Role of matrix metalloproteinases in kidney development and glomerulopathy: lessons from transgenic mice

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Abstract
Matrix metalloproteinase-9 (MMP9) is required for renal organogenesis in vitro and is increased in various nephropathies. We analysed the renal phenotype of MMP9-deficient mice and their susceptibility to a murine model of proliferative glomerulonephritis. MMP9 deficiency resulted in adult mice in a 12% nephronic reduction. Histological appearance and renal function of these mice was normal up to 12 months, at which time histological lesions appeared. In addition, glomerulonephritis was more severe in MMP9-deficient mice than in their control 3-month-old mates. In particular, the extent of crescent formation and fibrin deposition was greater, which led us to show that fibrin is a critical substrate for MMP9. These data provide the first demonstration in vivo that MMP9 is required for nephron mass formation and renal function in elderly mice, and further evidence of a novel protective effect of MMP9 on the development of fibrin-induced glomerular lesions.

Keywords: fibrinogen; glomerulonephritis; kidney development; matrix metalloproteinase

Introduction
Matrix metalloproteinase-9/gelatinase B (MMP9) has a restricted pattern of expression in developmental and adult tissues. Its principal substrate is type IV collagen, but it can degrade other basement membrane molecules as well as substrates that are not matrix components. We have shown previously that MMP9 is produced at the first stage of kidney embryogenesis in vivo and is required for branching morphogenesis in vitro [1]. We took advantage of the availability of MMP9-deficient mice [2] to analyse their renal phenotype under physiological conditions and in an accelerated model of crescentic proliferative glomerulonephritis [anti-glomerular basement membrane (GBM) nephritis].

MMP9-deficient mice have an abnormal renal phenotype
We postulated that MMP9 deficiency in mice would impede kidney growth. MMP9-deficient mice are actually viable, suggesting the absence of major kidney defects. However, we did observe two renal abnormalities. First, MMP9-deficient adult mice have a 12% reduction of nephron number, that most probably results from subtle defects of branching morphogenesis. Secondly, 12-month-old deficient mice develop renal impairment and atrophy compared with control mice. This is probably due to the addition of fibrotic lesions with inflammatory cell infiltration to the reduced nephron mass. This modest, but highly significant phenotype, is of the same order of magnitude as that observed in long bones [2].

MMP9 deficiency aggravates functional and histological features of anti-GBM nephritis
Since there is no renal alteration in 3-month-old MMP9-deficient mice, we used 3-month-old MMP9-deficient and control mice to induce the accelerated model of anti-GBM glomerulonephritis [3]. This model is characterized by a rapid development of severe crescentic proliferative glomerulonephritis in which fibrin is an important mediator of glomerular injury and renal impairment. By analogy with the results observed in bullous pemphigoid [3] and since MMP9 can degrade almost all GBM components, we expected that MMP9 deficiency would have a protective effect by inhibiting GBM degradation and, consequently, the issue of fibrin in the urinary space of the glomerulus.

However, MMP9 deficiency unexpectedly was associated with functional and histological exacerbation of glomerular injury. There was, in these mice, a...
Fig. 1. Fibrinolytic activity of MMP9. (A) Zymogram performed on a fibrin gel. Aliquots of 300, 150 and 50 ng of activated recombinant mouse MMP9 were loaded on 8% SDS–polyacrylamide gels that were applied to a fibrin gel. Note that the intensity of the lytic band was proportional to the quantity of MMP9. Lytic activity was inhibited when recombinant human TIMP1 (10 μg/ml) was incorporated in the gel.
significantly increased propensity to form crescents. Crescents result from accumulation, in the urinary space, of fibrin that is chemotactic for macrophages and favours proliferation of glomerular parietal epithelial cells [5,6]. Glomerular fibrin deposition was indeed significantly increased in MMP9-deficient mice. The increased renal impairment, fibrin deposition and crescent formation in these mice suggest that MMP9 plays a important role in protecting glomeruli against injury. The last step was to determine the mechanism.

MMP9 deficiency does not alter immune parameters, IL-1β and IL-10, in anti-GBM nephritis

The extent of immune response to the exogenous antigen could not account for the severity of injury since circulating levels of mouse anti-sheep IgG antibodies, and antibody deposition to the basement membrane were similar in MMP9 (+/−) and MMP9 (+/+ ) mice. Macrophages have a pivotal role in the transition between inflammation and repair through the balanced secretion of pro- and anti-inflammatory cytokines. Although the numbers of glomerular and periglomerular macrophages were not significantly altered by MMP9 deficiency, it was still possible that macrophage phenotype was altered. We analysed interleukin (IL)-1β and IL-10 for two reasons. First, IL-1β enhances glomerular fibrin deposition and crescent formation in this model [7] while IL-10 has a protective effect [8]. Secondly, MMP9 degrades IL-1β in vitro [9], and increases IL-10 production in a model of skin contact hypersensitivity [10]. We hypothesized that IL-1β degradation or IL-10 production could be altered in MMP9-deficient mice with anti-GBM pro-liferative glomerulonephritis. However, we could not find any significant differences in the production or degradation of these cytokines that could account for the increment of glomerular lesions.

MMP9 is a fibrinolytic enzyme

We finally asked whether MMP9 could exert a protective effect in the development of glomerular lesions by degrading fibrin. We performed three sets of experiments to answer this question. First, we tested MMP9 fibrinolytic activity by fibrin zymography and we found that MMP9 produced lysis bands in a fibrin gel, in a dose-dependent manner (Figure 1A). Secondly, we determined the sensitivity of fibrinogen and fibrin chains to MMP9 proteolysis. MMP9 degraded the Aα and Bβ chains of fibrinogen and the z chains and z polymers of fibrin. The action of MMP9 on fibrinogen and fibrin was direct, and not plasmin mediated, since aprotinin, a plasmin inhibitor, did not prevent MMP9-induced fibrin degradation (Figure 1B). Thirdly, we verified that MMP9 could degrade fibrin in situ. We incubated serial kidney cryosections from MMP9-deficient mice sacrificed 15 days after anti-GBM injection with glomerular extracts producing or not producing MMP9, then fibrin was revealed by immunofluorescence. Our data show that MMP9 contributes with plasmin [11] to the breakdown of fibrin caps observed in glomeruli during the course of anti-GBM glomerulonephritis (Figure 1C).

These results indicate that MMP9 is required for in vivo nephron mass formation and preservation of renal function, and further reveal a novel protective non-matrix effect of this enzyme on the development of fibrin-induced glomerular lesions. Thus, our data add to the increasing list of MMP9 substrates that are not conventional matrix components. Only one substrate, α1-proteinase inhibitor, had been identified previously as an MMP9 substrate in vivo [12].

References


Continued from p. 29 fibrin gel (300 ng + TIMP1). (B) SDS-PAGE analysis of fibrinogen (10 μg) and fibrin (3 μg) after a 24 or 72 h incubation, respectively, with 300 ng of activated recombinant mouse MMP9 alone or in the presence of a metalloprotease inhibitor (1 mM 1,10-phenanthroline; Phen.) or of a plasmin inhibitor (8 U of aprotinin; Aprot.). Note that MMP9 degraded Aα and Bβ chains of fibrinogen (compare MMP9 with Control), and that degradation was inhibited by 1,10-phenanthroline (MMP9 + Phen. vs MMP9) but not by aprotinin (MMP9 + Aprot vs MMP9). The z chain and z polymers of fibrin chains were degraded preferentially by MMP9 (MMP9 vs Control). Degradation was not inhibited by aprotinin (MMP9 + Aprot vs MMP9). (C) Fibrinolytic activity of MMP9 on tissue sections. (1) Gelatinase substrate buffer; (2) glomerular extracts from +/+ control mice (15 days after anti-GBM injection) treated with plasmin inhibitor (1000 U/ml aprotinin); (3) glomerular extracts from +/+ control mice (15 days after anti-GBM injection) treated with plasmin inhibitor and metalloprotease inhibitor (1 mM 1,10-phenanthroline); (4) glomerular extracts from MMP9 −/− mice (15 days after anti-GBM injection) treated with plasmin inhibitor. Fibrin deposits on glomerular sections were detected by immunofluorescence. Note that glomerular extracts containing MMP9 activity strongly reduced the intensity of glomerular fibrin deposits in the absence of plasmin activity (2). This effect was abolished when MMP9 activity was inhibited (3) or absent (4). Bar: 400 μm. Reproduced from The Journal of Experimental Medicine 2001; 193: 793–802 by copyright permission of The Rockefeller University Press.


