

Role of matrix metalloproteinases in the development of airway inflammation and remodeling

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Abstract

Matrix metalloproteinases (MMPs) are a major group of proteases known to regulate extracellular matrix (ECM) turnover and so they have been suggested to be important in the process of lung disease associated with tissue remodeling. This has led to the concept that modulation of airway remodeling including excessive proteolysis damage to the tissue may be of interest for future treatment. Within the MMP family, macrophage elastase (MMP-12) is able to degrade ECM components such as elastin and is involved in tissue remodeling processes in chronic obstructive pulmonary disease including emphysema. Pulmonary fibrosis has an aggressive course and is usually fatal within an average of 3 to 6 years after the onset of symptoms. Pulmonary fibrosis is associated with deposition of ECM components in the lung interstitium. The excessive airway remodeling as a result of an imbalance in the equilibrium of the normal processes of synthesis and degradation of ECM components could justify anti-protease treatments. Indeed, the correlation of the differences in hydroxyproline levels in the lungs of bleomycin-treated mice strongly suggests that a reduced molar pro-MMP-9/TIMP-1 ratio in bronchoalveolar lavage fluid is associated with collagen deposition, beginning as early as the inflammatory events at day 1 after bleomycin administration. Finally, these observations emphasize that effective treatment of these disorders must be started early during the natural history of the disease, prior to the development of extensive lung destruction and fibrosis.

Key words

- Fibrosis
- Inflammation
- Lung
- Matrix metalloproteinase
- Tissue inhibitor of matrix metalloproteinases
- Chronic obstructive pulmonary disease

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Introduction

Matrix metalloproteinases (MMPs), also called matrixins, are zinc-dependent endopeptidases, known for their ability to cleave one or several constituents of the extracellular matrix (ECM). They contain a large family of proteases that share common structural and functional elements and are produced by

different genes. These enzymes are primarily distinguished from other classes of proteinases by their dependence on metal ions and neutral pH for activity. Zymogen forms of the MMPs (pro-MMPs) are secreted into the matrix from a large number of cell types and activation of the pro-MMPs in the local microenvironment can result in discrete alterations in tissue architecture.

Matrix metalloproteinase family

Depending on substrate specificity, amino acid similarity and identifiable sequence modules, the MMP family can be classified into distinct subclasses: collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11), membrane-type MMP (MMP-14 to MMP-25), matrilysin (MMP-7), and macrophage metalloelastase (MMP-12; Table 1) (1). Comparative sequence analysis of the MMPs has revealed the molecular characteristics of these proteins: i) signal sequence, to direct secretion from the cell, ii) N-terminal pro-domain (pro-peptide), involved in maintaining the latency of proteins, including a highly conserved sequence PRCGVPDV, iii) catalytic domain, including the consensus HexxHxxGxxH zinc-binding motif, iv) C-terminal domain (hemopexin-like domain), implicated in macromolecular substrate recognition, and v) a cysteine residue in the pro-peptide of the enzyme (pRCGVPDV sequence) that maintains the latency of the zymogen by direct coordination with the active site zinc atom of the catalytic domain, blocking the access of the catalytic site to the substrate. Interruption of this interaction by aminophenyl mercuric acetate, HgCl_2 , HOCl, oxidized glutathione, by heat treatment, or by other proteases, including MMPs, initiates a cascade of events that alters the conformation of the protein. Following opening of the cysteine-Zn bond, a series of auto-catalytic cleavages result in excision of the remainder of the pro-peptide to yield a proteolytically truncated and catalytically competent enzyme (1).

The major physiologic inhibitors of the MMPs *in vivo* are α -2 macroglobulin and the family of specific tissue inhibitor of MMPs (TIMPs), naturally occurring proteins specifically inhibiting these proteases and produced by many cell types. The TIMP family at present comprises four structurally related members, TIMP-1, -2, and -3, and

the recently discovered TIMP-4. They have relative molecular masses ranging from 22 to 30 kDa, with 40-50% sequence identity. Of these, TIMP-1 and -2 are secreted in soluble form, whereas TIMP-3 is associated with the ECM. The TIMPs bind with high affinity in a 1:1 molar ratio to the catalytic site of active MMPs, resulting in loss of proteolytic activity. Moreover, TIMP-1 and TIMP-2 can form a specific complex with pro-MMP-9 and pro-MMP-2, respectively. This interaction has been suggested to provide an extra level of regulation by potentially preventing activation. However, it has recently been shown that TIMP-2 forms a tri-molecular complex on the surface of the cell with MT1-MMP and pro-MMP-2. This ternary complex is critical for the activation of pro-MMP-2 (2).

Role of MMP in the development of airway inflammation in chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is one of the major causes of mortality and morbidity all over the world and its prevalence is still increasing (3). The major triggering factor is cigarette smoking, which accounts for 80-90% of the COPD cases. However, in the population of smokers, only 15% of the subjects develop chronic airflow limitation (4).

COPD is characterized by the presence of a partially reversible airflow obstruction. This pathology is also associated with an airway inflammatory process characterized by an accumulation of inflammatory cells such as macrophages and neutrophils. Indeed, it has been shown that cigarette smoke consistently produces an increase in neutrophil numbers in bronchoalveolar lavage (BAL) fluid and in tissue (5,6). Macrophage numbers are also elevated in the lungs of smokers and of patients with COPD where they accumulate in the alveoli, bronchioli and small airways. Furthermore, there is a

Table 1. The family of matrix metalloproteinases and tissue inhibitors of metalloproteinases.

Subgroup	MMP	Enzyme	Molecular mass (kDa)		Known substrate(s)
			Latent	Active	
Collagenases	MMP-1	Interstitial collagenase-1	55	45	Collagens I, II, III, VII, VIII; gelatin; aggrecan; versican; proteoglycan link protein; casein; α -proteinase inhibitor; α -macroglobulin; ovostatin; nidogen; pro-TNF; L-selectin; MMP-2; MMP-9
	MMP-18	Interstitial collagenase-4 (<i>Xenopus</i>)	53	?	
	MMP-8	Neutrophil collagenase	75	58	Collagens I, II, III, V, VII, VIII, X; gelatin; aggrecan; α -proteinase inhibitor; fibronectin
	MMP-13	Collagenase-3	60	48	
	MMP-15	<i>Xenopus</i> collagenase	55	?	Collagens I, II, III, IV; gelatin; aggrecan; perlecan; tenascin
	MMP-21	Collagenase-4	72	?	(see MMP-24)
Stromelysins	MMP-3	Stromelysin-1	57	45	Collagens III, IV, IX, X; gelatin; aggrecan; versican; perlecan; fibronectin; laminin; elastin; casein; fibrinogen, α -macroglobulin; ovostatin; pro-TNF; MMP-1, 7, 8, 9, 13
	MMP-10	Stromelysin-2	57	44	Collagens III, IV, V; gelatin; casein; aggrecan; elastin; fibronectin; MMP-1, 8; α -proteinase inhibitor
	MMP-19	RASI or MMP-18 (human)	57	?	Aggrecan and cartilage oligomeric matrix protein; amelogenin
	MMP-20	Enamelysin	54	?	Amelogenin; enamel matrix; aggrecan
Gelatinases	MMP-2	Gelatinase A	72	66	Collagens I, IV, V, VII, X, XI, XIV; gelatin; elastin; fibronectin; aggrecan; perlecan; versican; β -amyloid; pro-TNF; α -proteinase inhibitor; MMP-9, 13
	MMP-9	Gelatinase B	92	86	Collagens IV, V, VII, X, XIV; gelatin; elastin; aggrecan; versican; fibronectin; nidogen; α -proteinase inhibitor; pro-TNF
Elastases	MMP-7	Matrilysin (PUMP-1)	28	19	Collagens I, II, III; gelatin; aggrecan; fibronectin; laminin; entactin; elastin; casein; transferrin; α -proteinase inhibitor; pro-TNF; MMP-1, 2, 9
	MMP-26	Matrilysin-2 or endometase	30	?	Collagen type IV; fibronectin; fibrinogen; beta-casein; type I gelatin; α -1 proteinase inhibitor. Is also able to activate pro-gelatinase B
	MMP-12	Macrophage metallo-elastase	54	45/22	Collagen IV; gelatin; elastin; α -proteinase inhibitor; fibronectin; vitronectin; laminin; pro-TNF
cAMP metalloproteinase ?	MMP-23		44	?	?
	MMP-28	Epilysin	59	?	Casein
Membrane type MMPs	MMP-14	MT1-MMP	66	56	Collagens I, II, III; gelatin; casein; elastin; fibronectin; laminin β chain; vitronectin; aggrecan; dermatan sulfate proteoglycan; MMP-2, 13; pro-TNF
	MMP-15	MT2-MMP	72	?	
	MMP-16	MT3-MMP	64	52	MMP-2; gelatin; fibronectin; tenascin; nidogen; laminin
	MMP-17	MT4-MMP	67	?	MMP-2
	MMP-24	MT5-MMP or MMP-21	70	?	Fibrin; pro-TNF; activation of pro-gelatinase A. Does not hydrolyze collagen types I, II, III, IV, and V, gelatin, fibronectin, laminin, decorin nor α -1-antitrypsin
	MMP-25	MT-MMP-6 or leukolysin	63	?	Specifically activates pro-gelatinase A and proteoglycans, such as dermatan sulfate and chondroitin sulfate proteoglycans. Cleaves partially fibronectin, but not collagen type I, nor laminin. Activation of pro-gelatinase A
Tissue inhibitor of MMPs	TIMP-1		28.5 (glycosylated)		All MMPs except MMP-14, MMP-19. Binds to pro-MMP-9
	TIMP-2		21 (unglycosylated)		All MMPs. Binds to pro-MMP-2
	TIMP-3		27 (glycosylated)		All MMPs. Binds to pro-MMP-2 and pro-MMP-9
	TIMP-4		24 (unglycosylated)		
			23 (unglycosylated)		MMP-1, 2, 3, 7, 9. Binds to pro-MMP-2

MMP = matrix metalloproteinase; TIMP = specific tissue inhibitor of MMP; TNF = tumor necrosis factor.

positive correlation between macrophage number in the alveolar walls and the mild-to-moderate emphysema status of patients with COPD (7). It is generally believed that the development of emphysema reflects a relative excess of cell-derived proteases that degrade the connective tissue of the lung and a relative paucity of anti-proteolytic defenses. This theory is often referred to as the “protease-antiprotease imbalance” hypothesis (Figure 1) and involves mainly serine proteases such as neutrophil elastase and MMPs.

There is increasing evidence for a role of MMPs in COPD (8). In patients with emphysema, there is an increase in BAL concentration and macrophage expression of MMP-1 (collagenase) and MMP-9 (gelatinase B) (9,10). There is an increase in MMP-9 activity in the lung parenchyma of patients with emphysema (11), and this is correlated with FEV1 (12). Alveolar macrophages from normal smokers express more MMP-9 than those from normal subjects (13), and there is an ever greater increase in cells in patients

with COPD (14), which greatly enhances elastolytic activity (15). Indeed, it was shown that MMPs account for most of the elastolytic activity released by alveolar macrophages of COPD patients over prolonged periods of time (15). MMP-9 and the MMP-9/TIMP-1 ratio are increased in induced sputum of patients with COPD (16). MMP-8 and MMP-9 do not only act as secreted enzymes, but are also bound to cells where they exert elastolytic activity.

MMP-12 is a 54-kDa proenzyme that is processed into a 45-kDa and then a 22-kDa active form. The human gene, which is designated human macrophage metalloelastase, produces a 1.8-kb transcript encoding a 470-amino acid protein that is 64% identical to the mouse protein. Both mRNA and the protein were detected in alveolar macrophages. As is the case for the mouse, the predicted human 54-kDa protein is processed by loss of both N- and C-terminal residues to a 22-kDa mature form (17).

MMP-12 seems to play a predominant role in the pathogenesis of chronic lung injury and particularly in emphysema. Indeed, MMP-12 is able to degrade different substrates, among them elastin (18). Elastin represents about 2.5% (weight/weight) of the dry weight of the lung and is distributed widely throughout the lungs (19). This protein is vital for the elastic recoil of the small airways and their ability to resist negative pressure collapse. In emphysema, the elastin content of the lung parenchyma is decreased and, ultrastructurally, elastic fibers are disorganized and probably nonfunctional (20). Moreover, elastin degradation products, such as desmosine, are increased in the urine of subjects with COPD (21) and correlate with the rate of lung function decline (22). *In vitro* studies on alveolar macrophages collected from COPD patients have shown their ability to degrade more elastin than macrophages collected from healthy volunteers (15). Using immunocytochemistry, we have observed MMP-12-positive macrophages in

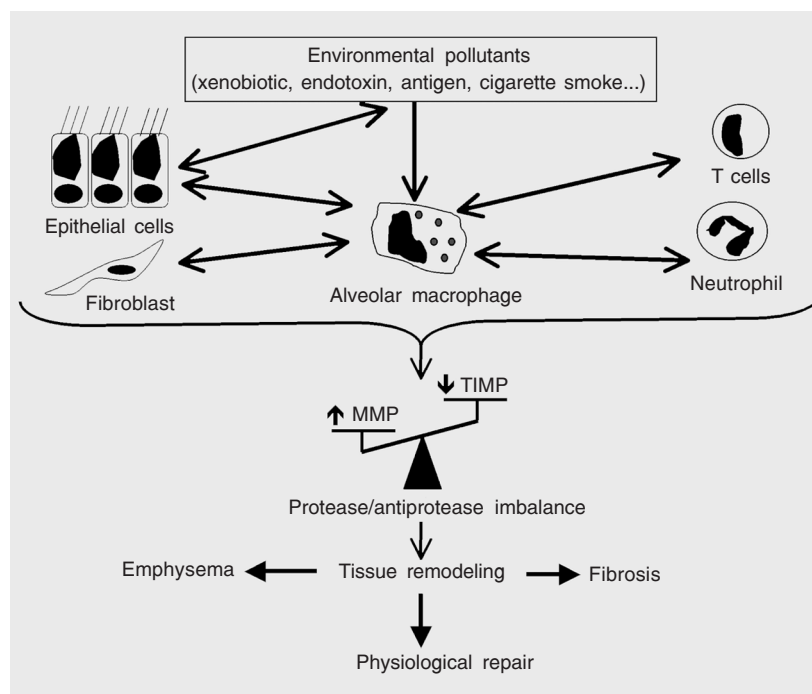


Figure 1. Role of inflammatory cells in the MMP/TIMP imbalance hypothesis and tissue remodeling. MMP = matrix metalloproteinase; TIMP = specific tissue inhibitor of MMPs.

both COPD and control samples. However, the number of MMP-12-expressing macrophages and staining intensity were higher in BAL samples from COPD patients than in control subjects. Similar results were observed in bronchial biopsies with higher MMP-12 expression in COPD subjects and in controls. Enhanced MMP-12 activity was also detected in BAL fluids from patients with COPD in comparison with control subjects. The study in question demonstrated that COPD patients produce greater quantities of MMP-12 than controls, which may be a critical step in the pathogenesis of COPD and emphysema (23).

Studies using MMP-12 knock-out (KO) mice have demonstrated that macrophage recruitment in lungs and emphysema induced by long-term exposure to cigarette smoke were linked to MMP-12 (24). MMP-12 KO mice were subjected to cigarette smoke over a 6-month period. In contrast to wild-type mice, MMP-12 KO mice did not have increased numbers of macrophages in their lungs and did not develop emphysema. A monthly intratracheal instillation of monocyte chemoattractant protein-1 into the lungs of MMP-12 smoke-exposed KO mice caused an increase in macrophage recruitment. However, despite the presence of the macrophages, these MMP-12 KO mice did not develop air space enlargement in response to smoke exposure. These data suggest that MMP-12 is probably sufficient for the development of emphysema that results from chronic inhalation of cigarette smoke (24). More recently, Selman et al. (25) showed that the use of a synthetic MMP inhibitor reduced the inflammatory response and the emphysematous-derived lesions mainly at 2 months in a cigarette smoke-guinea pig model.

The macrophage recruitment observed in response to cigarette smoke could be linked to the elastolytic properties of MMP-12. Indeed, MMP-12 generates elastin-derived peptides and experiments performed in modi-

fied Boyden chambers have shown that these elastin-derived peptides are chemotactic for monocytes (26). In a more recent study, it was reported that inflammatory lesions in the lungs of mice contained significantly more MMP-12 in macrophages at 10, 20, and 30 days of cigarette smoke exposure than in control mice exposed to 60 days (27). These results suggest that elastin degradation took place during the development of pulmonary change in mice exposed to cigarette smoke and activation of elastin-specific MMPs may be a determining factor for susceptibility to emphysema (27).

In an acute model of smoke exposure, neutrophils, desmosine and hydroxyproline, markers for elastin and collagen breakdown, respectively, were examined in BAL fluids from MMP-12 KO mice and wild-type mice 24 h after smoke exposure. None of these markers could be detected in MMP-12 KO mice, suggesting that acute smoke-induced connective tissue breakdown, the initial step towards emphysema, requires both neutrophils and MMP-12 and that the neutrophil influx is dependent on the presence of MMP-12 (28). We recently demonstrated that MMP-12 KO mice have a reduced airway inflammatory reaction following exposure to two cigarettes twice a day for 2 days. Moreover, a phosphodiesterase type 4 (PDE4) inhibitor, cilomilast, was able to block the neutrophilia induced by cigarette smoke whereas corticosteroid treatment with dexamethasone was ineffective (29).

The direct effect of MMP-12 on the development of the inflammatory process in mouse airways has been evaluated using a recombinant form of human MMP-12 (rhMMP-12). A single instillation of rhMMP-12 into the mouse airways elicited an intense inflammatory response characterized by the development of two successive phases. Indeed, rhMMP-12 induced an acute, severe and specific recruitment of neutrophils reaching a peak at 18 h, while the number of macrophages remained stable throughout this

period (30). The non-selective MMP inhibitor, marimastat, dexamethasone and the PDE4 inhibitor, rolipram, administered orally 1 h before rhMMP-12 instillation, were able to significantly decrease neutrophil recruitment at 4 and 24 h. However, only marimastat was effective regarding macrophage recruitment at day 7. In BAL fluids, marimastat, dexamethasone and rolipram significantly decreased IL-6, KC (IL-8), macrophage inflammatory protein-1 α , and MMP-9 levels. Recent results suggest that resident alveolar

macrophages and recruited neutrophils do not play a role in the delayed macrophage recruitment induced by rhMMP-12 (Nénan S, Bertrand C and Lagente V, unpublished data). Because of its ability to induce an inflammatory response and tissue remodeling, it may be possible to consider MMP-12 as an essential component of the process leading to the development of the disease (Figure 2). Finally, the development of selective MMP inhibitors would provide some answers and may be useful for the determination of new therapeutic opportunities.

Role of MMP in the development of pulmonary fibrosis

Pulmonary fibrosis is a severe and crippling disease with a poor prognosis. Its main histological features include alveolar septal lesions, abnormal reepithelialization, fibroblast proliferation and excessive deposition of ECM macromolecules due to abnormal wound healing, and inflammation characterized by an influx of macrophages, neutrophils and lymphocytes. Idiopathic pulmonary fibrosis (IPF) - usual interstitial pneumonia - is the most frequent form of interstitial pneumonia of unknown etiology (31,32).

It has been assumed that IPF is the consequence of chronic inflammation (33). Nevertheless, treatment with glucocorticoids, which are the most potent anti-inflammatory drugs, did not have the expected improving effects on the development of pulmonary fibrosis (34). Thus, the links between inflammation and fibrosis have been extensively debated and need to be clarified (31,35). Transforming growth factor β is a key fibrogenic mediator, promoting ECM synthesis by fibroblasts, and down-regulating collagen degradation through MMPs and TIMP (35-37).

MMPs are expressed at low levels in normal adult tissues, and their up-regulation appears to play an important role in the development of a number of pathological

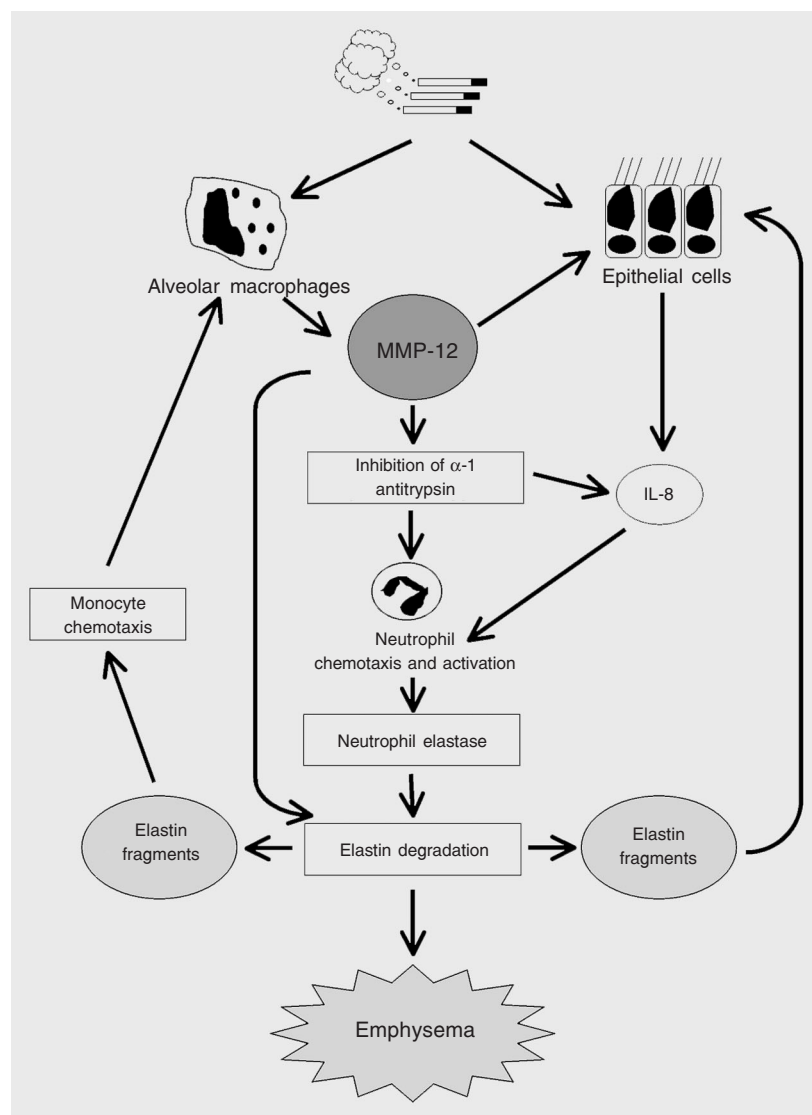


Figure 2. Role of inflammatory cells in the mechanism of action of macrophage metalloelastase (MMP-12). MMP = matrix metalloproteinase; IL-8 = interleukin 8.

processes including pulmonary fibrosis. Further studies have demonstrated the involvement of MMPs in the pathogenesis of pulmonary fibrosis. BAL fluids from individuals with sarcoidosis (38) or pulmonary fibrosis (39) contain high levels of collagenase, thought to be neutrophil-derived, a fact that has been suggested to be related to the development of fibrosis in these subjects. However, analysis of lung tissue samples from subjects with hypersensitivity pneumonitis before and after treatment with prednisone indicated that, when the collagenase levels in the lung tissue were low, the subjects developed fibrosis. In those who improved or recovered, interstitial collagenase levels were two or three times higher than in those with fibrosis (40). It has been suggested that this is the result of repression of collagenase production by fibroblasts during fibrosis (40), although increased production of TIMP and reduced activation of the latent enzyme could also account for a low collagenase activity. Fukuda et al. (41) investigated MMP activity in the lungs of patients with bronchiolitis obliterans organizing pneumonia and IPF. In bronchiolitis obliterans organizing pneumonia, predominant MMPs, mainly active MMP-2, may constitute the mechanisms of reversibility of fibrotic changes in this disease. Conversely, a TIMP-2 increase in IPF may contribute to the stable ECM deposition and the irreversible pulmonary structural remodeling. Hayashi et al. (42) have suggested the involvement of MMP-2 in collagen deposition in IPF. Moreover, Pardo et al. (43) reported that cultured fibroblasts derived from patients with IPF exhibited an increased MMPs/collagenase ratio. The involvement of MMP-2 in ECM deposition was also suggested in a model of bleomycin-induced pulmonary fibrosis in rabbits (44). It has been recently suggested that in IPF there is a higher expression of TIMPs compared with collagenases, supporting the hypothesis that no degrading fibrillar collagen microenvironment is prevailing (31). Such observa-

tions have been confirmed *in vivo* in a murine model of bleomycin-induced pulmonary fibrosis (45).

It has been reported that alveolar macrophages of patients with IPF are primed for MMP-9 expression and that MMP-9 activity secreted by macrophages tends to be normal in patients with IPF under glucocorticoid and immunosuppressive treatment (46). Although glucocorticoids appear to be efficient in inhibiting MMP activity, treatment with glucocorticoids did not appear to be correlated with clinical improvement of the patients. Lemjabbar et al. (46) concluded that MMP-9 may play a role in lung remodeling in IPF. However, the development of bleomycin-induced pulmonary fibrosis was not modified in MMP-9-deficient mice (47). Thus, this last study suggests that MMP-9 may not play an important role in the development of bleomycin-induced pulmonary fibrosis.

MMPs play a crucial role in the fibrogenic process, since it has been recently demonstrated that a selective MMP inhibitor, batimastat, markedly reduced bleomycin-induced pulmonary fibrosis in mice (48). Gelatinase A (MMP-2) and gelatinase B (MMP-9) seem to be involved in pulmonary fibrosis, but their particular roles in the process remain unclear. Whereas MMP-9 is rather released by inflammatory cells and may be linked to inflammatory process-induced tissue remodeling, MMP-2 is synthesized by structural cells including fibroblasts, endothelial and epithelial cells, and may be associated with chronically impaired tissue remodeling leading to abnormal collagen deposition. Moreover, strong evidence implies that TIMP/MMP imbalance is an important element of the fibrogenic process: TIMP-1 and, to a lesser extent, TIMP-2 are induced in bleomycin-induced pulmonary fibrosis and several investigators have proposed some events promoting a "non-degrading microenvironment" with an exaggerated TIMP/MMP ratio in lung tissue that

could lead to the fibrogenic process (31,34, 45). Besides, MMPs may be involved in the release and activation of profibrotic growth factors and cytokines and may therefore participate in the progression of pulmonary fibrosis (49).

The importance of early inflammatory components in the pathogenesis of pulmonary fibrosis has been extensively debated (35,50). Thus, arguments in favor or against the role of acute and chronic inflammation have been reported on the basis of the data of numerous studies. It has been proposed that a “fibrogenic microenvironment” including some tissue remodeling mediators interacting with the ECM could suffice to launch the fibrogenic process. It seems that the early inflammatory response is critical for the development of bleomycin-induced fibrosis. This assumption is supported by the development of pulmonary fibrosis following repeated exposure of mice to an “inflammatory stimulus” such as lipopolysaccharide (51).

Several studies have reported that antioxidant treatment attenuates the bleomycin-induced oxidative burden and subsequent pulmonary fibrosis (52-54). Moreover, the absence of extracellular superoxide dismutase exacerbates conditions that lead to inflammation and pulmonary fibrosis (55). Although these studies suggest that reactive oxygen species contribute to lung damage and fibrosis, they do not clearly indicate the mechanisms of the antioxidant effect. That is, antioxidant compounds may attenuate oxidative damage caused directly by bleomycin, or they may limit the impact of reactive oxygen species produced by phagocytes such as macrophages and neutrophils and thus interfere with the inflammatory process. We recently reported that mice deficient in the p47^{phox} subunit of the NADPH oxidase complex do not develop pulmonary fibrosis after intranasal administration of bleomycin (56). This also suggests that an imbalance of the molar MMP-9/TIMP-1 ra-

tio may influence the fibrogenic process in this model. Indeed, the MMP-9/TIMP-1 ratio did not change at day 1 after bleomycin administration in wild-type mice, but increased significantly in KO mice, leading to a “pro-fibrogenic environment” (56).

Over-expression of MMPs by lung fibroblasts has been blamed for much of the tissue destruction associated with airway inflammation. The potential involvement of MMPs in lung injury and remodeling raises the possibility of their regulation by therapeutic drugs and it was demonstrated that several MMPs appear to be down-regulated by steroids (14,57,58). Because cyclic AMP is known to regulate fibroblast proliferation, as well as cytokine and ECM protein production, we evaluated the ability of selective phosphodiesterase type 4 inhibitors to inhibit ECM degradation (59). PDE4 inhibitors induced inhibition of TNF- α -stimulated pro-MMP-2 release and prevented the increase in pro-MMP-1. These results underline a potential therapeutic benefit of selective PDE4 inhibitors in lung diseases associated with abnormal tissue remodeling.

Concluding remarks

Several studies have clearly shown that excessive levels of many MMPs are present in acute lung injury, COPD and pulmonary fibrosis associated with the inflammatory process. These results have led to the concept that modulation of airway remodeling including excessive proteolysis damage of the tissue may be of interest for future treatment. The excessive airway remodeling as a result of an imbalance in the equilibrium of the normal processes of synthesis and degradation of ECM components could be in favor of anti-proteases. Moreover, these observations emphasize that effective therapies for these disorders must be instituted early during the natural history of the disease, prior to the development of extensive lung destruction and fibrosis.

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