SERIES “MATRIX METALLOPROTEINASES IN LUNG HEALTH AND DISEASE”

Edited by J. Müller-Quernheim and O. Eickelberg
Number 5 in this Series

A therapeutic role for matrix metalloproteinase inhibitors in lung diseases?

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ABSTRACT: Disruption of the balance between matrix metalloproteinases (MMPs) and their endogenous inhibitors is considered a key event in the development of pulmonary diseases such as chronic obstructive pulmonary disease, asthma, interstitial lung diseases and lung cancer. This imbalance often results in elevated net MMP activity, making MMP inhibition an attractive therapeutic strategy. Although promising results have been obtained, the lack of selective MMP inhibitors, together with limited knowledge regarding the exact functions of a particular MMP, hampers clinical application. This review discusses the involvement of different MMPs in lung disorders and future opportunities and limitations of therapeutic MMP inhibition.

KEYWORDS: Inflammation, innate immune, matrix metalloproteinase

The family of matrix metalloproteinases (MMPs) is a protein family of zinc-dependent endopeptidases. They can be classified into subgroups based on structure (fig. 1), subcellular location and/or function [1, 2]. Although it was originally believed that they are mainly involved in extracellular matrix (ECM) cleavage, MMPs have a much wider substrate repertoire, and their specific processing of bioactive molecules is their most important *in vivo* role [3, 4]. They can degrade a wide variety of substrates, and they play crucial roles in diverse biological and, especially, pathological processes, such as wound healing, senescence, cancer, fibrosis and inflammation [5–8].

Net MMP activity is tightly regulated by both transcriptional and post-translational mechanisms. Additionally, once MMPs are released and activated, their activity is restricted by endogenous inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs) and α_2 -macroglobulin, which are present in the extracellular compartment. While MMP activity is rather limited in healthy adult lungs, several types of lung cells can release MMPs

in response to environmental stimuli, such as infectious pathogens, toxins, growth factors and cytokines. Subsequent disruption of the balance between MMPs and their endogenous inhibitors, called the protease/antiprotease imbalance, is a key event in the development of pulmonary diseases and is reflected in pulmonary architecture remodelling and in inflammation [9–11]. Consequently, several lung disorders, such as chronic obstructive pulmonary disease (COPD), asthma, interstitial lung diseases (ILDs) and lung cancer, are associated with MMP dysregulation. This observation and the extracellular localisation of most MMPs makes them appealing drug targets [12]. This article focuses on the dysregulation of different MMPs and the effect of MMP deficiency (table 1) or MMP inhibition (table 2 and fig. 2) in lung disorders. It also discusses the prospects for inhibiting these proteins therapeutically.

MMP INHIBITORS

There is increasing experimental evidence that MMP inhibition might be an effective strategy to treat respiratory diseases such as COPD, asthma,

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Received:

Feb 14 2011

Accepted after revision:

May 09 2011

First published online:

June 09 2011

Previous articles in this series: No. 1: Löffek S, Schilling O, Franzke C-W. Biological role of matrix metalloproteinases: a critical balance. *Eur Respir J* 2011; 38: 191–208. No. 2: Elkington PT, Ugarte-Gil CA, Friedland JS. Matrix metalloproteinases in tuberculosis. *Eur Respir J* 2011; 38: 456–464. No. 3: Gaggari A, Hector A, Bratcher PE, *et al.* The role of matrix metalloproteinases in cystic fibrosis lung disease. *Eur Respir J* 2011; 38: 721–727. No. 4: Davey A, McAuley DF, O’Kane CM. Matrix metalloproteinases in acute lung injury: mediators of injury and drivers of repair. *Eur Respir J* 2011; 38: 959–970.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

FIGURE 1. Schematic overview of matrix metalloproteinase (MMP) structure. MMPs share a common domain structure: the pre-domain, containing a signal peptide responsible for secretion, the pro-domain that keeps the enzyme inactive, by interaction between a cysteine residue and the Zn^{2+} ion group from the catalytic domain, and finally the haemopexin-like C-terminal domain, which is linked to the catalytic domain by a flexible hinge region. MMP7 lacks the hinge region and the haemopexin domain. MMP2 and MMP9 contain a fibronectin type II-like domain inserted into the catalytic site and membrane type MMPs have a transmembrane domain at the carboxy terminus.

ILDs and lung cancer. Among the MMP inhibition strategies under investigation are the use of TIMPs, small-molecule MMP inhibitors, blocking antibodies and anti-sense technologies, as recently reviewed [12, 78, 79].

The use of natural inhibitors, such as TIMPs, is associated with several negative aspects mainly linked to the difficulty of controlling the protease/antiprotease balance and, consequently, the net MMP activity. Moreover, TIMPs bind at a different rate and with different affinity to target MMPs. Sometimes they even promote MMP activity: TIMP2 activates proMMP2 after forming a complex with MT1-MMP [80]. Moreover, TIMPs have several MMP-independent functions. For example, TIMP2 inhibits the mitogenic response of human endothelial cells to growth factors such as vascular endothelial growth factor (VEGF)-A and fibroblast growth factor-2 *in vitro* and to angiogenesis *in vivo* [81], and TIMP3 promotes MMP-independent apoptosis [82]. Based on these observations, it is unlikely that TIMPs will ever be used therapeutically as MMP inhibitors.

Broad-range MMP inhibition can be useful to obtain maximal inhibition of ECM degradation. Examples are tetracycline and its non-antibiotic derivatives, which effectively inhibit a broad range of MMPs by direct binding of Ca^{2+} or Zn^{2+} ions in the active site [83]. However, these compounds are associated with several serious side-effects, such as musculoskeletal pain, probably caused by inhibition of the non-MMP metalloproteinases, such as adamalysins and tumour necrosis factor (TNF)- α converting enzyme [12]. The first generation synthetic MMP inhibitors were peptidic derivatives that mimicked the recognition site of natural MMP substrates such as collagen, combined with a hydroxamic acid Zn^{2+} binding group, which chelates the Zn^{2+} ion. As will be discussed below, several of these broad-spectrum MMP inhibitors significantly reduce lung damage and/or inflammation and so they have therapeutic potential. However, to avoid off-target effects, selective targeting of only that MMPs that cause the pathology is probably a more clinically appropriate approach, especially when long-term treatment is needed.

Next generation MMP inhibitors are no longer limited to substrate-like compounds and were designed with a variety of peptidomimetic and non-peptidomimetic structures. The preferred approach for obtaining more specific MMP inhibition is to avoid strong metal chelators. Therefore, the third generation of highly selective MMP inhibitors possess no zinc-binding group and exploit the deep S1' cavity present in some MMPs. So far, this has been successful especially for MMP13-specific inhibitors [84]. Monoclonal antibodies against MMPs appear to

be promising alternatives to synthetic inhibitors; an example is REGA-3G12, an antibody that selectively inhibits human MMP9 [85]. However, the difficulty of producing macromolecular proteins and the need for parenteral administration limit their therapeutic potential.

DYSREGULATION OF MMPs IN DIFFERENT LUNG DISORDERS AND ITS THERAPEUTIC IMPLICATIONS

COPD

COPD includes at least two different pathological manifestations: emphysema and chronic bronchitis. Emphysema is characterised by the permanent destruction of the alveolar septa and decreased lung plasticity, which results in gas trapping and subsequent decrease in pulmonary oxygenation. Chronic bronchitis is associated with inflamed and thickened airways, combined with increased mucus production by cells in the airways. This results in habitual cough and difficult breathing. Although the pathophysiological basis for COPD has not been fully elucidated, noxious particles, *e.g.* those present in cigarette smoke, are suspected of being the leading cause of this disease [86]. Additionally, it has been suggested that *Pneumocystis* colonisation might be a co-factor in progression of COPD [87].

MMPs can enzymatically induce morphological changes in the lung, which occur during obstructive pulmonary diseases. Several MMPs are known to be involved in the pathogenesis of COPD [88] and the key role of increased MMP expression in COPD development has been demonstrated in animal studies and preliminary studies in humans. Possible sources of MMPs during the course of COPD are lung parenchyma [89] and inflammatory cells invading the lung. For example, both neutrophils and macrophages, the predominant inflammatory cells in the lungs of COPD patients, can release several MMPs [90–92]. The use of different broad-spectrum MMP inhibitors demonstrated the importance of MMPs in COPD pathogenesis. Treatment with RS113456 resulted in inhibition of neutrophil but not macrophage infiltration after smoke induced emphysema in mice [46]. PKF242-484 dose-dependently reduced neutrophilia in BALB/C mice after cigarette smoke exposure. Strangely, PKF242-484 had no effect in C57BL/6 mice, suggesting that the role of MMPs during smoke-induced acute neutrophil inflammation is strain dependent [47]. Ilomastat also effectively attenuated the inflammation and emphysema induced by cigarette smoke in mice [48]. Treatment of guinea pigs with CP-471,474 delayed the emphysema induced by tobacco smoke [50].

MMP12 (macrophage elastase) is important during COPD pathogenesis. Genetic analysis of human COPD patients showed that the common serine variant at codon 357 of the MMP12 gene (rs652438) is associated with clinical manifestations consistent with more aggressive matrix degradation and subsequent increased disease severity [61]. In contrast, the minor allele of a single-nucleotide polymorphism in MMP12 (rs2276109) is associated with a positive effect on lung function in adult smokers and with a reduced risk of COPD [93]. Moreover, exaggerated MMP12 expression in alveolar macrophages was associated with smoking and with emphysema [94], and MMP12 was markedly increased in sputum from patients with stable COPD relative to controls [95]. An *in vivo* mouse study showed that deletion of MMP12 prevents cigarette smoke-induced inflammation, neutrophil influx and emphysema in the lung [13, 14]. This was attributed to diminished release of TNF from

TABLE 1 Phenotype of matrix metalloproteinase (MMP)-deficient mice in different lung pathologies

Lung pathology		Deficiency	Observations	[Ref.]
COPD	Cigarette smoke exposure	MMP12	Decreased inflammation, neutrophil influx and emphysema in the lung	[13, 14]
		MMP9	Normal development of cigarette smoke-induced inflammation and airspace enlargement compared	[15]
Asthma	<i>Aspergillus fumigatus</i> and OVA	MMP9	Disrupted trafficking of neutrophils and eosinophils into the BALF	[16]
	OVA		Massive accumulation of apoptotic cells in the lungs	
	German cockroach frass		Increased airway inflammation	[17]
	OVA		Increased airway inflammation	[18]
	OVA		Impaired cellular infiltration and bronchial hyperresponsiveness	[19]
	OVA		Impaired accumulation of airway wall dendritic cells	[20]
	<i>Aspergillus fumigatus</i> and OVA	MMP2	Disrupted trafficking of eosinophils into the BALF	[16, 21]
			Massive accumulation of apoptotic cells in the lungs	
			Increased susceptibility to asphyxiation	
	<i>Aspergillus fumigatus</i> and OVA	MMP9/MMP2	MMP9 is the dominant airway MMP controlling inflammatory cell egression	[16]
ILD	Cockroach antigen	MMP12	Reduced inflammatory injury	[22]
	House mite	MMP8	Airway hyperresponsiveness and decreased levels of soluble IL-13R α 2	[23]
	Bleomycin	MMP8	Less fibrosis	[24]
			Decreased IL-10 processing	
	Bleomycin	MMP12	No effect	[25]
	Fas-activating monoclonal antibody		Decreased expression of profibrotic genes	[26]
	Bleomycin	MMP9	Reduced alveolar bronchiolisation	[27]
	Bleomycin	MMP7	Impaired efflux of neutrophils into the alveolar space	[28]
			Reduced lethality	
	Bleomycin		Decreased alveolar fibrosis at later stages	
ALI	High-pressure ventilation	MMP8	Reduced fibrosis	[29]
			Decreased lung oedema and permeability	[30]
			Diminished histological injury	
	Mechanical ventilation		Increased alveolar permeability	[31]
	LPS		Accumulation of PMN cells in alveolar space	[32]
	Bleomycin		Accumulation of PMN cells in alveolar space	[33]
			Increased alveolar capillary barrier injury	
			Increased lung elastance	
			Increased mortality	
	LPS	MMP9	No protection	[34]
Lung cancer			Development of emphysema	
	Ozone		Increased protein content and numbers of neutrophils and epithelial cells in BALF	[35]
			Increased KC and MIP2 levels in BALF	
	Ozone	MMP7	No effect	[35]
	Hyperoxia	MMP13	Increased inflammation because of the lack of MCP1 cleavage	[36]
	Immunoglobulin G immune complex	MMP3	Reduced lung injury	[37]
			Reduction in leukocyte recruitment	
	MMTV-PyMT	MMP13	No effect on development of lung metastasis	[38]
	B16-BL6 melanoma	MMP9	Reduced number of metastatic colonies	[39]
	Lewis lung carcinoma			
BOS	MMTV-PyVT		Decreased angiogenesis	[40]
			Decrease in lung tumour burden	
	MMTV-PyVT	MMP7	No effect on development of lung metastases	[40]
	B16-BL6 melanoma	MMP2	Reduced tumour-induced angiogenesis	[41]
	Lewis lung carcinoma			
	Lewis lung carcinoma	MMP12	Increased number of pulmonary metastases	[42]
	Heterotopic airway transplant model	MMP8	Reduced neutrophil influx and collagen deposition	[43]
	Heterotopic murine tracheal allografts	MMP9	Reduced neutrophil influx and collagen deposition	[44]
CLD	Pulmonary overexpression of human mature IL-1 β	MMP9	Increased IL-1 β -induced lung injury	[45]

COPD: chronic obstructive pulmonary disease; ILD: interstitial lung diseases; ALI: acute lung injury; BOS: bronchiolitis obliterans syndrome; CLD: chronic lung disease of prematurity; OVA: ovalbumin; BALF: bronchoalveolar lavage fluid; IL: interleukin; LPS: lipopolysaccharide; PMN: polymorphonuclear; KC: keratinocyte chemoattractant; MIP2: macrophage inflammatory protein-2; MCP1: monocyte chemoattractant protein-1; MMTV-PyMT, MMTV-PyVT: mouse mammary tumour virus with polyoma middle T-antigen promoter, mouse mammary tumour virus.

TABLE 2 Effect of matrix metalloproteinase (MMP) inhibitors in different lung pathologies

Lung pathology		MMP inhibitor	Species	Observations	[Ref.]
COPD	Cigarette smoke	RS113456	Mice	Reduced neutrophil but not macrophage infiltration	[46]
	Cigarette smoke	PKF242-484	Mice	Reduced neutrophilia in BALB/C mice; no effects in C57BL/6 mice	[47]
	Cigarette smoke	Ilomastat GM6001	Mice	Reduced emphysema and inflammation	[48, 49]
	Cigarette smoke	CP 471,474	Guinea pigs	Delayed emphysema	[50]
	Cigarette smoke	AZ11557272	Mice	Reduced emphysema, inflammatory cell numbers and desmosome levels in BALF and TNF serum levels, reverted smoke-induced airspace enlargement	[51]
	Cigarette smoke	AS112108	Mice	Dampened early inflammatory process and inhibited increase in neutrophil numbers	[52]
	Cigarette smoke	AS111793	Mice	Dose-dependent reduction of the increase in the number of neutrophils and macrophages in BALF and the levels of several inflammation markers	[53]
	rhMMP12	MMP408	Mice	Reduced lung inflammation	[54]
	Acrolein	Doxycycline	Mice	Attenuation of mucin synthesis	[55]
	OVA	R-94138	Mice	Reduced development of allergic airway inflammation	[56]
Asthma	<i>Dermatophagoides pteronyssinus</i>	Marimastat	Human	Reduced bronchial hyperresponsiveness	[57]
	OVA	GM6001	Mice	Dose-dependent inhibition of inflammatory cell egression with concomitant accumulation of inflammatory cells in the lung parenchyma	[21]
	TDI	MMPI-I	Mice	Decreased number of inflammatory cells in BALF	[58, 59]
	TDI	MMPI-II	Mice	Decreased number of inflammatory cells in BALF	[59]
	OVA	C ₂₇ H ₃₃ N ₃ O ₅ S	Mice	Decreased severity of eosinophilic inflammation	[60]
	<i>Ascaris suum</i>	MMP408	Sheep	Inhibited early and late airway responses	[61, 62]
	OVA	S-1 (N-sulfonylamino acid derivative)	Mice	Inhibited increase in eosinophils in the BALF	[63]
	OVA	C ₁₇ H ₁₈ N ₂ O ₅ S	Mice	Reduced recruitment of Th cells and decreased severity of eosinophilic inflammation	[60]
	OVA	TIMP1	Mice	Reduced development of allergic asthma	[56]
	OVA	TIMP2	Mice	Reduced development of allergic asthma	[56]
ILD	Bleomycin	Batimastat	Mice	Reduced development of fibrosis	[64]
ALI	I/R	Ilomastat	Mice	Attenuated lung inflammation and injury	[65]
	Oleic acid	Ilomastat	Mice	Attenuated lung inflammation and injury	[65]
	I/R	ONO-4817	Mice	Attenuated lung injury	[66]
	LPS	COL-3	Yorkshire pigs	Attenuated lung injury	[67]
	High-pressure ventilation	Phosphonate derivative	Mice	Better gas exchange	[30]
				Decreased lung oedema and permeability	
				Diminished histological damage	
Lung cancer	Ventilation	CMT-3	Mice	Reduced neutrophilic inflammation	[68]
	Pancreatitis	Doxycycline	Mice	Reduced lung injury	[69]
	B16-F10 melanoma cells	MMI270	Mice	Decreased number of lung colonies	[70]
	NSCLC	BMS-275291	Human	No improvement in survival	[71]
	N-Nitrosodimethylamine	BAY 12-9566	Mice	Reduced neoplastic growth and development	[72]
	MMTV-PyMT	GM6001	Mice	Anti-metastatic effects	[73]
	MMTV-PyVT	SB-3CT	Mice	Huge decrease in lung tumour burden	[40]
	A549 cells	Ad-MMP2-si	Mice	Decreased tumour growth	[74]
	Lewis lung carcinoma	CH1104I	Mice	Inhibited pulmonary metastasis	[75]
BOS	Lung I/R	COL-3	Rats	Improved oxygenation	[76]
				Diminished polymorphonuclear leukocyte influx into the isograft	
	Lung transplantation		Rats	Suppression of delayed type hypersensitivity responses	[77]
				Abrogated local cytokine production	
				No prevention of rejection pathology	

COPD: chronic obstructive pulmonary disease; ILD: interstitial lung diseases; ALI: acute lung injury; BOS: bronchiolitis obliterans syndrome; BALF: bronchoalveolar lavage fluid; TNF: tumour necrosis factor; OVA: ovalbumin; TDI: toluene diisocyanate-induced; Th: T-helper; TIMP: tissue inhibitor of metalloproteinases; I/R: ischaemia/reperfusion; LPS: lipopolysaccharide; CMT-3, COL-3: chemically modified tetracycline 3; NSCLC: nonsmall cell lung carcinoma; MMTV-PyMT, MMTV-PyVT: mouse mammary tumour virus with polyoma middle T-antigen promoter, mouse mammary tumour virus; Ad-MMP2-si: adenoviral MMP2 siRNA.

macrophages, which normally accounts for endothelial activation, neutrophil influx and proteolytic matrix breakdown caused by neutrophil-derived proteases during COPD [96]. Furthermore, interferon- γ , a prominent product of CD8-positive cells, causes emphysema with alveolar enlargement, increased lung volume, enhanced pulmonary compliance, and macrophage- and neutrophil-rich inflammation; this occurs at least in part by the induction and activation of MMP12 [97]. Increased MMP12 activity leads to degradation of elastin, and the resultant monocyte chemotactic fragments further contribute to recruitment of eosinophils and macrophages [98] and to amplification of the inflammatory cascade [42]. Guinea pigs exposed daily to cigarette smoke for up to 6 months were protected from emphysema by the dual MMP9/MMP12 inhibitor, AZ11557272 [51]. This was reflected in a reduction of inflammatory cell numbers and desmosine levels in bronchoalveolar lavage (BAL) fluid and TNF levels in serum relative to non-treated mice. Also, AZ11557272 treatment reverted smoke-induced airspace enlargement by about 70%, demonstrating that MMP9 and/or MMP12 are potential targets for therapeutic intervention in COPD. AS112108, another dual inhibitor for MMP9 and MMP12, dampened the early inflammatory process in an experimental model of COPD, as reflected by inhibition of the increase in neutrophil numbers [52]. Similarly, the selective MMP12 inhibitor AS111793 dose-dependently limited the increase in the number of neutrophils and macrophages in BAL fluid and the levels of several inflammation markers after cigarette smoke exposure [53]. In contrast, lung inflammation elicited by lipopolysaccharide (LPS) challenge was not prevented [14]. Another selective MMP12 inhibitor, MMP408, blocked rhMMP12-induced lung inflammation in a mouse model [54].

Although elastase has been proposed to be the primary enzyme responsible for emphysematous lung damage, smoke-induced emphysema in guinea pigs was also associated with morphometric evidence of collagen breakdown and repair [99]. Furthermore, analysis of BAL fluid from patients with emphysema suggested that collagenase activity might be a better indicator of the presence of emphysema than elastase [91]. Analysis of COPD lung tissue revealed upregulation of the collagenases MMP1 and MMP8, but not MMP13 [100]. Moreover, transgenic mice expressing the human MMP1 collagenase displayed disruption of the alveolar walls and coalescence of the alveolar spaces [101] caused by the selective degradation of type III collagen within the alveolar wall [102]. This finding points to elastin-independent induction of emphysema. Indeed, MMP1 was shown to be present in type II pneumocytes in patients with emphysema but not in normal control subjects or smokers without emphysema [103]. Neutrophil-derived MMP8 levels increased remarkably in patients with COPD exacerbation compared with stable COPD patients and healthy controls [104]. Moreover, MMP8 might be used to differentiate healthy from symptomatic smokers, who have a high probability of developing COPD [105].

Patients with COPD also displayed an elevated gelatinolytic activity in sputum, and this elevation was linked to increased MMP9 levels and the induction and activation of the MMP2/MMP14/TIMP2 complex [106]. In contrast, acute exposure to tobacco smoke did not cause any substantial change in gelatinases; this finding is compatible with the many years needed for tobacco smoking to establish COPD [107]. ZHENG *et al.* [49] detected both

MMP2 and MMP9 induction during COPD pathogenesis and, by using the broad-spectrum MMP antagonist GM6001, they showed that these MMPs were important in emphysema and inflammation, but not in mucus formation. Additionally, MMP9 also plays a role in inhibiting neutrophil accumulation [98]. Sputum analysis revealed that MMP9 was expressed in both alveolar macrophages and neutrophils and that its activity correlates with the extent of airflow limitation [108]. Furthermore, an imbalance between MMP9 and TIMP1, caused by exogenous interleukin (IL)-10 [92], was associated with ECM remodelling and airflow obstruction [109]. Apparently, acrolein, a component of cigarette smoke that can also be endogenously generated in the airways of COPD patients, initiates cleavage of pro-MMP9 and activation of epidermal growth factor receptor/mitogen-activated protein kinase signalling, which leads to formation of more MMP9 [110]. MMP9 in sputum remained elevated 6 months after stopping smoking, and this sustained elevation might contribute to the continuous lung damage typical of COPD [111]. Doxycycline, a tetracycline antibiotic that inhibits MMPs, attenuated acrolein-induced mucin synthesis, in part by inhibiting expression of MMP9 [55]. Despite these encouraging data, a recent study using MMP9 knockout mice and human samples suggests that specific inhibition of MMP9 is unlikely to be an effective therapy against cigarette smoke-induced emphysema [15].

Finally, other MMPs were suggested to be important in COPD pathogenesis, such as a member of the membrane-type MMP (MT-MMP) subfamily, MMP14, which is induced by acrolein and subsequently leads to increased mucin production in COPD [112].

Asthma

Asthma, one of the most common chronic inflammatory diseases, affects about 300 million people worldwide [113]. It is a chronic inflammatory disease of the airways characterised by increases in epithelial mucin stores, caused by surface epithelial mucus metaplasia with modest hyperplasia and increased numbers of subepithelial bronchial microvessels that become leaky during inflammation. Changes in submucosal glands are not prominent, except in severe disease [114]. Although the available treatments, *i.e.* combinations of inhaled corticosteroids and long-acting β_2 -agonists, are effective for most asthmatic individuals, 5–10% of patients have severe disease that responds poorly [115]. MMPs might contribute to functional changes and the loss of airway–parenchyma interdependence observed in patients with fatal asthma, because MMP expression is increased at the outer region of the small airways and in the peribronchiolar parenchyma in asthmatic patients [116]. Moreover, the broad-spectrum MMP inhibitor R-94138 reduced the development of allergic airway inflammation in mice [56] and marimastat (another broad-spectrum MMP inhibitor) reduced bronchial hyperresponsiveness to inhaled allergen in atopic asthmatic subjects [57]. Although administration of GM6001 did not alter the ovalbumin (OVA)-induced asthma phenotype in mice, there was a dose-dependent inhibition of inflammatory cell egression with concomitant accumulation of inflammatory cells in the lung parenchyma [21]. Similarly, in a murine model of toluene diisocyanate-induced occupational asthma (TDI-OA), the broad-spectrum MMP inhibitors MMPI-I and MMPI-II decreased the number of inflammatory cells in BAL fluids [58, 59].

MMP9 was the first MMP to be studied in depth for its involvement in asthma pathology. Asthma patients have increased MMP9 levels in sputum, BAL fluid and serum [106, 117–119]. MMP9 was also shown to be elevated during spontaneous asthma exacerbations [120], in cases of TDI-OA among exposed workers [121], and in patients with nocturnal asthma [122]. Moreover, MMP9 immunoreactivity, identified in endobronchial biopsy specimens from all the tested asthmatic patients [123], was correlated with asthma severity [124] and with the number of macrophages and neutrophils [125]. Analysis of asthmatic human respiratory epithelia exposed to cigarette smoke indicated that asthmatics exposed to cigarette smoke may be more susceptible to MMP9-mediated airway remodelling [126]. Allergen challenge of MMP9-deficient mice with *Aspergillus fumigatus* [16], OVA [17] or German cockroach frass [18] resulted in heightened airway inflammation relative to wild-type mice, indicating a protective role for MMP9 in asthma, probably *via* its influence on efflux of inflammatory cells. Interestingly, another study reported reduced airway inflammation in MMP9-deficient animals sensitised to and exposed to OVA [19]. This reduction was linked to dendritic cell recruitment [20]. Similarly, systemic administration of an MMP9 selective inhibitor reduced T-helper (Th) cell recruitment to the airways after antigen challenge, and this decreased the severity of eosinophilic inflammation [60]. These contradictory results are difficult to interpret; a possible explanation is the variation in antigen and/or administration protocol. Also, the studied end-points are different; for example, while some studies focus on the lung tissue, others focus on the BAL fluid. Anyway, it is clear that MMP9 plays an important role in inflammatory cell migration during asthma. Whether this role is protective or sensitising is still under debate and needs further research.

MMP2 was also increased in sputum of asthmatic patients [106]. Like MMP9, MMP2 dampens inflammation by promoting the egress of inflammatory cells into the airway lumen. Consequently, lack of MMP2 results in a robust asthma phenotype and increased susceptibility to asphyxiation induced by allergens, but a reduced influx of cells into the BAL fluid [21]. However, studies with MMP9 and MMP2 double knockout mice revealed that MMP9, and not MMP2, is the dominant airway MMP controlling inflammatory cell egression [16].

MMP12-mediated pathologic degradation of the ECM is associated not only with COPD, but also with asthma. The MMP12 gene variant (rs652438) results in more aggressive matrix degradation and was linked to increased asthma severity [61], while the rs227610 variant was associated with a positive effect on lung function in asthmatic children [93]. In a model of allergic airway inflammation induced by cockroach antigen, MMP12-deficient mice showed significant reduction in inflammatory injury [22]. Oral delivery of a potent and selective MMP12 inhibitor led to significant inhibition of both early and late airway responses to allergen challenge in an *Ascaris suum*-sensitised sheep asthma model [61, 62]. Similarly, the MMP12 selective inhibitor S-1, an N-sulfonylamino acid derivative, inhibited the increase in eosinophils in the BAL fluid after antigen challenge in a mouse model of allergic airway inflammation [63].

MMP8 seems to play a protective role in asthma, since its deficiency promotes allergen-induced airway inflammation,

mainly by delaying clearance of recruited neutrophils [127]. Additionally, following house mite challenge, MMP8-deficient mice displayed airway hyperresponsiveness and decreased levels of soluble IL-13R α 2, the decoy receptor for the central mediator of asthma IL-13 [23]. In contrast, systemic administration of an MMP8-selective inhibitor reduced recruitment of Th cells to the airways after antigen challenge and thereby resulted in decreased severity of eosinophilic inflammation [60]. However, it cannot be excluded that the effects observed with the MMP8 specific inhibitor are dependent on other MMPs, as no information on inhibition constant (K_i) or half maximal inhibitory concentration values is currently available (fig. 2).

Other MMPs suggested to be involved in asthma are MMP1, 3, 7 and 19. MMP1-mediated insulin-like growth factor binding protein proteolysis was shown to induce airway smooth muscle hyperplasia and airway obstruction by modulating the insulin-like growth factor axis [128]. Moreover, bronchial lavages from asthmatic patients contained not only MMP9 and TIMP1, but also MMP3 [129]. MMP3, together with MMP2, is associated with increased synthesis of procollagen I in patients with stable mild-to-moderate asthma in bronchial fibroblasts, resulting in reduced lung function and airway hyperreactivity [130]. In patients with severe asthma, MMP7 levels are increased in basal epithelial cells, causing FasL cleavage and release of soluble FasL, which subsequently contributes to airway epithelial damage and inflammation [131]. Recently, GUEDETS *et al.* [132] proposed that MMP19 is another new mediator in asthma, because its deficiency in mice prevented tenascin-C accumulation, Th2-driven airway eosinophilia, and airway hyperreactivity in a murine asthma model.

Net MMP activity can also be dysregulated by TIMPs. For example TIMP1, which inhibits amongst others, MMP1, 3 and 9, is increased in sputum of asthmatic patients [106]. The importance of the MMP/TIMP balance in asthma was demonstrated in an OVA mouse asthma model by the finding that TIMP1-deficiency results in increased airway inflammation associated with enhanced MMP9 activity [133], while pulmonary administration of TIMP1 or TIMP2 reduced the development of allergic asthma, possibly by inhibiting MMP2 and MMP9 [56]. Both findings support the hypothesis that TIMP1 plays a protective role by preventing airway hyperresponsiveness and modulating inflammation, remodelling, and cytokine expression in an animal model of asthma.

Interstitial lung disease

ILD refers to a broad category of lung diseases with diverse causes affecting the interstitium. Exposure-related ILD can be caused by exposure to asbestos (and other forms of occupational exposure), tobacco or radiation or by certain drugs and antigens, including bacteria and fungi (called hypersensitivity pneumonitis). ILDs are also often associated with systemic diseases, such as rheumatoid arthritis and systemic lupus erythematosus. Examples of systemic-associated ILDs are connective tissue disease and sarcoidosis. But the causes of several ILDs are not known, *e.g.* idiopathic interstitial pneumonia, idiopathic pulmonary fibrosis (IPF), nonspecific interstitial pneumonia, cryptogenic organising pneumonia and lymphocytic interstitial pneumonia. Finally, some ILDs have a distinct pathology, such as lymphangioleiomyomatosis (LAM).

MMP inhibitor	Structure	MMP inhibition
RS113456		Ki MMP1 = 70 nM Ki MMP3 = 5.2 nM Ki MMP9 = 0.065 nM Ki MMP13 = 0.17 nM Ki MT-MMP1 = 0.089 nM
PKF242-484		Ki MMP1 = 3.6 nM Ki MMP2 = 0.1 nM Ki MMP3 = 0.9 nM Ki MMP9 = 1 nM Ki MMP13 = 4.5 nM
CP 471,474 C ₁₆ H ₁₆ FN ₂ O ₅ S		IC ₅₀ MMP1 = 1170 nM IC ₅₀ MMP2 = 0.7 nM IC ₅₀ MMP3 = 16 nM IC ₅₀ MMP9 = 13 nM IC ₅₀ MMP13 = 0.9 nM
AZ11557272	NA	NA; MMP9/12 specific
AS112108	NA	NA; MMP9/12 specific
AS111793#		IC ₅₀ MMP12 = 20 nM Selectivity ratio: MMP1 = 1:30 MMP2 = 1:30 MMP9 = 1:40
MMP408		IC ₅₀ MMP1 and 7 > 6 μM IC ₅₀ MMP3 = 351 nM IC ₅₀ MMP12 = 2 nM IC ₅₀ mMMP12 = 160 nM IC ₅₀ rMMP12 = 320 nM IC ₅₀ shMMP12 = 22.3 nM IC ₅₀ MMP13 = 120 nM IC ₅₀ MMP14 = 1100 nM
GM6001 Ilomastat Galardin®		IC ₅₀ MMP1 = 0.4 nM IC ₅₀ MMP2 = 0.4 nM Ki MMP3 = 27 nM Ki MMP8 = 0.1 nM Ki MMP9 = 0.2 nM IC ₅₀ MMP14 = 5.2 nM
Doxycycline		IC ₅₀ MMP1 > 400 μM IC ₅₀ MMP7 = 28 μM IC ₅₀ MMP8 = 30 μM IC ₅₀ MMP13 = 2 μM
R-94138		IC ₅₀ MMP2 = 38 nM IC ₅₀ MMP3 = 28 nM or 120 nM IC ₅₀ MMP7 = 23 μM IC ₅₀ MMP9 = 1.2 nM IC ₅₀ MMP13 = 38 nM
Marimastat BB-2516 C ₁₅ H ₂₉ N ₃ O ₅		IC ₅₀ MMP1 = 5 nM IC ₅₀ MMP2 = 6 nM IC ₅₀ MMP3 = 200 nM IC ₅₀ MMP7 = 20 nM IC ₅₀ MMP8 = 2 nM IC ₅₀ MMP9 = 3 nM IC ₅₀ MMP14 = 1.8 nM

MMP inhibitor	Structure	MMP inhibition
MMPI-I C ₂₃ H ₃₄ N ₆ O ₆		IC ₅₀ MMP1 = 1.0 μM IC ₅₀ MMP3 = 150 μM IC ₅₀ MMP8 = 1.0 μM IC ₅₀ MMP9 = 30 μM
MMPI-II MMP2/MMP9 inhibitor II C ₂₁ H ₂₀ N ₂ O ₄ S		IC ₅₀ MMP2 = 17 nM IC ₅₀ MMP9 = 30 nM
MMP9 inhibitor I C ₂₇ H ₃₃ N ₃ O ₅ S		IC ₅₀ MMP1 = 1.05 nM IC ₅₀ MMP9 = 5 nM IC ₅₀ MMP13 = 113 nM
MMP8 inhibitor I C ₁₇ H ₁₈ N ₂ O ₅ S		IC ₅₀ MMP8 = 4 nM
Batimastat BB-94		IC ₅₀ MMP1 = 3 nM IC ₅₀ MMP2 = 4 nM IC ₅₀ MMP3 = 20 nM IC ₅₀ MMP7 = 6 nM IC ₅₀ MMP8 = 10 nM IC ₅₀ MMP9 = 1 nM
ONO-4817 C ₂₂ H ₂₈ N ₂ O ₆		IC ₅₀ MMP1 = 1600 nM Ki MMP2 = 0.73 nM Ki MMP3 = 42 nM Ki MMP7 = 2500 nM Ki MMP8 = 1.1 nM IC ₅₀ MMP9 = 2.1 nM Ki MMP12 = 0.45 nM Ki MMP13 = 1.1 nM
COL-3 Metastat CMT-3		IC ₅₀ MMP1 = 34 μg·mL ⁻¹ IC ₅₀ MMP8 = 48 μg·mL ⁻¹ IC ₅₀ MMP13 = 0.3 μg·mL ⁻¹
Cyclohexylamine salt of (R)-1-(3'- methylbiphenyl- 4-sulfonylamino)- methylpropyl phosphonic acid		IC ₅₀ MMP1 = 150 nM IC ₅₀ MMP2 = 1.5 nM IC ₅₀ MMP3 = 52 nM IC ₅₀ MMP7 = 460 nM IC ₅₀ MMP8 = 1.4 nM IC ₅₀ MMP9 = 8.0 nM IC ₅₀ MMP13 = 2.6 nM IC ₅₀ MMP14 = 79 nM
MMI270		IC ₅₀ MMP1 = 33 nM IC ₅₀ MMP2 = 11 nM IC ₅₀ MMP3 = 13 nM IC ₅₀ MMP9 = 8 nM IC ₅₀ MMP13 = 6 nM

MMP inhibitor	Structure	MMP inhibition
BMS-275291 Rebimastat		IC ₅₀ MMP1 = 25 nM IC ₅₀ MMP2 = 41 nM IC ₅₀ MMP3 = 157 nM IC ₅₀ MMP9 = 25 nM IC ₅₀ MMP13 = 4 nM
BAY 12-9566		K _i MMP1 >5000 nM K _i MMP2 = 11 nM K _i MMP3 = 134 nM K _i MMP9 = 301 nM K _i MMP13 = 1470 nM
SB-3CT		K _i MMP1 = 206 μM K _i MMP2 = 14 nM K _i MMP3 = 15 μM K _i MMP7 = 96 μM K _i MMP9 = 600 nM
CH11041†		NA

FIGURE 2. Structure and matrix metalloproteinase (MMP) inhibition of synthetic MMP inhibitors studied in lung pathologies. CMT: chemically modified tetracyclin; NA: not available; K_i: inhibition constant; IC₅₀: half maximal inhibitory concentration. #: 2-hydroxy-3-(1-(thiophenyl-oxadiazolyl)-2,2-dimethyl-propylcarbamoyl)-methyl-hexanohydroxamic acid; †: (S)-methyl 6-(benzyloxycarbonylamino)-2-(2-((S)-2,6-dioxo-3-(3,4,5-trimethoxybenzamido)-piperidin-1-yl)-acetamido) hexanoate.

Most ILDs are associated with pulmonary fibrosis and are characterised by collagen deposition in the lung interstitium. As in COPD, this results in loss of elasticity and decreased gas exchange. It is believed that TIMP/MMP imbalance is a determinant of fibrogenesis [134–137]. For example, TIMP1 was upregulated in a mouse model of bleomycin-induced pulmonary fibrosis [138] and TIMP3 was upregulated in patients with IPF [139]. Upregulation of TIMP1 or TIMP3 generates a “non-collagenolytic microenvironment” and leads to excessive deposition of extracellular matrix [137]. Conversely, if MMP activity is enhanced, increased ECM degradation might facilitate VEGF release, resulting in neoangiogenesis and capillary permeability, both of which are seen in pulmonary fibrosis [140]. Indeed, lavage fluid from IPF patients shows increased concentrations of several MMPs (e.g. MMP3, 7, 8 and 9), abnormal capillary permeability and increased VEGF levels [141–144]. Fibrocytes, which are progenitor cells characterised by the simultaneous expression of mesenchymal, monocytic and haematopoietic stem cell markers, were recently proposed to be an important source of MMPs during pulmonary fibrosis pathogenesis [145]. In mice, inhibition of MMPs by the non-selective MMP inhibitor batimastat reduced the development of bleomycin-induced fibrosis [64]. MMP8 deficiency, but not MMP12 deficiency [25], protects against bleomycin-induced pulmonary fibrosis [24, 146]. MMP12-deficient mice were protected against Fas-induced pulmonary fibrosis, and this was reflected in decreased expression of the profibrotic genes *egr1* and *cyr61*, even though the inflammatory responses in the lungs were similar to those in wild-type mice [26]. MMP9-deficient

mice developed fibrosing alveolitis after intratracheal treatment with bleomycin, but alveolar bronchiolisation was reduced, perhaps by limited migration of Clara cells and other bronchiolar cells into the regions of alveolar injury [27]. MMP7-deficient mice showed reduced fibrosis after bleomycin treatment [28, 29], probably caused by E-cadherin cleavage and the subsequent regulation of a population of CD103-positive dendritic cells that limit acute inflammation and inhibit progression of pulmonary fibrosis [147].

Several MMPs and TIMPs were detected in lung biopsies of patients with sarcoidosis, a granulomatous inflammatory disease. MMP activity was more prevalent in the parenchyma than in the bronchi, and this is indirect evidence for the involvement of MMPs and/or TIMPs in the sarcoid inflammation of the distal airways [148].

LAM is a rare disease caused by proliferation of disordered smooth muscle growth (leiomyoma) throughout the bronchioles, alveolar septa, perivascular spaces and lymphatics. The disease primarily affects females of childbearing age with an incidence of approximately 1.0–2.6 per million [149]. Levels of different MMPs were elevated in urine and lung nodules of LAM patients [150], and analysis of LAM tissue from lung biopsies indicated elevated reactivity of MMP2 and MMP9, but not of TIMP1 and TIMP2 [151]. MMP1 was suggested as a modifier gene affecting changes in the ECM that result in greater susceptibility to pneumothorax and rate of decline in lung function [152]. Consequently, MT1-MMP, an activator of MMP1, was also associated with proliferating LAM cells [153].

Acute lung injury

Acute lung injury (ALI) and its most severe manifestation, the acute respiratory distress syndrome (ARDS) [154], are defined by acute hypoxaemic respiratory failure, bilateral pulmonary infiltrates consistent with oedema, and normal cardiac filling pressures [155, 156]. ARDS is characterised by damage to the alveolar capillary barrier and can be initiated by trauma, pancreatitis, burns or sepsis. This damage precipitates a systemic inflammatory response involving numerous mediators.

Levels of MMP8, 9, 2, 3, 11 and 12 were higher in lung secretions of paediatric ALI patients compared with controls [157] and these MMPs were produced mainly by neutrophils and macrophages [158]. In BAL fluid of patients with hospital-acquired pneumonia, MMP8 and MMP9 levels are elevated [159] and correlate with the state of ventilation and degree of systemic inflammation [160]. Additionally, the kinetics of MMP8 and 9 expression in ventilator-associated pneumonia seems to be strongly influenced by *Pseudomonas aeruginosa* infection, and imbalance between MMP9 and TIMP1 at end of therapy may determine the intensity of lung injury and the ultimate outcome of ventilator-associated pneumonia [161].

Different animal models can be used to unravel the role and therapeutic potential of MMPs in ALI. These models are mostly based on clinical disorders that are associated with ALI/ARDS in humans, but none of them fully reproduces the features of human lung injury [162]. Possible triggers of ALI in mice are mechanical ventilation, pulmonary ischaemia/reperfusion (I/R), LPS, live bacteria, hyperoxia, bleomycin, oleic acid, caecal ligation and puncture, and acid aspiration. Increased MMP expression is observed in lung injury induced by LPS, oleic acid

and I/R [163–165]. Ilomastat attenuated lung inflammation and injury caused by I/R or oleic acid, which was reflected in a decrease in leukocyte influx [65]. Similarly, ONO-4817 attenuated I/R-induced lung injury [66]. Additionally, a chemically modified tetracycline (COL-3) prevented lung injury in endotoxin-induced ARDS in Yorkshire pigs [67].

MMP8-deficient mice, and mice treated with an MMP8-specific inhibitor, showed better gas exchange, decreased lung oedema and permeability, and diminished histological damage after high-pressure ventilator-induced lung injury [30]. In contrast, another study showed that MMP8-deficient mice develop more alveolar permeability than their wild-type counterparts after mechanical ventilation [31]. The fact that different ALI stimuli were used makes it difficult to compare both studies, but it is important to realise that the MMP8 inhibitor not only inhibits MMP8, but also MMP2 and MMP13 with similar K_i (fig. 2). Similarly, LPS- or bleomycin-induced ALI in MMP8-deficient mice resulted in the accumulation of polymorphonuclear (PMN) cells relative to wild-type mice. This accumulation was caused by a reduction in MMP8-mediated MIP1 α inactivation and resulted in increased mortality [32, 33].

MMP9-deficient mice developed LPS-induced emphysema [34], while MMP9 inhibition with CMT-3 significantly reduced neutrophilic inflammation in a ventilator-induced lung injury mouse model [68]. Similarly, in a model of ALI induced by immune complexes, MMP9-deficient mice displayed less severe lung injury than wild-type controls [37]. In contrast, more recent data indicate that MMP9 might have an unrecognised beneficial role in reducing pulmonary oedema in ARDS by improving alveolar epithelial healing [165]. Inhibition of MMP9 activity with doxycycline reduced pancreatitis-associated lung injury and diminished expression of MMP9 in pulmonary tissue [69]. BAL from MMP9-deficient mice exposed to ozone had increased protein content and greater numbers of neutrophils and epithelial cells compared to wild-type mice; this finding points to a protective role of MMP9 in ozone-induced inflammation [35]. This protective effect was not observed in MMP7-deficient mice [35]. It is important to note that, although often neglected by the authors, none of the presented MMP9 inhibition strategies was specific for MMP9 (fig. 2).

MMP13-deficient mice subjected to hyperoxia-induced ALI showed increased inflammation after ALI, probably because of the lack of monocyte chemotactic protein-1 cleavage, resulting in excessive attraction of macrophages to the BAL fluid [36]. Also MMP3 plays a role in the development of lung injury induced by acute inflammation, as evidenced by a reduction in the degree of lung injury in MMP3-deficient animals [37].

Lung cancer

Lung cancer is the most common cause of cancer-related death in males and females and can be divided into small cell lung carcinoma and nonsmall cell lung carcinoma (NSCLC). Since MMPs proteolytically cleave several components of the ECM and basement membranes, they play a fundamental role in cancer progression. Furthermore, almost all invasive malignant tumours, including lung cancers, express high levels of MMPs. For example, expression of MMP7 and MMP9 was stronger in NSCLC tissue than in the surrounding tissue or in benign lung disease [166], and the functional polymorphism in the MMP7

promoter (-181A/G) has been associated with susceptibility to NSCLC [167]. Certain MMP1 and MMP3 promoter polymorphisms have been linked to modified susceptibility to NSCLC and to increased risk of lymphatic metastasis of these tumours, respectively [168]. MMP2 and MMP9 have been associated with increased tumour spread and poor prognosis in lung cancer [169]. MMP2 expression showed prognostic value in patients with NSCLC [170], and it probably plays an important role in invasion of lung cancer into lymphatic tissue [171]. Moreover, gain of function of MMP2 due to genetic polymorphisms plays an important role in susceptibility to lung cancer [172].

The broad-spectrum, orally bioavailable MMP inhibitor MMI270 significantly decreased the number of colonies in the lung after intravenous injection of mouse B16-F10 melanoma cells [70]. The broad-spectrum MMP inhibitor BMS-275291 is of particular therapeutic interest because it has no musculoskeletal side-effects; it also was evaluated in advanced lung cancer [173]. However, a randomised phase III study, in which BMS-275291 was added to chemotherapy, increased toxicity but did not improve survival in advanced NSCLC [71]. In contrast, treatment with the broad-spectrum MMP inhibitor BAY 12-9566N counteracted the genotoxic carcinogen *N*-nitrosodimethylamine and reduced neoplastic growth and development [72]. Similarly, GM6001, a potent MMP inhibitor that reacts with most MMPs, displayed very potent anti-metastatic effects in the MMTV-PyMT cancer model [73].

In the same mouse model, the absence of MMP13 did not influence tumour growth, vascularisation, progression to more advanced tumour stages, or lung metastasis, though MMP13 mRNA was strongly upregulated with the transition to invasive and metastatic carcinomas [38]. Similarly, lack of MMP7 had no effect on the development of lung metastases, while MMP9 deficiency or administration of a highly selective pharmacological MMP9 inhibitor was associated with a huge decrease in lung tumour burden in mice [39, 40]. Surprisingly, the anti-metastatic outcome of MMP9 ablation seemed to be strain dependent [40]. MMP2 also plays a role in cancer development, because tumour-induced angiogenesis is reduced in MMP2-deficient mice [41]. Indeed, *in vivo* adenovirus-mediated knock-down of MMP2 decreased tumour growth and prevented formation of lung nodules in a spontaneous lung metastasis model [74]. *In vitro* studies further revealed that MMP2 inhibition leads to decreased induction of VEGF and consequent inhibition of angiogenesis and endothelial apoptosis [174]. Additionally, MMP2 siRNA inhibited lung cancer cell-induced tube formation of endothelial cells, and addition of recombinant human MMP2 restored angiogenesis [175]. Treatment of mice with CH1104I significantly inhibited pulmonary metastasis of carcinoma cells, which suggests that inhibition of MMP2 and 9 might effectively suppress tumour invasion and metastasis [75]. Overexpression of MMP1 significantly induces formation of lung metastases [176]. In contrast, MMP12-deficient mice develop significantly more gross Lewis lung carcinoma pulmonary metastases than their wild-type counterparts, both in spontaneous and experimental metastasis models, which indicates that MMP12 has a tumour-suppressive role [42]. MMP3-deficiency did not significantly affect tumour growth and metastasis in the MMTV-PyMT model [177].

Other lung disorders

Ageing lung

MMPs play a role not only in pulmonary diseases, but also in homeostasis. Ageing of the lung results in changes in lung morphology, physiology and defence mechanisms. Several MMPs and TIMPs are associated with impaired lung function during ageing. For example, increased MMP9, MMP12, TIMP1 and TIMP2 levels and decreased MMP1 and 2 levels were detected in ageing lungs [178–180]. The resulting persistent injury may contribute to the pathology of diseases such as asthma, COPD and pulmonary fibrosis [181].

Cystic fibrosis

Cystic fibrosis (CF) is another lung disease in which imbalanced protease activity could damage the airway architecture and contribute to progressive bronchiectasis [182]. There is emerging evidence that MMPs could play a key role in the pathogenesis of CF [183]. MMP levels are elevated in the sputum of CF individuals undergoing acute pulmonary exacerbation [184, 185]. These MMPs generate proline-glycine-proline (PGP), an extracellular matrix-derived neutrophil chemoattractant by which they regulate the immune response during CF [186].

Bronchiolitis obliterans syndrome

Bronchiolitis obliterans syndrome (BOS) (or chronic rejection) is the main long-term complication after lung transplantation and affects 60% of lung transplant patients. Elevated levels of MMP8, MMP9 and TIMP1 can be observed in obliterative bronchiolitis patients in the 2 yrs after transplantation [187]. Similarly, an unopposed increase in gelatinase activity observed in BAL fluid from BOS patients might be caused by MMP9 secretion by local neutrophils [188]. Genetic polymorphisms of MMP7 that result in lower MMP7 levels predispose to the development of BOS [189]. In the lung transplant model, inhibiting MMPs in the donor before lung harvest and in the recipient after lung transplantation improved oxygenation and diminished PMN leukocyte influx into the isograft [76]. Both MMP8- and MMP9-deficient mice were shown to be protected from obliterative bronchiolitis, reflected by reduced neutrophil influx and collagen deposition [43, 44]. However, absence of the endogenous MMP inhibitor TIMP1 *in vivo* provided direct evidence that TIMP1 contributes to the development of airway fibrosis in the heterotopic tracheal transplant model [190]. Apparently, MMPs and TIMPs play a complex role during the immunopathogenesis of lung allograft rejection. Indeed, while TIMP1 or TIMP2 overexpression had no consistent effect on cytokine profiles or rejection pathology, MMP inhibition *via* systemic administration of the broad-spectrum MMP inhibitor COL-3 was associated with striking reductions in allograft rejection [77].

Chronic lung disease of prematurity

Chronic lung disease of prematurity (CLD) (or bronchopulmonary dysplasia) causes respiratory morbidity and mortality in preterm infants. Serial BAL fluid obtained from ventilated newborn preterm infants suffering from CLD revealed that MMP9, the MMP9/TIMP1 complex, and cell counts were all statistically increased in infants developing CLD [191], which resulted in an increased MMP9 activity [192]. Another study detected significantly increased MMP3 and decreased TIMP1 levels in the CLD group compared to the non-BPD group [193].

In a mouse model of bronchopulmonary dysplasia, MMP9 deficiency worsened IL-1 β -induced lung injury [45].

FUTURE PERSPECTIVES

Proteolysis, such as cleavage by MMPs, is a very effective mechanism that enables an organism to react very rapidly to a physiological stimulus when fast changes are needed. Consequently, MMP activity is tightly controlled and disturbance of this delicate balance can have dramatic effects. Indeed, different lung disorders have been associated with upregulated MMP activity. This, together with the fact that most of their actions are extracellular, makes MMPs attractive therapeutic targets. However, several issues should be addressed before therapeutic MMP inhibition will ever be successful.

Although increased MMP expression in a particular disease does make these MMPs candidate diagnostic biomarkers, this does not necessarily mean that inhibiting them will have a beneficial therapeutic effect. The use of MMP-deficient mice can provide more evidence on the specific role of a particular MMP during disease progression. However, most available MMP-deficient mice are constitutively null, sometimes resulting in compensatory increases in other MMPs, which makes the observed phenotypes difficult to interpret. Additionally, none of the available animal models resembles perfectly the complex human situation. This makes it extremely difficult to extrapolate the outcome of MMP inhibition in animal models to humans. Nevertheless, a greater understanding of the involvement of MMPs in lung disorders is indispensable, in order to further explore the possibilities of therapeutic MMP inhibition. For example, it is extremely important to unravel the MMP degradome. Until now, most of the substrates have been identified *in vitro*. However, since the *in vivo* environment is much more complex, several of these substrates might not be physiological substrates.

It is clear that several MMPs have both detrimental and beneficial activities. To make the situation even more complex, this is often context dependent. For example, several MMPs are considered to have tumour-inhibitory effects. Therefore, therapeutic MMP inhibition strategies should ideally maintain the optimum balance between MMPs and TIMPs to avoid complications of MMP over- or under-activity. In this context, gathering more information about the specific MMP/TIMP pattern during disease progression is of extreme importance.

Clinical trials with the currently available MMP inhibitors have been very disappointing. However, the detrimental off-target effects caused by broad-spectrum MMP inhibitors might be avoided by using more specific MMP inhibitors. A third generation of MMP inhibitors is currently under investigation and they are especially designed to block only the target MMP, while sparing the anti-target ones. Additionally, off-target effects can be avoided by trying to achieve the optimum level of MMP activity for normal physiological processes. This again stresses the need to understand in more detail the involvement and activity levels of MMPs during different diseases, but this requires the development of specific and sensitive quantitative assays to determine MMP activity.

In conclusion, we believe that MMP inhibition is a feasible therapeutic approach for treatment of lung disorders, but only if there is a better understanding of the physiological and

pathological roles of MMPs and if more selective MMP inhibitors are made available.

STATEMENT OF INTEREST

None declared.

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