Commentary

Plasminogen Activation/Plasmin in Rheumatoid Arthritis

Matrix Degradation and More

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Rheumatoid arthritis (RA) is one of the most common and most debilitating joint diseases, producing high personal, social, and economic costs.^{1,2} Patients with RA suffer pain and functional losses, often followed by disability and premature death. Although the etiology of RA is unknown, substantial efforts have been directed toward studying the transition of the joint from normal to diseased. The maintenance of normal joint architecture and function requires equilibrium between the synthesis and degradation of the specialized extracellular matrices that make up bone, cartilage, and tendons. In RA, the balance of tissue remodeling is disturbed,³ and excessive proteolytic degradation of the joint matrices leads to joint destruction.^{4,5} The early phase of RA is characterized by the infiltration of immune and inflammatory cells into the synovium and by hyperplasia of the fibroblasts in the synovial lining. This leads to pannus formation, cartilage breakdown, and eventual bone destruction.^{1,2} Through the use of an experimental mouse model of RA, Li and colleagues⁶ have implicated plasmin as an essential component of the early phase of RA, as reported in this issue of *The American Journal of Pathology*. (*Am J Pathol* 2005, 166:645–647)

Plasminogen, the zymogen of the serine protease plasmin, is primarily, but not exclusively, synthesized in the liver⁷ and is present at micromolar concentrations in human plasma and in extracellular fluids.⁸ Although plasminogen/plasmin is best known as the key molecule in the fibrinolytic system and is critical for clot lysis,⁹ plasmin also directly degrades extracellular matrix components including the glycoproteins fibronectin, laminin,

and elastin, as well as proteoglycans.^{8,10,11} Plasmin indirectly contributes to matrix degradation by proteolytically activating matrix metalloproteinases and proteoglycanases.^{10–12} Other plasmin substrates have been described and suggest that (cell surface-bound) plasmin modulates intracellular signal transduction pathways. For example, plasmin can activate latent transforming growth factor- β ¹³ and cleave insulin-like growth factor binding proteins, releasing active insulin-like growth factor.¹⁴ Plasmin also activates protease-activated receptors (PAR), which are a family of G-protein-coupled receptors in which proteolytic cleavage unmasks a tethered ligand. Plasmin activates PAR-1 in fibroblasts and induces the PAR-mediated transcription of motility factors.¹⁵ In a different study, integrin-bound plasmin enhanced cell migration by a PAR-1-dependent mechanism.¹⁶ Plasmin also cleaves components of the complement system and releases chemotactic complement fragments.^{17,18} In monocytes, plasmin has been shown to induce cytokine expression,¹⁹ but the plasmin substrate in this system is currently unknown.

Because of the many functions performed by plasmin, its generation and activity are tightly controlled by specific activators and inhibitors. In mammals, there are two well characterized plasminogen activators (PA): urokinase-type PA (uPA) and tissue PA (tPA).⁹ The activity of PAs in turn is regulated by activating proteases and specific PA inhibitors. Plasmin itself is inhibited by several protease inhibitors present in human plasma, including α 2-macroglobulin, α 1-anti-trypsin, and α 2-anti-plasmin. Binding of plasminogen to cell surfaces via a number of different bind-

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ing proteins accelerates plasmin generation and can protect plasmin from inactivation by its inhibitors.²⁰

The PA/plasmin system plays many roles in physiology and pathophysiology. In addition to clot lysis, PA/plasmin has been implicated in ovulation,²¹ wound healing,²² adipose tissue development,^{23,24} and tumor invasion and metastasis.^{8,10,11} Indeed, the role of PA in malignancy is underscored by the fact that uPA and PA inhibitor-1 are independent markers for a poor prognosis in breast cancer and other solid tumors.²⁵ PA/plasmin is also important for the recruitment of inflammatory cells in atherosclerosis and restenosis.²⁶ Furthermore, the contribution of PA/plasmin to inflammatory joint disease and particularly to RA has been appreciated for some time^{5,27,28} and is usually viewed in the context of cartilage and bone matrix degradation.

In this issue of *The American Journal of Pathology* Li and colleagues⁶ report that plasmin is essential in the early phase of RA. The authors compared plasminogen-deficient ($plg^{-/-}$) and plasminogen-expressing mice in a model for chronic erosive inflammatory arthritis, collagen type II-induced arthritis (CIA), and found a complete absence of joint inflammation or destruction in $plg^{-/-}$ animals. Importantly, this study used mice bred into the CIA-susceptible DBA/1 mouse strain, and more than 80% of the matched congenic $plg^{+/+}$ littermate controls developed full blown arthritis with inflammatory infiltrates, pannus formation, cartilage degradation, and bone erosion. In contrast, collagen type II injection resulted in high titers of anti-collagen antibodies in the $plg^{-/-}$ mice; however, there was a lack of inflammatory cells in the joints, suggesting a role for plasminogen/plasmin in the inflammatory phase of arthritis. Interestingly, there was a dose effect, and $plg^{+/-}$ animals, which have ~50% of the normal serum level of plasminogen, developed less severe arthritis in fewer joints with a later onset than matched $plg^{+/+}$ mice.

To further examine the role of plasminogen/plasmin in the early phase of arthritis, Li and colleagues⁶ studied the effect of plasminogen deficiency in anti-collagen type II antibody-induced arthritis, a model for the acute inflammatory phase of the disease. Again, $plg^{-/-}$ mice developed no joint inflammation, whereas all $plg^{-/+}$ and $plg^{+/+}$ mice developed inflammatory collagen type II antibody-induced arthritis. The absence of arthritis in $plg^{-/-}$ mice was not because of a defect in the posttranslational modification of collagen because intravenously injected anti-collagen type II antibodies bound to the joint cartilage of $plg^{+/+}$ and $plg^{-/-}$ mice. Finally, to ultimately prove the role of plasminogen in the effector phase of arthritis, the authors injected purified human plasminogen into both $plg^{-/-}$ mice and controls. They demonstrated that reconstituted $plg^{-/-}$ mice develop arthritis, albeit a less severe form, likely because of the more rapid clearance of the exogenous plasminogen. Together, the data provided by Li and colleagues⁶ demonstrate that plasminogen/plasmin plays a critical role in the early, inflammatory phase of arthritis.

The study also raises several interesting questions. Currently, there are no known functions for the zymogen plasminogen, so plasminogen is presumably cleaved

and activated to promote arthritis. In the present study,⁶ uPA-deficient mice developed arthritis with reduced severity compared to uPA-expressing mice, but the uPA-dependent difference in CIA development was markedly less dramatic than the difference between $plg^{-/-}$ and $plg^{+/+}$ mice. This suggests the involvement of an additional plasminogen activator, with tPA being an obvious candidate. Interestingly, a previous study by a different group also demonstrates that uPA deficiency reduces the severity of CIA,²⁹ whereas tPA deficiency results in an aggravated form. Together, these data suggest that in arthritis plasminogen may be activated by serine protease(s) other than uPA and tPA. In this regard, alternate plasminogen activators have been proposed to regulate plasminogen activation in nonfibrinolytic contexts. For example, recent work demonstrates that the serine protease plasma kallikrein can activate plasminogen in adipogenesis.²⁴

Another open question concerns the mechanism by which plasminogen/plasmin contributes to arthritis. The present study clearly points to an early role distinct from joint destruction. The target of plasmin in CIA, however, remains unknown. Li and colleagues⁶ propose that the plasmin substrate is a complement component. Complement factors are plasmin substrates^{17,18} and complement, particularly the complement factor C5, is critical for the development of arthritis in mice.³⁰⁻³² Complement has also been implicated in the pathogenesis of human RA.^{33,34} There is to date, however, no *in vivo* evidence for complement activation by plasmin in arthritis, and additional work is required to test the complement hypothesis proposed by Li and colleagues.⁶ Alternative or additional plasmin substrates, including fibrin and PARs, may also contribute to disease progression in arthritis. Plasminogen-deficient mice are severely impaired in clearing fibrin deposits,³⁵ which are present in the early phase of CIA³⁶ and eliminated as the disease progresses. Impaired fibrin clearance may modulate the inflammatory responses in CIA, although current evidence points to a proinflammatory role of fibrinogen/fibrin.³⁷ PAR signaling results in the production of proinflammatory cytokines^{38,39} and PARs have been implicated in the development of arthritis in mice.⁴⁰ PARs or other receptor/ligand systems may require cleavage by plasmin to induce the inflammatory phase of CIA. In this regard, the fact that synovial fibroblasts from patients with RA have a substantially higher capacity for plasminogen binding and express a different class of plasminogen receptors than normal synovial fibroblasts⁴¹ suggests that plasminogen/plasmin may directly influence signal transduction events in this cell type.

Finally, it remains to be determined whether PA/plasmin is critically important in the early phase of RA in humans. If this is the case, plasmin may be an attractive target for interfering with early stage disease. Currently, specific small molecule plasmin inhibitors are not available, and their long-term systemic application would likely have severe thrombotic complications. This being said, the striking absence of arthritis development in plasminogen/plasmin-deficient mice should encourage the exploration of strategies that inhibit plasmin activity selectively in the joint.

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