

# Commentary

## MMP-12, An Old Enzyme Plays a New Role in the Pathogenesis of Rheumatoid Arthritis?

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Rheumatoid arthritis (RA) is a chronic inflammatory disorder of unknown cause that is notorious for the chronic polyarticular synovial inflammation and progressive destruction of affected joints. The clinical outcome caused by irreparable degradation of cartilage, tendon, and bone and, if untreated, is usually aggressive, which is well known for both physicians and patients. Although the etiology for RA has not been completely understood, it is generally believed that the joint destruction process is mediated by the concerted action of various proteinases including metalloproteinases (MMPs) (see reviews<sup>1-2</sup>). Although emerging data suggest that MMPs may be associated with the severity of RA, it is not clear (1) whether elevation of these enzymes are causative for or consequence of the disease, (2) how these enzymes work collaboratively, and (3) whether inhibition of these MMPs may be conclusively beneficial for the treatment of RA. Since many MMPs are generated by a variety of cells in RA lesions, it is nearly impossible to investigate which individual MMP is critical among the others in human RA. Thus, development of MMP transgenic and knockout animals provides a powerful tool that will allow scientists to dissect and elucidate possible functional roles of each MMP in RA. To date, deletion of gelatinases, MMP-2, and MMP-9 in KO mice have shown that these MMPs play opposite roles in antibody-induced arthritis.<sup>3</sup>

MMPs are a family of zinc endopeptidases that are structurally and functionally related. The MMPs are capable of degrading a variety of extracellular matrix protein components including the collagens, proteoglycans, fibronectin, and laminin, all of which are present in the joint connective tissues.<sup>2</sup> Until now, more than 25 different MMPs have been identified and many of them have been shown to be associated with RA. The MMP family can be classified into five major groups based on the substrates: (1) the collagenases (MMP-1, -8, -13), (2) gelatinases (MMP-2, -9), (3) the stromelysins (MMP-3, -10, -11), (4) a heterogeneous subgroup including matrilysin (MMP-7), enamelysin (MMP-20), macrophage metalloelastase (MMP-12), and MMP-19, and (5) the membrane-type MMPs

(MMP-14 to MMP-17 and -24, -25 or MT1-6-MMP). The first four groups are considered to be the classical MMPs.

Human macrophage elastase (HME; MMP-12) was first identified as an elastolytic metalloproteinase secreted by inflammatory macrophages 30 years ago.<sup>4,5</sup> MMP-12 shares many features typical of MMPs, including its domain structure, chromosomal location within the MMP gene cluster on human chromosome 11q22, and capacity to degrade extracellular matrix components.<sup>6,7</sup> Like other MMPs, MMP-12 is composed of three distinct domains: an amino-terminal propeptide domain that is involved in the maintenance of enzyme latency; a catalytic domain that binds zinc and calcium ion and hemopexin-like domain at the carboxy terminal which determines substrate specificity. MMP-12 is secreted as a 54-kd pro-form protein that undergoes self-activation through autolytic processing to produce 45- and 22-kd active forms of the enzyme.<sup>6,7</sup> The major substrate for MMP-12 is elastin, which is abundant in the lung and arterial wall. Abnormal regulation of MMP-12 expression has been implicated in abdominal aortic aneurysm,<sup>8</sup> atherosclerosis,<sup>9</sup> and emphysema.<sup>10</sup> In addition to elastolytic activity, MMP-12 has been shown to be capable of degrading a broad spectrum of other extracellular matrix components, including type IV collagen, fibronectin, laminin, vitronectin, proteoglycans, chondroitin sulfate, and myelin basic protein. One apparently important function of catalytic MMP-12 *in vivo* is its ability to activate other MMPs such as MMP-2 and MMP-3, by which MMP-12 exaggerates the cascade of proteolytic processes.<sup>9</sup> For a long time, MMP-12 functional roles in RA have been neglected since the elastin, a major substrate for MMP-12, is not normally present in the articular connective tissues. Recently, MMP-12 expression in mononucleated cells from the rheumatoid synovium was reported, although its physiological implications have not been disclosed.<sup>11</sup>

In this issue of *The American Journal of Pathology*, Wang and colleagues<sup>12</sup> have reported that increased MMP-12 expression in macrophages significantly exacerbated the development of experimentally inflammatory arthritis in

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transgenic rabbits. Using transgenic rabbits (rather than murine) that overexpress high levels of MMP-12 in macrophage lineage directed by the human scavenger receptor promoter, these authors applied the carrageenan-induced arthritis model and compared the arthritis lesion formations between transgenic and control rabbits at 7, 14 and 35 days. Their results showed for the first time, that overexpression of MMP-12 in macrophages (predominately present in arthritis lesions) significantly resulted in increased synovial thickening, pannus formation, and cartilage destruction, a feature similar to those of RA. It is of particular interest that they demonstrated that macrophage-derived MMP-12 plays a pivotal role in these changes because the lesions in transgenic rabbits are enriched in macrophages and subsequently associated with MMP-3. These findings by Wang et al<sup>12</sup> have provided several substantial implications for the pathogenesis of RA. First, accumulating evidence has revealed that an increased number of macrophages in the synovial tissue strongly correlate with the severity of joint damage, although the molecular mechanisms have not been fully defined. Therefore, it may be reasonable to speculate that macrophages abundantly present in the lesions of RA participate in the lesion progression through MMP-12 secretion. Several inflammatory factors have been shown to up-regulate the expression of MMP-12, such as GM-CSF, IL-1, and MCP-1 whereas TGF- $\beta$  can suppress MMP-12 expression.<sup>13</sup> Thus, there may be a coordinate regulation among these inflammatory factors. Secondly, MMP-12 can either directly degrade the extracellular matrix in the joint since it can digest a broad spectrum of substrates as mentioned above or indirectly participate in the destruction process through activating other MMPs. The latter functions may be particularly important considering that MMP-12 essentially digest elastin. Finally, degradation of the extracellular matrix caused by MMP-12 may facilitate the migration of inflammatory macrophages, leading to a chronic viscous circle of the disease. These results shed a new light on the understanding on the pathogenesis of RA and provide a clue to develop MMP-12 inhibitor for treatment of RA in the future.

Although the findings reported by Wang et al<sup>12</sup> are intriguing and impressive, several issues still remain unsolved. First, it has not been defined whether such effects indicated in MMP-12 found in transgenic rabbits is also present in human RA patients. Indeed, there is an immediate need to investigate the correlation between MMP-12 expression and RA. It is still not clear, however, whether increased MMP-12 activity is associated with the duration of disease, disease activity, or therapeutic regimen. Apparently, a large number of patients are required to address these issues in clinical studies. It will be of great interest to elucidate whether MMP-12 knockout mice have less susceptibility to collagen- or antigen-induced arthritis in future. Secondly, although MMP-12 is potentially important, other MMPs such as MMP-1, -2, -3, and -9 cannot be underestimated. How these MMPs are cooperatively and coordinately regulated is still mysterious. Finally, there are no commercial kits currently available for physicians to measure MMP-12 activity in either plasma or synovial fluid, therefore, the development of

such methods may be necessary to determine MMP-12 activity as an adjunct test method for the evaluation of RA activity. In sum, it still seems premature to conclude that blocking a single MMP-12 will be sufficient to halt the destructive process in RA patients before these questions are clearly answered.

For the last decade, many MMP inhibitors, either natural or synthetic compounds, have been under clinical trials,<sup>2,14</sup> unfortunately, the results are still not satisfactory and their efficacy in treating RA is largely unknown because most MMP inhibitors have the toxic side effects and lack enzyme specificity. It will be interesting to develop MMP-12-specific inhibitors and determine whether inhibition of MMP-12 is really effective in treating RA. Apparently, using either transgenic or knockout animals such as the transgenic rabbits reported in Wang et al<sup>12</sup> should certainly pave the way to elucidate both mechanistic and treatment efficiency of MMP-12 in RA.

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