMP is a key mechanistic driver of IPF. Such work is hindered by reliance on models, such as bleomycin-induced injury, which fail to properly model human disease [61].

CONCLUDING REMARKS

IPF is a devastating disease, and current/emerging therapy is unsatisfactory due to toxicity and limited efficacy. Current theories of the pathogenesis of IPF suggest that alveolar epithelial injury provokes the migration and proliferation of mesenchymal cells with fibroblast focus formation. Pathologically, this results in areas of exaggerated collagen deposition in some parts of the lung, with the loss of epithelial structures and honeycomb formation. Despite the progressive scarring that is seen, evidence has emerged that there is augmented production of metalloproteinases. The roles of these enzymes are currently unclear, as they have pleiotropic effects upon both the extracellular matrix and in the processing of chemokines, cytokines and growth factors. It is possible that upregulated matrix degradation is, therefore, a mechanistic driver of progressive fibrosis in IPF. Research in this area is hindered by the lack of good animal models of IPF but a better understanding of the pathophysiology of IPF and collagen turnover should identify novel therapeutic targets for this devastating disease.

STATEMENT OF INTEREST

A statement of interest for D.R. Thickett can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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