Matrix metalloproteases and lung disease

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Development, tissue remodelling, cell migration, inflammation, angiogenesis, and wound healing are just some of the physiological processes in which the matrix metalloproteases (MMPs), a family of highly homologous endopeptidases, play a crucial part. Collectively, the MMPs can cleave most, if not all, the protein constituents of the extracellular matrix including collagen, proteoglycan, laminin, fibronectin, and elastin. In so doing they facilitate cell movement, reorientation, and dispersal. The production and action of the MMPs are subject to strict control, ensuring limited proteolysis of the extracellular matrix during cell migration, tissue remodelling and repair. Inadequate regulation of MMP production or action has, however, been implicated in a wide range of pathological processes including several lung diseases, rheumatoid arthritis, periodontal and renal diseases, osteoarthritis, fibrotic disorders, and tumour invasion.

Recent studies elucidating (1) the sequence homologies between members of the MMP family, (2) factors involved in the regulation of MMP expression, and (3) mechanisms of MMP activation and inhibition have added considerably to our knowledge of the crucial control mechanisms which can tip the balance of MMP action from normal homeostasis to pathological process. In this review the salient features of the action and regulation of MMPs which contribute to their role in normal and diseased states will be outlined and evidence for their participation in lung disease discussed.

The matrix metalloproteases (MMPs) Nine members of the MMP family have been identified. All are produced and secreted in a latent, proenzyme form. They require Zn$^{2+}$ and Ca$^{2+}$ ions for activity and are inhibited by a group of specific inhibitors — the tissue inhibitors of metalloproteases (TIMPs). Initially characterised and classified on the basis of their different substrate specificities and molecular weights (table 1), individual MMPs have been given various names by different groups. The International Union of Biochemistry and Molecular Biology has recently recommended a standardised system of nomenclature which will be used in this review. Alternative names are cited in table 1. The cDNA predicted amino acid sequence of the MMPs reflects a high degree of homology between family members. Three functional domains are evident: a propeptide domain which codes for the peptide released upon activation of the proenzyme; a catalytic domain containing the Zn$^{2+}$ binding site; and a C-terminal domain which exhibits some homology with hemopexin and vironectin (fig

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Molecular weight (kDa)</th>
<th>ECM substrates</th>
<th>Other names†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial collagenase (EC 3.4.24.7)</td>
<td>55</td>
<td>Collagens I, II, III (III &gt; I &gt; VII, VII, X, gelatin, proteoglycan (PG)-core protein</td>
<td>MMP-1 Fibroblast-type collagenase</td>
</tr>
<tr>
<td>Neutrophil collagenase (EC 3.4.24.34)</td>
<td>75</td>
<td>Same as interstitial collagenase but I &gt; III</td>
<td>MMP-8 PMN-type collagenase</td>
</tr>
<tr>
<td>Gelatinase A (EC 3.4.24.24)</td>
<td>72</td>
<td>Gelatins, collagens IV, V, VII, X, XII, fibronectin, elastin, PG-core protein</td>
<td></td>
</tr>
<tr>
<td>Gelatinase B (EC 3.4.24.35)</td>
<td>92</td>
<td>Gelatins, collagens IV and V, elastin, PG-core protein</td>
<td></td>
</tr>
<tr>
<td>Stromelysin-1 (EC 3.4.24.17)</td>
<td>57</td>
<td>PG-core protein, laminin, fibronectin, gelatins, elastin, collagens III, IV, V, IX, X, interstitial collagenase proenzyme</td>
<td>MMP-3 Transin Proteoglycanase Proteoglycanase activator</td>
</tr>
<tr>
<td>Stromelysin-2 (EC 3.4.24.22)</td>
<td>57</td>
<td>Same as stromelysin-1</td>
<td>MMP-10 MMP-11</td>
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<tr>
<td>Stromelysin-3</td>
<td>ND</td>
<td>ND</td>
<td>MMP-7 MMP-9</td>
</tr>
<tr>
<td>Matrilysin (EC 3.4.24.23)</td>
<td>28</td>
<td>PG-core protein, laminin, fibronectin, collagen IV, gelatin, interstitial collagenase proenzyme</td>
<td>PUMP-1 Urokinase metalloproteinase (None)</td>
</tr>
<tr>
<td>Macrophage metalloelastase</td>
<td>55</td>
<td>Elastin, fibronectin</td>
<td></td>
</tr>
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</table>

ECM = extracellular matrix.
* Molecular weight of proenzyme form.
† MMP numbering according to Nagase et al. 18
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1. The propeptide domain contains a highly conserved PROG(V/N)PD sequence which is involved in maintaining the stability of the latent proenzyme. In the catalytic two conserved histidines are thought to act as Zn ligands. Matrilysin, the smallest of the MMPs, is composed of the propeptide and catalytic domains only. Other family members contain a C-terminal domain which plays a part in determining the substrate specificity of the collagenases and stromelysins, and is also involved in the interaction of MMPs with the inhibitors TIMP-1 and TIMP-2. The two gelatinases contain an additional 175 residue insert in the catalytic region which is similar to the collagen binding region of fibronectin. The larger of these two enzymes, gelatinase B, contains a second insert at the C-terminal end of the catalytic domain which exhibits some homology to the $\alpha_2$ chain of type V collagen. These inserted domains are involved in determining the substrate binding capabilities of the gelatinases.

DEGRADATION OF EXTRACELLULAR MATRIX COMPONENTS

Between them the MMPs are capable of digesting all the major components of the extracellular matrix and basement membrane (table 1). The two collagenases display a unique ability to cleave the native helix of fibrillar collagen types I, II, and III. They hydrolyse a single bond located one quarter length from the collagen C-terminus to yield one quarter and three quarter fragments of the original helical molecule. At body temperature these released fragments spontaneously denature into randomly coiled gelatin peptides which are further degraded by a range of proteases, including other members of the MMP family. The two collagenases digest a similar range of collagen substrates (table 1) but at different rates. The neurophil enzyme preferentially degrades type I collagen whereas interstitial collagenase prefers a type III substrate.

The major in vivo role of the gelatinases was initially thought to be the digestion of gelatin peptides released from fibrillar collagens by the collagenases. It has since been shown that gelatinases degrade other collagen types, including native type IV collagen, the major constituent of cellular basement membranes. This has led to the suggestion that their major in vivo role may be the digestion of basement membranes during inflammatory cell migration and tumour invasion.

While the gelatinases can also degrade elastin and fibronectin, the MMPs with the broadest substrate range are the stromelysins. They digest fibronectin, laminin, elastin, proteoglycan core protein, and several collagen types. An additional feature of stromelysin-1 is its ability to potentiate the activation of latent interstitial collagenase, suggesting a synergistic interaction between these MMPs.

SOURCE OF MMPS

Intersetional collagenase is produced by a range of stromal cells including fibroblasts, endothelial cells, osteoblasts, keratinocytes, chondrocytes, and hepatocytes. Cells of the monocyte/macrophage lineage also produce this enzyme. In contrast, neutrophil collagenase is found only in polymorphonuclear leucocytes where it is produced during maturation and stored in intracellular granules prior to release.

Gelatinase A and B also differ in their cellular distribution. Gelatinase B is the only form found in polymorphonuclear leucocytes and is also the major gelatinase produced by macrophages. By comparison, connective tissue cells preferentially produce gelatinase A, although transformed fibroblasts, cells of neoplastic origin and stromal cells surrounding tumours can produce both enzymes. It is suggested that fibroblasts may be induced to express gelatinase B by tumour derived factors.

Stromelysins-1 and 2 are produced by various connective tissue cells and by macrophages. Distribution of stromelysin-3 is less well characterised. Similarly, while expression of matrilysin has been observed in monocytes and cells of the involuting uterus, its distribution is, as yet, poorly defined.

REGULATION OF MMP PRODUCTION BY CYTOKINES

In stromal cells MMP production is regulated at transcriptional level by a range of cytokines which influence the production and stability of MMP mRNAs (table 2). Expression of interstitial collagenase and stromelysin-1 is coordinately upregulated by interleukin 1 (IL-1), tumour necrosis factor $\alpha$ (TNF$\alpha$), platelet derived growth factor (PDGF), endothelial growth factor (EGF), basic fibroblast growth factor (bFGF), and the phorbol ester tumour promoters.

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**Table 2 Cytokine and pharmacological regulation of MMP and TIMP expression in stromal cells**

<table>
<thead>
<tr>
<th>Enzyme/inhibitor</th>
<th>Uptregulation</th>
<th>Repression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial collagenase</td>
<td>IL-1, TNF$\alpha$, PDGF, EGF, bFGF, TGF$\beta$, NGF</td>
<td>TGF$\beta$, IFN$\gamma$, IL-4, glucocorticoids, retinoids</td>
</tr>
<tr>
<td>Gelatinase A</td>
<td>TGF$\beta$,</td>
<td>IL-10, IFN$\gamma$, TGF$\beta$, TIMP-1</td>
</tr>
<tr>
<td>Gelatinase B</td>
<td>TGF$\beta$,</td>
<td>IL-10, IFN$\gamma$, TGF$\beta$, TIMP-1</td>
</tr>
<tr>
<td>Stromelysin-1</td>
<td>IL-1, TNF$\alpha$, PDGF, EGF, bFGF, TGF$\beta$, NGF</td>
<td>TGF$\beta$, TIMP-2</td>
</tr>
<tr>
<td>TIMP$^*$</td>
<td>IL-1, IL-10, TIMP-1</td>
<td>TIMP-2</td>
</tr>
</tbody>
</table>

IL = interleukin; TNF = tumour necrosis factor; PDGF = platelet derived growth factor; EGF = epidermal growth factor; bFGF = basic fibroblast growth factor; TGF = transforming growth factor; NGF = nerve growth factor; IFN = interferon.

* Most studies do not distinguish between TIMP-1 and TIMP-2. TGF$\beta$ is reported to have differing effects on the expression of these inhibitors, causing upregulation of TIMP-1 and repression of TIMP-2. **

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**Figure 1** Prototype of domain structure of matrix metalloproteases

- N-Terminal
- Catalytic domain
- Hemopexin-like domain
- C-Terminal
- Gelatinase inserts
- Cys
- Zn

*TIMP = TIMP-1 and TIMP-2.*
Many of these cytokines also enhance production of TIMP-1 (table 2). This co-expression of enzyme and inhibitor may be important in confining degradation of extracellular matrix to areas close to the protease producing cell. In polarised cells MMPs exhibit a directional preference for secretion—for example, endothelial cells secrete MMPs from their basal compartment and not into the apical lumen.37 In cell migration it is suggested that enzyme and inhibitor may be generated at different sites to enhance directional movement of the cell.6 Most authors report the downregulation of interstitial collagenase and stromelysin-1 gene expression by transforming growth factor β (TGFβ), interferon γ (IFNγ) and IL-4. TGFβ also abrogates the effects of IL-1, TNFα, EGF, and bFGF on the expression of both these genes.38 TIMP-1 expression is enhanced by TGFβ, as is the production of collagen, fibronectin, proteoglycans, and glycosaminoglycans.39 Thus, the overall effects of TGFβ is to enhance interstitial matrix formation by increasing production of matrix components, decreasing production of interstitial collagenase and stromelysin-1, and increasing production of TIMP-1. The effect of TGFβ on basement membrane components may be somewhat different. Recent studies indicate that expression of the gelatinases by fibroblasts and keratinocytes is significantly increased by TGFβ.39-41 Expression of TIMP-2, which is a more effective inhibitor of the gelatinases than TIMP-1, is also upregulated by TGFβ.42,43 These effects may be of significance in regulating cell detachment from basement membranes during wound healing and tumour invasion.

Pharmacological modulation of stromal cell MMP production is observed with retinoids and corticosteroids.44-46 Both decrease transcription of interstitial collagenase and stromelysin-1. The retinoids also enhance TIMP-1 production.44 The effects of these agents on gelatinase expression is not, as yet, defined.

MMP RELEASE FROM NEUTROPHILS
Neutrophil MMPs are stored in intracellular granules and released upon stimulation. Control of neutrophil MMP production is therefore governed by factors which affect neutrophil activation rather than gene transcription. Available evidence indicates that neutrophil collagenase and gelatinase B are stored in separate granules—collagenase in the secondary (or specific) granules and the gelatinase B in the tertiary granules.45-47 Although the contents of both granules are released upon neutrophil stimulation by a range of chemotactic and activation factors (table 3), gelatinase B release is observed at stimulus concentrations lower than those required to promote release of secondary granule contents. Sequential MMP release along a chemotactic gradient is therefore proposed.45-49 Exocytosis of MMP-containing granules is observed during neutrophil adherence and exudation suggesting that these enzymes play a part in neutrophil migration and connective tissue remodelling in early inflammation and wound healing.40

MMP ACTIVATION
Activation of latent MMPs is a stepwise process involving destabilisation of the coordinate bond between the active site Zn2+ and a free cysteine in the conserved PRCG(V/N)PD propeptide sequence. This destabilisation induces a conformational change which unmasks the active site, leading to autolytic cleavage of the N-terminal propeptide (fig 2).51 In vitro a range of disparate reagents, including organomercurials and detergents, activate MMPs by disrupting the cysteine–Zn2+ bond. Serine proteases activate MMPs by digesting part of the propeptide sequence which stabilises the cysteine–Zn2+ bond. In the case of interstitial collagenase, the enzyme produced following activation by these agents can be further activated by stromelysin-1.51 The identity of the in vivo activators of the MMPs is still uncertain. The serine protease, plasmin, has been implicated in the activation of stromelysin-1 and interstitial collagenase, suggesting a cascade mechanism involving plasminogen, plasminogen activator, and stromelysin-1 in the activation of interstitial collagenase.52 Unlike the other MMPs, gelatinase A is not activated by serine proteases.16 This enzyme is activated by binding to a membrane associated protein which is sensitive to metalloprotease inhibitors and is induced by phorbol esters and TGFβ.33 Whether the membrane associated protein is itself a protease or acts by enhancing the autolytic activation of the gelatinase is not clear. Neutrophil collagenase and gelatinase can be activated by hypochlorous acid (HOCl), a pro-
duct of the respiratory burst which oxidises the Zn$^{2+}$ chelating cystine. However, as activation of neutrophil MMPs can occur prior to, and independently of, the respiratory burst, the in vivo relevance of this activation mechanism remains speculative.

**MMP INHIBITION**

The coordinated production of MMPs and their specific tissue inhibitors in a range of developing and remodelling situations suggest that TIMPs play a significant role in the regulation of MMP activation in vivo. Four TIMPs have been reported and two – TIMP-1 and TIMP-2 – have been characterised. Both characterised TIMPs inhibit the active forms of all MMPs, binding in a non-covalent manner to form a 1:1 complex. Interestingly, the inhibitors can also bind to the latent form of both gelatinases – TIMP-1 binding to the gelatinase B proenzyme and TIMP-2 to latent gelatinase A. In this manner TIMPs may exert additional control on MMP action by inhibiting the auto-activation of the gelatinases.

The plasma protein $\alpha_1$-macroglobulin is an effective inhibitor of MMP action in vitro. Given the large size of this protein, however, its role in inhibiting MMPs released by stromal cells is probably minor except at inflammatory sites where increased capillary permeability causes leakage of plasma proteins. In these instances inhibition of $\alpha_1$-macroglobulin may be important as TIMP activity is destroyed by the action of serine proteases released by infiltrating neutrophils.

**Matrix metalloproteases and the lung**

Maintenance of the extracellular matrix scaffold which supports the alveolar structure is essential for normal lung function and is achieved by a dynamic balance between synthesis and degradation of extracellular matrix components. In the healthy lung MMPs – which are produced by virtually all resident lung cells including fibroblasts, alveolar macrophages, epithelial and endothelial cells – are involved in normal extracellular matrix turnover and also participate in the wound healing response following injury. Given their role in normal lung homeostasis, it is not surprising that the MMPs are implicated in a range of pulmonary diseases characterised by alterations in alveolar structure, or abnormal wound healing responses, including emphysema, adult respiratory distress syndrome (ARDS), interstitial fibrosis, granulomatous disease, lung cancer, and pleural disease. In subsequent sections of this review evidence for the participation of MMPs in the pathogenesis of these diseases is outlined (with the exception of emphysema, which has been the subject of a recent review in *Thorax*).

**MMPs and ARDS**

Neutrophil collagenase has been detected in bronchoalveolar lavage (BAL) fluid from patients with ARDS. The enzyme is present in active form, indicating that it has been activated subsequent to release and has evaded in vivo inhibition by TIMPs and $\alpha_1$-macroglobulin. The destructive potential of this unopposed activity in the lung is considerable and may contribute to the tissue damage observed during the acute inflammatory stage of disease. To our knowledge, lung tissue or BAL fluid from patients with ARDS have not been assessed for the presence of gelatinase B, the second MMP stored in polymorphonuclear leucocytes. Indirect evidence would, however, suggest that this enzyme is likely to be released during the process of neutrophil adhesion and migration into the lung in ARDS. The tertiary granules which contain both gelatinase B and the adhesion molecule Mac-1 (CD11b/CD18) are rapidly transported to the cell surface following stimulation of neutrophils by chemotactic and activation factors. In addition, IL-8, which appears to have a significant role in promoting neutrophil-mediated inflammation and injury in ARDS, stimulates release of gelatinase B from neutrophils. Release of this MMP could contribute to the breakdown of alveolar basement membrane observed in ARDS. Morphological studies indicate that preservation of basement membrane is vital for effective repair, as this provides tissue cells with migratory pathways that ensure the maintenance of the original alveolar architecture. Thus, the extent of basement membrane digestion by gelatinase B may be a significant factor in determining disease outcome in ARDS.

Evidence from wound healing systems indicate that regulated MMP expression and production by stromal cells is required for tissue remodelling – a process which presumably takes place in the lungs of ARDS survivors. It is suggested that the excessive fibrosis observed in the lungs of non-survivors is a result of an unregulated repair process. Whether the outcome is repair or fibrosis, MMPs produced by alveolar fibroblasts, macrophages, epithelial and endothelial cells are likely to have a significant role. To date, however, the participation of these MMPs in ARDS has received little attention. A recent study which demonstrates increased interstitial collagenase expression in the lungs of rats following hyperoxic injury (a model with similarities to ARDS) suggests that this may prove a fruitful area for future investigation.

**MMPs and Interstitial Fibrosis**

As indicated above, fibrosis – the common end stage of several interstitial lung diseases – is thought to reflect an aberrant wound healing response. The injury which triggers the response varies from neutrophil mediated tissue damage (cryptogenic fibrosing alveolitis) to granuloma formation in response to known (extrinsic allergic alveolitis) and unknown (sarcoidosis) antigens, direct tissue damage (asbestos, silica), and injury resulting from abnormal immune responses (collagen vascular diseases). Whatever the initial cause, a
A unifying hypothesis on the involvement of MMPs in fibrosis encompassing all the above observations would involve (1) collagenase (and probably gelatinase B) released from inflammatory polymorphonuclear leucocytes contributing to initial tissue injury, and (2) a decrease in interstitial collagenase expression by fibroblasts and/or other stromal cells contributing to subsequent aberrant repair and accumulation of collagen. In this regard, Selman and colleagues have noted that lung fibroblast lines from patients with cryptogenic fibrosing alveolitis constitutively produce less interstitial collagenase than lines from normal lung. They have also observed decreased collagenase production in a fibroblast line derived from BAL cells recovered from a patient with systemic lupus erythematosus (unpublished observations). In vitro studies indicate that several cytokines known to be present in inflammatory situations, including TGFβ, IL-4 and IFNγ, are capable of reducing expression of interstitial collagenase by fibroblasts and monocytes/macrophages (table 2). As previously indicated, TGFβ also increases collagen production and production of TIMP-1. Studies which indicate that TGFβ promotes gelatinase expression in fibroblasts94-96 raise the intriguing possibility that, while "driving" fibroblasts to produce and deposit components of the interstitial matrix, this cytokine also enhances their capability to degrade basement membrane. Thus, under the influence of this cytokine the action of fibroblasts would ideally "fit" that required for producing fibrosis in situations where the extent of injury and/or continued inflammation prevented healing.

While most studies have concentrated on the role of interstitial and neutrophil collagenases in interstitial fibrosis, the potential involvement of other MMPs cannot be ignored. We have detected gelatinase B in BAL fluids from patients with cryptogenic fibrosing alveolitis, extrinsic allergic alveolitis and sarcoidosis (unpublished results), and the transient expression and activity of gelatinase A by alveolar macrophages from patients with sarcoidosis has been observed by another group.84 As these MMPs are particularly potent in degrading basement membrane collagen, their release or production in the lung may contribute to tissue injury in these diseases.

MMPs AND LUNG CANCER
Tumour invasion and metastasis involves degradation of tissue basement membrane and interstitial matrix. In recent years considerable attention has focused on the role of MMPs in these processes, as increased MMP activity has been shown to be associated with the metastatic potential of both cultured tumour cells and malignant tissue.12,27 In the lung, expression of both gelatinases, stromelysin-3 and interstitial collagenase has been observed in various primary and metastatic tumours including adenocarcinomas, squamous cell, and small cell carcinomas.49,49 Because of their role in basement membrane digestion, the expression and production of the two forms of gelatinase has...
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received particular attention. While initial immunohistochemical localisation of gelatinase A suggested its production by malignant cells, subsequent in situ hybridisation studies have revealed that stromal cells are the major source of this enzyme in many tumours, including lung carcinomas. Emond et al have recently reported the presence of a binding site for gelatinase A on the surface of breast adenocarcinoma cells. These observations suggest a parasitic type interaction between malignant and stromal cells with the malignant cell binding gelatinase produced by stromal cells, hence gaining the capacity to migrate through tissue basement membrane. In situ hybridisation experiments indicate that gelatinase B is produced by both tumour and stromal cells, particularly tumour associated macrophages. Decreased production of MMP inhibitors may also contribute to tumour metastatic potential. Khokha et al have demonstrated that antisenes DNA RNA knockdown modulation of TIMP transforms normal Swiss 3T3 fibroblasts into malignant cells capable of forming metastatic tumours. This transformation is accomplished by an increase in MMP expression. The opposite effect was observed by DeClerck et al. These investigators transfect malignant cells with TIMP-2 cDNA and noted a decreased capacity for invasion in the transfected cells. Direct inhibition of MMP activity by a synthetic peptide containing a modified "copy" of the MMP Zn-binding sequence has also been shown to inhibit tumour cell invasion. While still at an early stage, these studies suggest that therapies aimed at decreasing MMP expression by tumour and stromal cells and/or inhibiting MMP activity may be of value in limiting the metastatic spread of a range of tumours.

**MMPs in Pleural Fluid**

A role for MMPs in maintaining the integrity of the pleural space has recently been suggested by Hurewitz and colleagues. These investigators noted high levels of both gelatinases in pleural effusions from patients with malignant and non-malignant inflammatory diseases. The enzyme activity observed was attributed to tetracyclinases. The authors suggest that gelatinases in pleural fluid may serve to dissolve adhesions and accumulate connective tissue following pleural injury, and that the action of tetracycinases in pleuritis may be attributable to their ability to inhibit these enzymes.

**Future directions**

Research in recent years has highlighted the involvement of common cytokines and growth factors in a range of seemingly diverse processes including cell migration, proliferation and transformation; destructive inflammatory disorders; resorptive processes; fibrosis and tumorigenesis. The involvement of common mediators suggests similarities in underlying cellular mechanisms. In vivo, all of these processes require or are accompanied by change and reorganisation of the surrounding extracellular matrix. The most likely mechanism by which cytokines and growth factors exert their modulating effects on the extracellular matrix is via the inductive or repression of MMPs and their inhibitors. Thus, the potential application of therapies targeted at MMP regulation is of considerable interest. As outlined in this review, recent advances in our understanding of these regulatory mechanisms now make it possible to design and examine such therapies.
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