Matrix Metalloproteinases in Kidney Disease Progression and Repair: A Case of Flipping the Coin

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Summary: Matrix metalloproteinases (MMPs) have pleiotropic enzymatic actions that go far beyond degradation of extracellular matrix. Both the multiplicity of their targets and the complexity of their regulation account for a variety of biological effects. In renal diseases, MMP effects may be different and/or opposite during the different phases of the pathology evolution. The major challenge with future therapeutic interventions using MMP inhibitors remains how to accomplish temporal and spatial control of their activity without flipping the coin.
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Matrix metalloproteinases (MMPs) were discovered more than 40 years ago when Gross and Lapiere[1] identified an activity that was present during metamorphosis in tadpoles, which had the ability to degrade rigid rods of collagen. This activity turned out to be interstitial collagenase, an enzyme with an almost singular ability to degrade the collagen triple helix at neutral pH. Interstitial collagenases (MMP-1, -8, -13, -18) are the first members of an ever-growing large family of zinc-requiring enzymes, which also include gelatinases (MMP-2,-9), stromelysins (MMP-3,-10), matrilysins (MMP-7, -11,-26), macrophage elastase (MMP-12), and membrane-type MMPs (MMP-14 to -17, -24, -25). Those family members have dedicated substrate specificities that allow the MMPs to degrade synergistically all components of the extracellular matrix (ECM) in a concerted manner. MMPs thus are involved in a variety of pathophysiologic processes in which tissue remodeling plays a major role such as embryonic development, angiogenesis, invasive cell behavior, inflammation, wound healing, and fibrosis.

However, we have learned that MMP roles are not just limited to matrix degradation.[2] MMPs are involved in proteolytic activation or degradation of nonmatrix susbstrates including cytokines, chemokines, and growth factors. Their regulation also is complex because it occurs at different levels including gene transcription, glycosylation, cell trafficking and secretion, activation of latent forms, specific inhibition by the tissue inhibitors of metalloproteases (TIMP), and binding to cell receptors that concentrate their proteolytic activity in discrete areas of the cell membrane (Fig. 1). Both the multiplicity of their targets and the complexity of their regulation account for a variety of biological effects, which sometimes could be opposite and make it difficult to predict the role of MMPs in a given pathophysiologic condition. Thus, it is important to realize that in renal diseases MMP effects may be different and/or opposite during the different phases of the pathology evolution.

In this review, we focus on gelatinases (MMP-2, 72 kd; MMP-9, 92 kd), a subfamily of
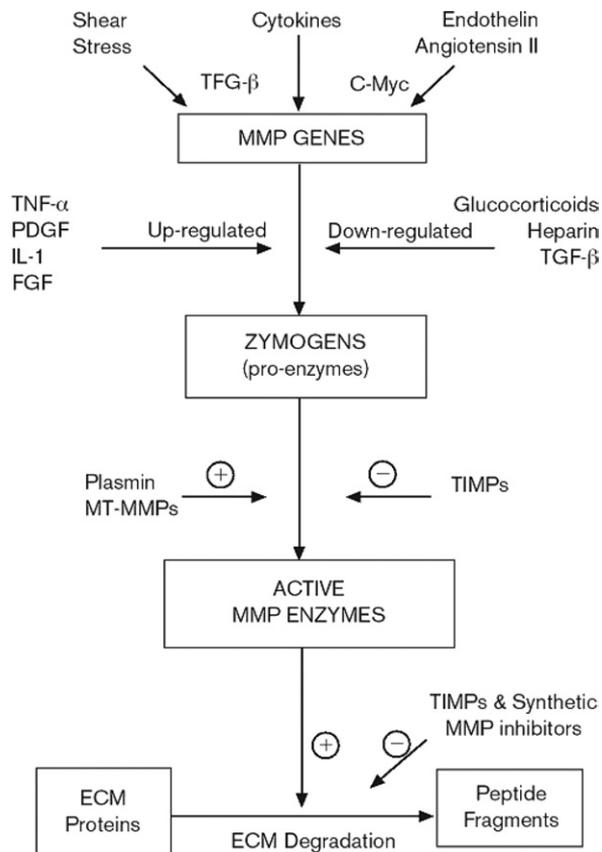
Figure 1. Regulation of MMPs at 3 levels: gene transcription, activation of latent proenzyme, and via TIMPs. TNF, tumor necrosis factor. Reprinted with permission from Donnelly R, Collinson DJ, Manning G. Hypertension, metalloproteinases and target organ damage. J Hypertens. 2003;21:1627-30.[45]

MMPs that degrade gelatins (denatured collagens), and basement membrane (BM) components, mostly type IV collagen. Glomerular and tubular BMs are known to play a key role in induction, progression, and repair of renal disease because BM damage is a major event in crescentic glomerulonephritis, Alport's syndrome (AS), and epithelial to mesenchymal transition (EMT) of epithelial tubular cells. In addition, MMP-9 can cleave type V collagen, aggrecan, and elastin,[3] whereas MMP-2 can degrade fibronectin and laminin.

Unlike MMP-2, which mostly is expressed constitutively, MMP-9 has a restricted pattern of expression in developmental and adult tissues,[3] and is highly regulated by many cytokines and growth factors. MMP-2 and MMP-9 also differ by their mode of activation because pro–MMP-2 is activated at the cell surface by a membrane-type MMP, whereas the activation of pro–MMP-9 mostly occurs via plasmin(ogen)-dependent and -independent mechanisms.[4]

Both MMP-2 and MMP-9 also have the ability to form proenzyme complexes with their endogenous inhibitors, the TIMPs. TIMP-1 and TIMP-2 are the main inhibitors of MMP-9 and MMP-2, respectively.[5] A greater degree of complexity results from the fact that MMP-2 activation requires the binding of TIMP-2. However, TIMP-1 and TIMP-2 functions are not limited to MMP regulation, but also involve apoptosis and cell growth–modulating activity independently of MMP inhibition.[6,7] In addition to TIMPs, α2-macroglobulin, tissue factor protease inhibitor-2, and thrombospondins[8] are natural inhibitors of gelatinases.

HEADS: ROLE OF MMPS IN ECM REMODELING

Matrix homeostasis in normal tissues is a balance between matrix production and degradation. The excessive matrix accumulation seen in fibrotic kidney results from a combination of overproduction of matrix components and defective degradation. This notion is supported by many observations showing that TIMP-1 and plasminogen activator inhibitor-1, a potent inhibitor of the fibrinolytic cascade, are up-regulated in a diseased kidney. About 30 matrix proteins have been characterized as substrates of MMP in vitro. Nonetheless, the dogma that the main role of the MMPs is to cleave matrix proteins is still controversial for at least 3 reasons. First, only 2 matrix proteins, collagen type IV and aggrecan, were shown to be cleaved in vivo.[9-11] Second, recent studies using knockout mice have painted a different and complex picture of the function of MMPs (and of the plasminogen/plasmin system as well) in relation to fibrotic lesions in vivo. Except for the membrane type 1 MMP–deficient mice that develop fibrosis of soft tissues,[12] other MMP knockout mice do not spontaneously develop tissue fibrosis, although this may be explained in some cases by redundancy (eg, compensation by other MMPs).[11] Even more, transgenic mice overexpressing MMP-2 display fibrotic lesions.[13] Last but not least, no side effects rela-

tive to connective tissue were reported in tumor treatment studies using MMP inhibitors. These results raise the question: can MMP really help to prevent or resolve fibrosis in pathologic conditions?

Because gelatinases degrade BM components, it initially was suggested that they could have a deleterious effect in inflammatory glomerulonephritis involving lesions of the glomerular BM (GBM), but that they could prevent accumulation of BM material in chronic renal disease. From a theoretic point of view, gelatinases can have opposite effects on the development of renal fibrosis and progression of renal diseases. On the one hand, MMP-9 usually is considered as an antifibrotic enzyme because it cleaves type IV collagen and particularly denatured collagens that result from the action of interstitial collagenases. On the other hand, MMP-9 also can be viewed as a profibrotic agent because it activates transforming growth factor β (TGF-β) and endothelin-1[3] and the degradation of type IV collagen may favor EMT of tubule epithelial cells.[14] Furthermore, MMP-2 was found to be necessary and sufficient to induce tubular cell EMT in vitro.[15] The role of MMP-2 and MMP-9 in renal disease progression in vivo was investigated recently by using pharmacologic agents and knockout mice.

Role of Gelatinases in Hypertensive Nephrosclerosis and Vascular Remodeling

To address the question regarding the role of gelatinases in hypertensive nephrosclerosis and vascular remodeling, hypertension and renal failure were induced by inhibiting nitric oxide (NO) synthesis in rats.[16] After 1 month of hypertension, animals displayed a decrease of renal function (evidenced by increased levels of plasma creatinine with proteinuria), an exaggerated gene and protein expression of TGF-β, collagen I and collagen IV within the renal vasculature, and an abnormal accumulation of ECM in glomeruli. These structural and functional alterations were accompanied by increased activities of MMP-2 and MMP-9. The administration of an angiotensin II (AngII)-receptor antagonist immediately decreased collagen I, collagen IV, and TGF-β gene and protein

expressions without affecting the activities of MMP-2 and MMP-9. These cellular alterations were accompanied by a gradual regression of glomerulosclerosis and restoration of renal function; after 1 month of antihypertensive treatment, all functional and structural parameters of the kidney were normalized.

The mechanism of regression, at least in the NO model, appears to be dual: inhibition of collagen synthesis as a result of angiotensin II type 1 (AT1)-receptor antagonism and activation of MMP that probably is associated with the degree of fibrosis independently of AT1 blockade. These results are corroborated by the observation that in experimental models and human diseases in which ECM accumulation is observed, fibrosis most often is accompanied by a sustained increase in MMP expression. The up-regulated MMP expression presumably reflects cellular compensatory mechanisms aimed at limiting the rate of matrix accumulation. It is likely but still not proved that integrins and other ECM receptors, including the discoidin domain receptor of collagen, inform the cells that ECM is accumulating in the cellular microenvironment, thus triggering a cascade of events that leads to sustained MMP synthesis and activity.[17]

Because MMP-9–deficient mice show a mild renal phenotype characterized by a 12% reduction in nephron number without renal impairment in the first 6 months of life,[18] we reasoned that these mice could be used to evaluate how MMP-9 contributes to the progression of hypertensive nephrosclerosis in vivo. We expected that MMP-9 deficiency would have no impact on blood pressure, which is a prerequisite to investigate the protective effect of MMP-9 on renal fibrosis irrespective of systemic hemodynamics. Although the baseline blood pressure was similar in wild-type and MMP-9$^{-/-}$ mice, the increase in blood pressure was significantly greater in MMP-9$^{-/-}$ mice treated with NG-nitro-L-arginine methyl ester (L-NAME) (which inhibits NO synthase), AngII, or L-NAME plus salt (which increases mouse sensitivity to NO inhibition)[19] (Fig. 2). In the last 2 models, the systolic pressure increment was more important in MMP-9$^{-/-}$ animals than in the wild-type animals as early as 3 days, and only knockout animals
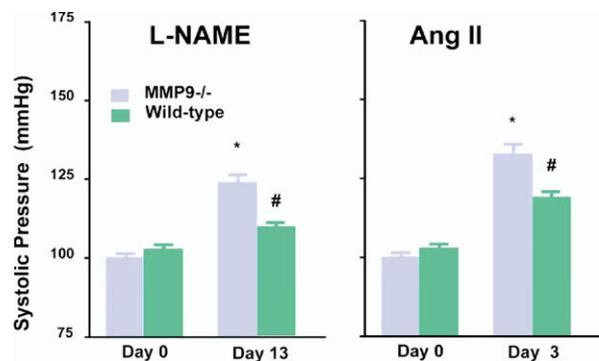
Figure 2. The lack of MMP-9 is accompanied by an early increase of systolic pressure. Rats were treated with L-NAME, an inhibitor of NO synthase, or AngII, for 13 and 3 days, respectively. *P < .01 vs control; #P < .01 wild-type vs knockout (original data).

displayed an increment in pulse pressure after 10 days of AngII infusion.[19]

To investigate the blood-pressure effect of MMP-9 deficiency further, animals were treated with AngII (1 μg/kg/min, 10 days) plus 5% NaCl diet. In wild-type mice, this regimen increased the carotid artery pressure–diameter relationship significantly (eg, the vessel wall compliance), whereas in MMP-9$^{-/-}$ mice carotid artery compliance actually was reduced after AngII treatment.[19] In comparison, compliance of resistance vessels was unaffected by AngII treatment in either phenotype. AngII induced MMP-2 and increased carotid media thickness equally in both phenotypes. However, only wild-type mice showed MMP-9 induction and enhanced in situ gelatinase activity on AngII treatment, and vessels from these mice also produced more collagen breakdown products (CITP) than their MMP-9$^{-/-}$ counterparts. Inversely, staining for collagen IV was particularly enhanced in vessels from MMP-9$^{-/-}$ mice treated with AngII. These results show that (1) the onset of AngII-induced hypertension is accompanied by increased MMP-9 activity in conductance vessels, concurrent with an increase in vessel distensibility; (2) absence of MMP-9 activity results in vessel stiffness and increased pulse pressure; and (3) MMP-9 activation plays a beneficial role early on hypertension by preserving vessel structure and alleviating blood pressure increase. This suggests that, at least in the short term, MMP activation exerts a beneficial effect by counteracting the prohypertensive stimulus. Of note, hypertension-induced vessel thickening and medial hypertrophy were similar in wild-type and knockout animals, indicating that organization of the vessel wall components, rather than medial mass, is the primary determinant of compliance. The involvement of MMP-9 in the reorganization of a collagenous matrix also was observed in experiments using MMP-9–deficient aortic smooth muscle cells that showed decreased migratory activity and reduced capacity to contract collagen, compared with wild-type cells.[20]

In the setting of long-term NO synthase inhibition, plasminogen activator inhibitor-1 deficiency alone is sufficient to protect against the structural vascular changes that accompany hypertension.[21] Whether this effect is mediated, at least partly, by overactivation of gelatinases, particularly MMP-9, remains to be established.

Dual Role of Gelatinases in Progressive Kidney Disease: Case of Experimental AS

Significant advances were made recently in understanding the role of MMPs, particularly gelatinases, in the progression of renal disease by using an animal model of AS deficient in the α3 chain of type IV collagen (α3[IV]).[11] GBM, a key component of the blood-filtration system, is formed through the assembly of type IV collagen with laminins, nidogen, and sulfated proteoglycans. Mutations or deletions involving α3(IV), α4(IV), or α5(IV) chains of type IV collagen have been identified as the cause for AS in human beings, a progressive hereditary disease associated with deafness. Previous studies have suggested that abnormal persistence of α1(IV) and α2(IV) isoforms instead of α3(IV), α4(IV), and α5(IV) chains is associated with an increased susceptibility to proteases.[22] However, studies using mice that are deficient in both the α3(IV) chain of type IV collagen and MMP-9 failed to provide evidence for the role of MMP-9 in the initiation and progression of renal disease.[23]

Zeisberg et al[11] confirmed that proteolysis of human as well as mouse Alport BM was enhanced significantly compared with normal BM by MMP-2, MMP-3, and MMP-9. At 4 weeks (eg, before the onset of proteinuria in the α3(IV)$^{-/-}$

mice), MMP-2, MMP-3, and MMP-9 levels were up-regulated significantly in glomeruli, whereas after 8 weeks of age (when glomerular kidney disease is established and progresses toward tubulointerstitial fibrosis), MMP expression spread from the glomeruli into the tubulointerstitial compartment. Genetic ablation of either MMP-2 or MMP-9, or both MMP-2 and MMP-9, led to compensatory up-regulation of other MMPs in the kidney glomerulus, which most likely accounts for the lack of aggravated phenotype in the double-knockout MMP-9$^{-/-}$ and α3(IV)$^{-/-}$ mice as compared with the α3(IV)$^{-/-}$ mice that express normal levels of MMP-9.[23] To evaluate the impact of type IV collagen–degrading proteases on the progression of kidney disease associated with AS, combined pharmacologic inhibitors of MMP-2, MMP-3, and MMP-9 were administered orally to the α3(IV)$^{-/-}$ mice. Pharmacologic ablation of enzymatic activity before the onset of proteinuria and GBM structural defects led to significant attenuation in the disease progression associated with delayed proteinuria and markedly prolonged survival (Fig. 3). In contrast, inhibition of MMPs after induction of proteinuria led to the acceleration of disease associated with extensive interstitial fibrosis and early death.

These important findings suggest that at early stages MMPs help to break down the GBM, whereas at later stages they may help to remove ECM associated with scarring and fibrosis. This probably may apply to a number of pathologic conditions.

EMT: A Deleterious Side Effect of Gelatinase-Induced BM Degradation

The beneficial effect of MMP-9 on renal disease progression at later stages of AS may represent only part of the picture. By degrading type IV collagen, gelatinases also theoretically may aggravate disease progression via EMT, a central mechanism for diversifying the cells found in complex tissues. Studies on renal fibrosis indeed suggest that a third of all disease-related fibroblasts originate from tubular epithelia at the side of injury after unilateral ureteral obstruction,[24] although this proportion may be much lower in other pathologic settings. In the past 5 years, experimental evidence has accu-
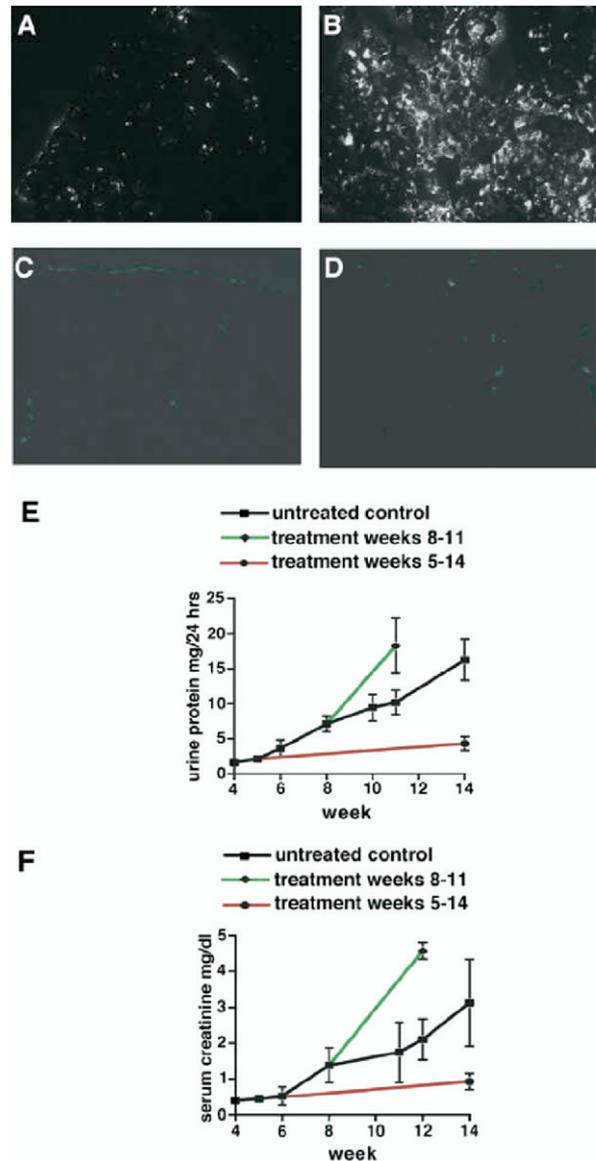

Figure 3. Effect of combined MMP inhibition on the progression of renal disease in α3(IV)$^{-/-}$ mice. (A–D) In situ zymography displayed gelatin-degrading activity in normal kidney (A), which was increased substantially in kidneys of 14-week-old COL4A3$^{-/-}$ mice (B). Incubation with the MMP-2, MMP-3, or MMP-9–inhibitor cocktail significantly blocked MMP activities in kidneys from (C) wild-type mice and (D) kidneys from COL4A3$^{-/-}$ mice. (E and F) Renal function in α3(IV)$^{-/-}$ mice. (E) Urinary protein excretion was measured in 24-hour metabolic cage collections. (F) Serum creatinine level was determined biweekly. The graphs display the progression of urinary protein excretion and serum creatinine levels from 4 to 14 weeks of age. In each panel, disease progression is shown in untreated control mice (black), in mice that received MMP-inhibitors starting at 5 weeks of age (red), and in mice that received MMP-inhibitors starting at 8 weeks of age (green). Reprinted with permission from Zeisberg et al.[46]

Table 1. Cleavage of Nonmatrix Substrates by MMP-9

In Vitro	In Vivo
Plasminogen → angiostatin	α1-proteinase inhibitor
Cytokines (*IL-1β, tumor necrosis factor-α, IL-10*)	Fibrin
IL-2 receptor (α chain)	*β1-crystallin*
CXC-chemokines (*IL-8*, Gro-α, SDF-1, PF-4)	*Stem cell factor*
TGF-β	
Insulin, substance P	
Big ET-1 → *ET1*	
Aβ amyloid peptide	

NOTE. Italics denote activation or induction by MMP-9.

mulated in favor of the implication of both MMP-2 and MMP-9 in this process.

Cheng and Lovett[15] showed that active MMP-2 is absolutely required for EMT induced by TGF-β1. In addition, purified active MMP-2 alone is sufficient to induce EMT in the absence of exogenous TGF-β1. MMP-2 also may mediate EMT in a paracrine manner through the proteolytic generation of active TGF-β1 peptide. These results obtained in cell culture were confirmed recently by the same group[13] in mice overexpressing constitutively active MMP-2 specifically in the renal proximal tubule. This MMP-2 expression is sufficient to generate the entire spectrum of pathologic and functional changes characteristic of human chronic kidney disease. At the earliest point, MMP-2 leads to structural alterations in the tubular BM (TBM), a process that triggers tubular EMT, with resultant tubular atrophy, fibrosis, and renal failure.

A role for MMP-9 also has been established in mice with ablation of tissue-type plasminogen activator, which actually protects the kidney from developing interstitial fibrosis in obstructive nephropathy.[14] The pathogenic effect of tissue-type plasminogen activator in this model primarily depends on its ability to induce *MMP-9* gene expression. Increased MMP-9 disrupts the integrity of tubular BM, which promotes EMT.

A recent study also showed that MMP-3 induced Rac1 expression, which causes an increase in cellular reactive oxygen species and promotes EMT.[25]

Overall, these data suggest that in conditions such as unilateral ureter obstruction, in which EMT seems quantitatively important, gelatinases may have the potential to induce EMT and aggravate the renal disease. In contrast, in settings in which EMT is low, gelatinases may slow down renal disease progression by preventing the accumulation of ECM.

TAILS: NONMATRICIAL EFFECTS OF MMPS: TOWARD NEW SUBSTRATES AND BIOLOGICAL ACTIVITIES

In the past few years, it has become clear that MMP substrates are far from being limited to ECM components.[2] The ability of these enzymes to cleave biologically active peptides including cytokines and chemokines can indeed override their ECM proteolytic actions in some, if not many, pathologic conditions. It is beyond the scope of this article to review all nonmatricial effects of MMPs. We concentrate on gelatinases, and particularly MMP-9, to illustrate the multiplicity of potential substrates and of resulting biological activities.

The number of nonmatrix molecules that may serve as substrates for MMP-9 is increasing continuously (Table 1). In vitro, MMP-9 was shown to activate and/or induce interleukin (IL)-1β, tumor necrosis factor-α, IL-8, IL-10, TGF-β, and endothelin-1, whereas it cleaves the IL-2 receptor α chain, several CXC-chemokines, insulin, substance P, and Aβ-amyloid peptide.[3] More importantly, it was shown that MMP-9

could degrade in vivo the $\alpha 1$-proteinase inhibitor,[26] which is a major antagonist of neutrophil elastase, fibrinogen and fibrin,[27] and lens $\beta B1$ crystallin.[28] MMP-9 also can activate the stem cell factor in vivo by releasing its soluble form from the membrane precursor, which triggers hematopoietic reconstitution after bone marrow ablation.[29]

Similar to its dual effects on fibrogenesis, MMP-9 can exert antagonistic effects in renal inflammation and injury. On one hand, MMP-9 can behave as a proinflammatory mediator and damage the kidney through activation of proinflammatory molecules, as indicated in the previous paragraph. On the other hand, MMP-9 can exert opposite anti-inflammatory protective effects through activation of TGF-β,[30] degradation of IL-1β, and cleavage of the IL-2 receptor. Therefore, MMP-9 effects may vary from one model/pathologic condition to another, depending on the inflammatory pathways that are involved.

By using MMP-9–deficient mice, it was shown that the absence of MMP-9 protected mice from experimental autoimmune encephalomyelitis[31] and bullous pemphigoid (BP).[26,32] Three- to 4-week-old mice were resistant to autoimmune encephalomyelitis, whereas no differences in mortality, incidence, mean day of onset, and mean disease score were observed in adult MMP-9$^{-/-}$ versus control mice, suggesting the action of alternative or compensatory pathways by other MMPs. Young MMP-9$^{-/-}$ mice showed a significant reduction in infiltrated neutrophils in comparison with controls, which may be compatible with the observation that MMP-9 activates IL-8, a potent neutrophil chemoattractant. Cleaved α-1-proteinase inhibitor, a product of MMP-9 action, also is a potent chemoattractant for neutrophils. Experimental BP nicely illustrates the point that despite broad substrate specificity of MMP-9 against ECM substrates in vitro, specificity in vivo is yet highly restricted, which may represent a general paradigm for other MMPs. BP is an immune inflammatory skin disease that is initiated by deposition of autoantibodies and complement components at the BM zone. The 180-kd transmembrane hemidesmosomal protein BP180 (type XVII collagen), a target of autoantibodies, is cleaved during BP,

and this is accompanied by separation of the epidermis from the dermis and intense neutrophil infiltration. Mice deficient in either MMP-9 or neutrophil elastase (NE) are resistant to blister formation in response to anti-BP180 antibodies in a mouse model. Disease develops on complementation of MMP-9$^{-/-}$ mice with NE$^{-/-}$ neutrophils or NE$^{-/-}$ mice with MMP-9$^{-/-}$ neutrophils.[26] Although MMP-9 has been shown to cleave BP180 in vitro, only NE degrades BP180 and produces dermal-epidermal separation in vivo. Instead, MMP-9 acts upstream to regulate NE activity by inactivating $\alpha 1$-proteinase inhibitor.

Role of MMPs in Crescentic Glomerulonephritis: Case of Experimental Anti-GBM Nephritis

By analogy with the results observed in experimental BP, in which the initial lesion affects the BM, we asked whether MMP-9 deficiency also could protect mice in the accelerated model of anti-GBM nephritis. We speculated that crescent formation would be less in MMP-9$^{-/-}$ mice because MMP-9 can cleave almost all GBM components, particularly type IV collagen, and thus favor the issue of fibrin in the urinary space. Surprisingly, renal failure, protein loss, and glomerular lesions were more severe in MMP-9$^{-/-}$ mice that showed extensive crescent formation with more abundant fibrin deposits as compared with control mice.[27] The increased severity of the disease could not be accounted for by increased antibody deposition at the GBM, increased macrophage infiltration, or changes in IL-1β and IL-10 production by the diseased glomeruli. The greater extent of fibrin deposits and crescents in MMP-9$^{-/-}$ mice led us to identify fibrin as a novel non-ECM substrate of MMP-9 (Fig. 4).[27] We showed that MMP-9 could degrade fibrinogen and fibrin in vitro by using zymography, as well as fibrin deposits in situ in crescentic glomeruli. These results indicate that MMP-9, similar to plasmin, contributes to fibrinolytic activity in this model, thus reducing accumulation of fibrin, an important mediator of glomerular injury.

In contrast to MMP-9, macrophage metalloelastase MMP-12 seems to play a protective
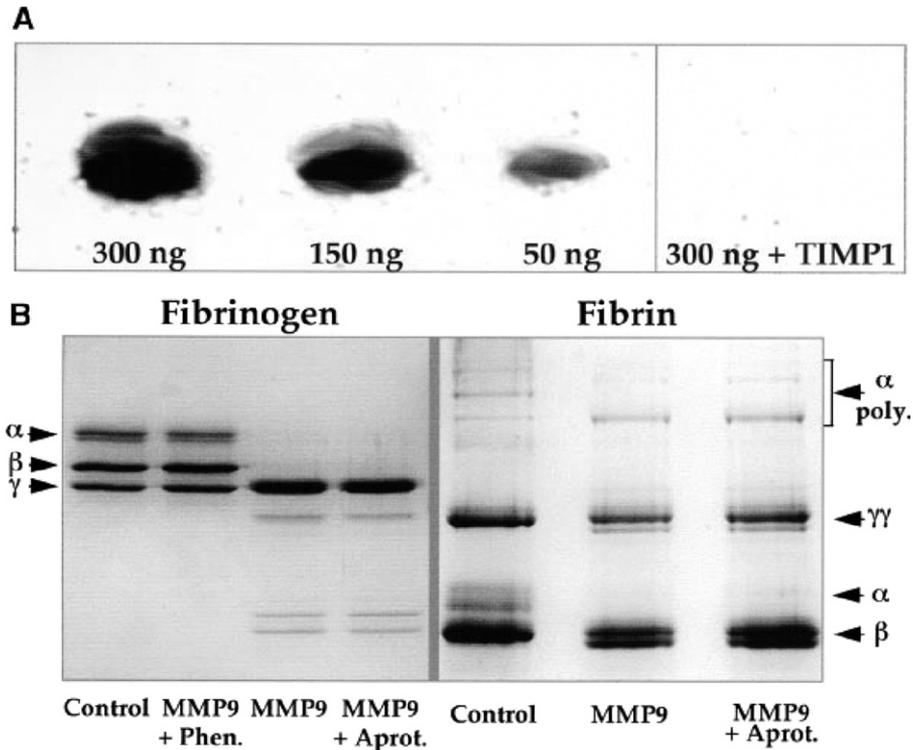
Figure 4. Fibrinolytic activity of MMP-9. (A) Zymogram performed on fibrin gel. A total of 300 ng, 150 ng, and 50 ng of activated recombinant mouse MMP-9 were loaded on 8% sodium dodecyl sulfate polyacrylamide gels that were applied to a fibrin gel. Note a lytic band at the expected molecular size for the active form of MMP-9. Its intensity was proportional to the quantity of MMP-9. Lytic activity was inhibited when 10 μg/mL of recombinant human TIMP1 was incorporated into the fibrin gel (300 ng plus TIMP1). (B) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of 10 μg fibrinogen and 3 μg fibrin after a 24- or 72-hour incubation, respectively, with 300 ng of activated recombinant mouse MMP-9 alone or in the presence of a metalloprotease inhibitor (1 mmol/L 1,10-phenanthroline [Phen.]) or of a plasmin inhibitor (8 U aprotinin [Aprot.]). Note that MMP-9 degraded Aα and Bβ chains of fibrinogen (compare MMP-9 with control), and that degradation was inhibited by 1,10-phenanthroline (MMP-9 + Phen versus MMP-9) but not by aprotinin (MMP-9 + Aprot. versus MMP-9). The achain and apolymers of fibrin chains were preferentially degraded by MMP9 (MMP-9 versus control). Degradation was not inhibited by aprotinin (MMP-9 + Aprot. versus MMP-9). Reproduced from Journal of Experimental Medicine, 2001, 193:793-802. Copyright 2001 Rockefeller University Press.[27]

role in a similar anti-GBM model in the rat. Crescent formation and macrophage infiltration were reduced significantly in rats treated with antirat recombinant MMP-12 antibody, and the amount of urinary protein also was decreased.[33]

In human antineutrophil cytoplasmic antibody–associated glomerulonephritis, in which fibrin deposits often are extensive, MMP-2-, MMP-3-, MMP-9-, and TIMP-1–positive cells were detected in crescentic glomeruli, with the number of MMP-9–expressing cells per glomerulus correlating to the percentage of crescentic glomeruli.[34] Increased glomerular expression of MMP-9, but not of MMP-2, also has been reported in human im-munoglobulin A nephropathy, mesangial prolifer-ative glomerulonephritis, and lupus nephritis.[35]

It is clear that all of the substrates of MMPs have not been discovered yet, and that the many ways in which these enzymes can influence complex biological processes is just beginning to be unveiled. It also has become evident that the ECM degradation products released by MMPs can have important biological actions, as previously shown for laminin-5[36] and tumstatin.[37] Previous assumptions about the antifibrotic role of MMPs in renal disease progression appear to be overly simplistic. The effect of these enzymes probably depends on the nature

and etiologies of the diseases as well. The availability of double- and triple-MMP knockout mice hopefully will provide valuable information about which MMP family members to target for therapeutic interventions.

THERAPEUTIC PERSPECTIVES AND FUTURE DIRECTIONS

The presence of MMP-2 and MMP-9 in malignant and metastatic cells generated increased interest in the 1990s in developing pharmaceutical inhibitors. These inhibitors can be divided into 3 categories: peptidomimetics, nonpeptidomimetics, and tetracycline derivatives. Peptidomimetics (eg, Batimastat and Marmistat) contain a sequence that resembles MMP substrates but are relatively nonspecific. They have shown limited benefit in advanced cancer, with intolerance as a result of joint and muscle pain. The nonpeptidomimetics (eg, Bay 12-9566 and Prinomastat) were synthesized to resemble the catalytic pocket of MMPs. Some of these compounds are more selective for gelatinases[38] and for MMP-3.[39] They have been tested on patients with advanced cancer. Although side effects are not an issue, phase III trials were stopped because of a lack of efficacy.[40] Tetracycline, doxycycline, and chemically modified tetracyclines all can decrease MMP production and activity. Doxycycline has been used successfully to prevent periodontitis, a disease in which the inflammatory cytokines that are elicited by bacteria in the gingival tissues result in substantial connective-tissue destruction by MMPs and loss of teeth.[41]

In renal diseases, MMP nonpeptidomimetic inhibitors have been used recently in experimental models of AS and chronic allograft nephropathy. In AS, MMP inhibitors induced significant attenuation of renal disease when administered at an early phase before the onset of proteinuria.[11] But if this window of opportunity was lost, MMP inhibition at the later stages led to significant acceleration of glomerular and interstitial fibrosis. Similarly, inhibition of MMPs early after transplantation reduced the development and progression of chronic allograft nephropathy, but promoted chronic allograft nephropathy if initiated at later stages.[42] There is, to our knowledge, no information on the effect of MMP inhibitors in acute renal failure related to tubular cell injury, but interesting lessons may be drawn from their use in stroke. Earlier studies supported the view that inhibiting MMP-9 after stroke is neuroprotective.[43] MMP inhibitors ameliorate blood-brain barrier disruption and reperfusion injury. In contrast, a recent study suggested that late inhibition of MMP-9 is detrimental[44] because it prevents activation of vascular endothelial growth factor, which has an important role during neurovascular repair after stroke.

Theses findings illustrate that the major challenge with therapeutic interventions using MMP inhibitors remains how to accomplish temporal and spatial control of their activity in a target organ, tissue, or system. The kidney is no exception. Given the multiple targets of MMPs, and their variable implications depending on the stage and etiology of the nephropathy, it is premature to predict the therapeutic usefulness of the matrix-degrading enzymes or their inhibitors in human renal diseases, and particularly in those leading to renal fibrosis. It seems mandatory to first identify stage-specific biomarkers and to further unravel the pathways in which MMPs are involved in a given nephropathy before transferring the use of MMP inhibitors to the renal patient.

Acknowledgment

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