0022-3565/06/3183-933–938$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 318, No. 3
103465/3123928
Printed in U.S.A.

JPET 318:933–938, 2006

Perspectives in Pharmacology

Role of Matrix Metalloproteinases in Intestinal Inflammation

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Received March 1, 2006; accepted April 24, 2006

ABSTRACT

Matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of MMPs (TIMPs), are produced in the gastrointestinal tract by several structural cells. The balance between MMPs and TIMPs is essential for many physiological processes in the gut. However, imbalance between MMPs and TIMPs plays an important role in the pathophysiology of diverse intestinal inflammatory conditions. We reviewed the role of the MMP/TIMP system in the pathogenesis of intestinal inflammatory diseases and pharmacologic perspectives for the use of compounds that restore the MMP/TIMP balance.

Matrix metalloproteinases (MMPs) are a class of structurally related proteins that are collectively responsible for the metabolism of extracellular matrix (ECM) of the connective tissue (Visse and Nagase, 2003). These zinc- and calcium-dependent endopeptidases degrade most components of the ECM and are involved in the remodeling and degradation of the matrix, such as collagen, proteoglycans, and glycoproteins. All MMPs have a similar domain structure, with a "predomain" to target for secretion, a "prodomain" to maintain latency, and an "active catalytic region" that contains the zinc-binding active site. Moreover, some MMPs have an additional domain, such as the hemopexin region, implicated in substrate recognition (Visse and Nagase, 2003). Similar to other proteinases, MMPs are secreted as latent enzymes and become activated by the action of other MMPs or serine proteases, which cleave peptide bond within the prodomain (Sternlicht and Werb, 2001).

To date, more than 24 human MMPs have been identified. Based on substrate specificity and structural homology, MMPs have been divided into collagenases (MMP-1, -8, -13, and -18), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -7, -10, and -11), elastase (MMP-12), and others (MMP-19, -20, -23, -26, -27, and -28) (Visse and Nagase, 2003). These proteases are secreted as latent enzymes by various cell types, including neutrophils, platelets, mesenchymal cells, T cells, monocytes, macrophages, and cancer cells, and the conversion to active form requires proteolytic cleavage to release the active catalytic enzyme. However, a subset of MMPs known as membrane-type MMPs (MT-MMPs, including MMP-14, -15, -16, -17, -24, and -25), are not secreted but remain attached to cell surfaces, thus functioning in the perimembrane environment.

The MMP activity is tightly controlled at diverse levels. The MMP genes are transcriptionally responsive to several factors, including cytokines such as TNF-α and IL-1, mitogens, and growth factors. In addition, their enzymatic activity is regulated by tissue endogenous inhibitors of MMPs (TIMPs), which act by forming a 1:1 complex with the highly conserved zinc binding site of MMPs. The resultant MMP-inhibitor complex is inactive and unable to bind substrate. The TIMP gene family consists, at least, of four members (TIMP-1, -2, -3, and -4), and the interactions of these proteins with MMPs are generally nonselective. In fact, all MMPs can be inhibited by diverse TIMPs. Furthermore, in plasma, the

This work was supported in part by a grant from the Secretaria de Estado de Educacion y Universidades fellowship, cofunded by the European Social Fund (to C.M.), and by Instituto de Salud Carlos III (C03/02) (Madrid, Spain). C.M. is a postdoctoral fellow of Spanish Ministry of Education.
Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
doi:10.1124/jpet.106.103465.

ABBREVIATIONS: MMP, matrix metalloproteinases; TIMP, endogenous inhibitor of matrix metalloproteinases; ECM, extracellular matrix; IBD, inflammatory bowel disease; TNBS, trinitrobenzene sulfonic acid; CD, Crohn's disease; IL, interleukin; UC, ulcerative colitis; TNF-α, tumor necrosis factor-α; DSS, dextran sulfate sodium; CT1399, N^4-hydroxy-N'-[1-(*S*)-morpholiosulfonylaminoethyl aminocarbonyl-2-cyclohexylethyl]-2-(*R*)-(4-chlorophenylpropyl) succinamide; CGS-27023-A, [*N*-hydroxyl-2(*R*)-(4-methoxysulfonyl) 3-piconyl)-(amino-3 methylbutaneamide hydrochloride monohydrate)]; ONO-4817, (2*S*,4*S*)-*N*-hydroxy-5-ethoxy-methyloxy-2-methyl-4-(4-phenoxylbenzoyl) aminopentanamide.

circulating general protease inhibitor, α-2 macroglobulin, may also block the enzymatic activity of MMPs. Under physiological conditions, MMPs are present at low levels, usually in the latent form, and when activated, they are responsible for normal ECM turnover. However, under pathologic situations, the increased amount of active MMPs cannot be controlled by TIMPs, resulting in the ECM breakdown and tissue injury. The important role of MMPs in several pathologic situations, such as tumor progression and metastasis, atherosclerotic plaque rupture, and hemorrhagic transformation of stroke, has encouraged the development of diverse synthetic MMP inhibitors that block enzyme activity. However, MMPs have been also implicated in the release of several growth factors by the ECM, such as transforming growth factor-β, which is a multifunctional cytokine important for maintaining tissue homeostasis (Mott and Werb, 2004).

There is also growing evidence that MMPs can be implicated in the pathophysiology of several intestinal inflammatory disorders. In fact, MMPs can be released from almost all connective tissue cells present in the bowel in response to inflammatory stimuli. In this review, we will focus on molecular, basic, and clinical pharmacology aspects of MMPs in intestinal inflammation, including inflammatory bowel disease, necrotizing enterocolitis, collagenous colitis, and diverticulitis.

Association between MMPs and Human Inflammatory Bowel Disease

Inflammatory bowel disease (IBD)—both ulcerative colitis (UC) and Crohn's disease (CD)—is a chronic, relapsing condition with inflammation and tissue remodeling of the gastrointestinal tract. Clinically, it is characterized by abdominal pain, diarrhea, rectal bleeding, and fever. UC only affects rectum and colon; the inflammatory process is limited to the mucosa and is histologically characterized by the presence of crypt abscesses and ulceration. CD may affect any region of the gastrointestinal tract; the inflammatory process may extend throughout the intestinal wall, (transmural) narrowing the intestinal lumen, and is histologically characterized by the formation of granulomas, fibrosis, and fistulae. The IBD etiology remains still unclear, although environmental, microbiological, immunological, and genetic factors have been implicated in the pathogenesis of the disease. According to the currently accepted hypothesis, both UC and CD result from a dysregulated response of the intestinal immune system toward intraluminal antigens of bacterial origin in genetically predisposed patients, leading to the activation and release of several factors, which initiate a cascade of events resulting in intestinal injury, including cytokines, nitric oxide, eicosanoids, and proteolytic enzymes (Fiocchi, 1997; Podolsky, 2002). Interestingly, MMPs can be released by different connective tissue cells in response to proinflammatory cytokines, such as TNF-α and IL-1β (Sternlicht and Werb, 2001). In fact, TNF-α is one of the most important inducers of MMP protein production (Gan et al., 2001; Nee et al., 2004). Recently, increased levels of MMPs have been found in homogenates of inflamed tissues of IBD patients, suggesting a role for these enzymes in the increased proteolysis of the mucosa, leading to ulceration, inflammation, and fistula formation (Naito and Yoshikawa, 2005). In fact, MMP-9 was the most abundantly expressed protease in inflamed tissues, and polymorphonuclear neutrophils were proposed as the most likely cellular source of this enzyme (Baugh et al., 1999). It has been also shown that the ratio between MMP-1/TIMP-1 and MMP-3/TIMP-1 is increased in inflamed colons of IBD patients compared with noninflamed tissue samples, whereas TIMP-2 mRNA remained unaltered (von Lampe et al., 2000). In addition, MMP-1 and MMP-3 were overexpressed in CD68-positive mononuclear cells within the mucosa of inflamed colon biopsies (von Lampe et al., 2000). Moreover, an imbalance between MMP-3 and TIMP-1 transcripts has also been found in this inflammatory condition (Heuschkel et al., 2000). The elevated MMP-1 and MMP-2 protein and transcript levels have been shown in patients suffering from pouchitis, inflammation of the ileoanal pouch anastomosis after proctocolectomy in UC patients, where mesenchymal cells were identified as the major cellular source of these enzymes. Interestingly, when patients were treated with metronidazole for six weeks, there was a significant decrease in MMP-1 and MMP-2 levels, with the corresponding improvement of clinical symptoms and histological findings of IBD (Stallmach et al., 2000). Furthermore, the levels of MMP-3 are increased in mononuclear macrophage-like cells and fibroblasts with low levels of TIMP-1, TIMP-2, and TIMP-3 in intestinal fistulae in CD patients, whereas the MMP-9 protein is highly expressed in small mononuclear leukocytes, granulocytes, and giant cells, suggesting an important role for these enzymes in the fistula formation in CD (Kirkegaard et al., 2004). However, an increased level of TIMP-1 protein has been related to fibrotic strictures in this condition (McKaig et al., 2003). In addition, the MMP-3 gene 5A/6A polymorphism, which confers a higher gene transcriptional promoter activity, has been associated with CD susceptibility (Pender et al., 2004). In the same study, when patients were stratified for CARD15 genotype, transmission of the 5A allele in CARD15 mutation carriers was associated with previous surgical resection, stenosis, and fistulizing patterns of the disease. All of these studies suggest that MMPs play an important role in the process of tissue remodeling and destruction, which is detected in patients suffering from IBD. However, clinical studies using selective inhibitors of MMP are required to prove pathogenetic role of MMPs in human IBD.

MMPs and the Intestinal Immune System

There is increasing evidence that several MMPs are involved in the host immune response under normal conditions. In fact, MMPs can be involved in the recruitment of inflammatory cells into the intestinal wall and other organs. It has been shown that the presence of MMP activity is necessary for lymphocyte transmigration across the endothelial venules from bloodstream into lymph nodes (Faveeuw et al., 2001). In addition, MMP activation in the endothelium is required for VCAM-1-dependent lymphocyte migration (Deem and Cook-Mills, 2004). Although the precise role of MMP-9 in polymorphonuclear neutrophil migration across the endothelial cells is still unclear, there is evidence that this protease is secreted during neutrophil migration across the basement membrane (Delclaux et al., 1996). In addition, TIMP-1 was able to inhibit the trans-basement membrane neutrophil migration. Recently, it has been found that myofibroblast MMPs are able to enhance the neutrophil chemoattractant capacity of intestinal epithelial cells via activation of the neutrophil chemoattractant CXCL7 (neutrophil-activat-

ing peptide 2) (Kruidenier et al., 2006). Furthermore, MMP-9 and MMP-2 are needed for the migration of Langerhans and dendritic cells from the skin to the draining lymph nodes to initiate cutaneous sensitivity (Ratzinger et al., 2002). Moreover, in several experimental models of colitis, MMP inhibition has been shown to reduce the neutrophil accumulation into the intestinal wall (Sykes et al., 1999; Di Sebastiano et al., 2001; Naito et al., 2004). Finally, MMP-3 plays an important role in the migration of $CD4^+$ T lymphocytes into the intestinal mucosa, thus controlling pathogenic bacteria in the colon. Indeed, MMP-3-deficient mice orally inoculated with *Citrobacter rodentium* have delayed clearance of bacteria and delayed appearance of $CD4^+$ T lymphocytes into intestinal lamina propria compared with wild-type animals (Li et al., 2004). In addition, labeled $CD4^+$ T lymphocytes from infected wild-type animals showed an impaired ability to migrate to mesenteric lymph nodes and colonic lamina propria when injected to MMP-3-deficient animals.

However, enhanced immune responses, such as activation of lamina propria T cells elicited by luminal antigens, are implicated in the pathophysiology of IBD. Indeed, activated T cells induce a pathologic chronic inflammatory response leading to intestinal damage. The molecular mechanisms of T cell-mediated intestinal injury in the gut have been carefully studied in a human fetal intestinal culture explant model. Activation of lamina propria T cells by pokeweed mitogen showed a dramatic increase in the concentration of MMP-1 and MMP-3, leading to severe tissue damage with epithelial cell shedding and loss of villi, whereas pokeweed mitogen-induced mucosa injury was abolished by CT1399, a synthetic MMP inhibitor (Pender et al., 1997). Nanomolar concentrations of recombinant MMP-3 also induced severe tissue damage when added directly to the fetal explants. In the same model, a p55 TNF receptor immunoadhesin prevented T cell-mediated intestinal injury by inhibiting MMP production from mesenchymal cells, suggesting that one of the major pathways in which TNF-α induces intestinal damage is by stimulating MMP secretion (Pender et al., 1998). In addition, gene array analysis and in situ hybridization have also shown a marked up-regulation of other MMPs, such as MMP-10 and MMP-12, in the fetal explants associated with mucosal destruction (Salmela et al., 2002). Moreover, activated T cells release IL-22 that can induce increased mRNA MMP expression by colonic subepithelial fibroblasts (Andoh et al., 2005). Taken together, these results suggest an important role for lamina propria T cells in the loss of mucosa organization in the gut that is mediated by MMPs.

MMPs and Intestinal Epithelial Cells

It is now apparent that the intestinal epithelium may play an important role in the immunomodulatory response of the intestinal mucosa. It has been shown that intestinal epithelial cells may produce several immunomodulatory substances, such as cytokines, complement factors, and immune receptors (Su et al., 1999). In addition, the loss of intestinal epithelium integrity could facilitate interactions between the luminal antigenic stimuli and the mucosal immune system, resulting in chronic intestinal inflammation (Rath et al., 2001). We have found that Caco-2 cells, an intestinal epithelial cell line, when stimulated with TNF-α showed increased activity and expression of MMP-9 but not MMP-2 (A. Santana, C. Medina, M. C. Paz, F. Diaz-Gonzales, E. Farre, A. Salas, M. W. Radomski, and E. Quintero, unpublished data). Our results are consistent with the finding of increased levels of MMP-9 transcripts in Caco-2 cells in response to diverse inflammatory stimuli (Gan et al., 2001). In addition, it has been shown using different models of mice colitis that epithelial-derived MMP-9 is crucial in intestinal damage (Castaneda et al., 2005). Therefore, MMP-9 released from intestinal epithelial cells during inflammation could be responsible for degradation of ECM components with subsequent loss of mucosal integrity. This could lead to enhanced penetration of inflammatory cells and facilitation of cellular interactions with luminal antigens. However, studies performed in humans have usually demonstrated an up-regulation of MMP-9 mainly in leukocytes (Baugh et al., 1999; Kirkegaard et al., 2004; Gao et al., 2005). On the other hand, other epithelial-derived MMPs may be responsible for epithelial migration in intestinal inflammation, probably playing an important role in wound healing. In fact, MMP-7 has been shown to be expressed by migrating enterocytes bordering intestinal ulcers in specimens of patients suffering from ischemic colitis (Salmela et al., 2004). In addition, overexpression of epithelial-derived MMP-7 by immunohistochemistry has been also observed in necrotizing enterocolitis (Bister et al., 2005). Therefore, intestinal epithelium is also an important source of MMPs in intestinal inflammation and no longer considered an intestinal barrier only.

MMPs and Animal Models of IBD

In the last decade, several experimental models of colitis have been developed in an attempt to unravel different pathophysiological mechanisms implicated in colonic inflammation. Among all of them, colitis induced by trinitrobenzene sulfonic acid (TNBS) and by dextran sulfate sodium (DSS) are the most widely studied experimental models. TNBS-induced colitis is a hapten-induced model of chronic inflammation characterized by segmental lesions, mucosal ulceration with granulation tissue at the base, and mixed transmural infiltration by neutrophils, lymphocytes and macrophages, and occasionally, small granulomas are observed in this model. When TNBS-induced colitis is severe, strictures of the lumen, resulting from intestinal fibrosis and fistulae formation, are also seen (Morris et al., 1989). The DSS model exhibits clinical and morphological features resembling human UC, including diarrhea and rectal bleeding. DSS-induced colitis is histologically characterized by infiltration of inflammatory cells, crypt loss, and extensive mucosal erosions, with predominance of distal involvement of the large intestine. Occasionally, crypt abscesses and regenerated epithelium are seen (Okayasu et al., 1990).

Similar to human studies that observed elevated levels of MMPs in IBD, there is strong evidence implicating MMPs in the pathogenesis of TNBS- and DSS-induced colitis. Most of these studies were mechanistic in nature and used selective pharmacologic inhibitors of MMPs to investigate their effects on colitis. Table 1 shows the list of MMP inhibitors, which have been used in several experimental models of colitis. Marimastat, a synthetic hydroxamate-derived MMP inhibitor, has been tested in an acute model of colitis induced by TNBS. In this study, marimastat (40 mg/kg) was dosed by oral gavage for 3 days before and 3 days after rectal administration of TNBS. It was found that colitic samples from rats dosed with marimastat showed significantly less myeloper-

TABLE 1
Different classes of MMP inhibitors tested in animal experimental models of colitis

Drug	Chemical Class	MMP Inhibition	Model of Colitis
1,10-Phenantroline	Phenantroline	Broad spectrum	DSS, TNBS, bacteria-induced colitis
Marimastat	Hydroxamate	Broad spectrum	TNBS
Batimastat	Hydroxamate	Broad spectrum	TNBS
CGS27023A	Hydroxamate	Collagenase gelatinase stromelysin	DSS, TNBS
ONO-4817	Hydroxamate	Broad spectrum except for MMP-1 and MMP-7	DSS

oxidase activity (MPO), which is an index of neutrophils trapped into intestinal tissue, and histological colitis score compared with control animals (Sykes et al., 1999). Using the same model of colitis, it was found that batimastat (10 to 40 mg/kg) administered 30 min before induction of colitis and twice daily over 7 days dose-dependently reduced colitis scores and MPO activity in the rat-colonic tissue (Di Sebastiano et al., 2001). We also found that 1,10-phenantroline, a zinc-chelator compound that inhibits MMP activity, dosed at 20 mg/kg over 7 days led to a significant improvement of morphological and histological scores in a chronic rat model of colitis induced by TNBS (Medina et al., 2001). In addition, we have recently investigated which MMP is involved in this experimental model (Medina et al., 2006). We have found that MMP-9 is the main MMP involved in TNBS-induced colitis, especially on days 7 and 10 after TNBS instillation, whereas MMP-2 levels remain unaltered by the TNBS treatment. Moreover, CGS-27023-A, a potent synthetic MMP inhibitor, abolished MMP-9 activity and attenuated the histological score when started at the early phase of colitis. Furthermore, neutrophils were found to be the major source of MMP-9 in this rat experimental model.

We also showed that MMP-9 was implicated in DSS-induced colitis (Medina et al., 2003). In this study, MMP-9 levels were up-regulated after 5 days of DSS administration, and the treatment with CGS-27023-A (20 mg/kg) significantly improved the histological score of colitis in rats. Interestingly, the compound did not seem to influence mucosal repair processes in DSS-induced colitis, neither in terms of crypt distortion nor epithelial regeneration (Medina et al., 2003). Another hydroxamate-derived MMP inhibitor, ONO-4817, has also been tested in DSS-induced colitis in mice. ONO-4817 (30 mg/kg) given by oral gavage twice per day for 7 days resulted in a significant improvement of histological score as well as significant decrease in tissue-associated MPO activity and inflammatory cytokines (TNF-α and interferon-γ) compared with control animals (Naito et al., 2004). Castaneda et al. (2005) found that epithelial-derived MMP-9 plays a crucial role in DSS-induced colitis and in *Salmonella typhimurium*-induced enterocolitis in mice. Using neutrophil transmigration studies and bone marrow chimeras, they found that neutrophil MMP-9 is neither required for its migration nor sufficient to induce tissue damage during both experimental models of colitis, whereas epithelial MMP-9 is important for tissue damage. In addition, they have shown that MMP-9 inhibited cell attachment and wound healing in an in vitro model using Caco-2 intestinal cell line (Castaneda et al., 2005). Other MMPs have been found in inflamed colonic tissues from mice treated with DSS, including MMP-3, -7, -8, and -12 (Pirila et al., 2003; Naito et al., 2004).

MMPs have been also implicated in the pathophysiology of other models of experimental colitis, such as colitis induced in immunodeficient mice by transfer of $CD4^+$ T lymphocytes. In this model, up-regulation of MMP-9 and MMP-2 has been also shown (Tarlton et al., 2000). Interestingly, in this study, mucosal and epithelial matrix degradation was clearly associated with infiltrating leukocytes. In addition, serine proteases were also up-regulated, but studies with MMP inhibitors showed that proteolytic activity on the injured tissue was due more to the action of MMPs than serine proteases.

There is increasing evidence that bacteria may also be implicated in the pathophysiology of IBD. Evidence from human and animal studies supports the idea that patients suffering from IBD have a nonphysiological immune response to intestinal flora. For instance, it has been found that bacteria from common rat flora may invade the colonic wall immediately after colonic instillation of TNBS, suggesting a significant role for enteric microorganisms in colonic tissue damage (Garcia-Lafuente et al., 1997). In addition, wide-spectrum antibiotic treatment may decrease the bacterial load and alleviate intestinal inflammation in this experimental model of colitis (Garcia-Lafuente et al., 1997) and also in human IBD (Casellas et al., 1998). We have recently elucidated the role of bacterial MMPs in colonic tissue damage in rats (Medina et al., 2005). When studying mechanisms implicated in transmural inflammation induced by *Bacteroides fragilis*, we found that suspensions of this bacterium, but not *Escherichia coli*, showed significant MMP activity. Pharmacologic inhibition of MMPs with phenanthroline reduced both the activity of MMPs in *B. fragilis* and the ability of these bacteria to induce colitis, suggesting that bacterial MMPs may play an important role in the induction of chronic transmural colonic inflammation (Medina et al., 2005). These data provide evidence that MMPs are implicated in the pathophysiology of intestinal inflammation in diverse experimental models of colitis and, therefore, MMP inhibitors could be considered as potential candidates for the treatment of IBD.

MMPs and Other Intestinal Disorders

Necrotizing enterocolitis (NEC) is a severe gastrointestinal disease affecting predominantly premature infants. It is characterized by rapid hemorrhagic inflammatory necrosis involving largely the distal ileum and proximal colon, although in severe cases, the total bowel may be involved. It may present with a wide spectrum of clinical symptoms, ranging from benign gastrointestinal disturbance to a rapidly fulminant course characterized by intestinal gangrene, perforation, sepsis, and shock. The etiology of the disease remains unclear. Because NEC is characterized by a rapid intestinal mucosal degradation, MMP activity and expression have been studied. It has been shown that MMP-3 and TIMP-1 transcripts were up-regulated in NEC compared with control samples, whereas MMP-1, MMP-2, MMP-9, and TIMP-2 transcripts remained unaltered. In addition, Western blotting confirmed the increased MMP-3 and TIMP-1 protein production. Furthermore, the α-smooth muscle actin-

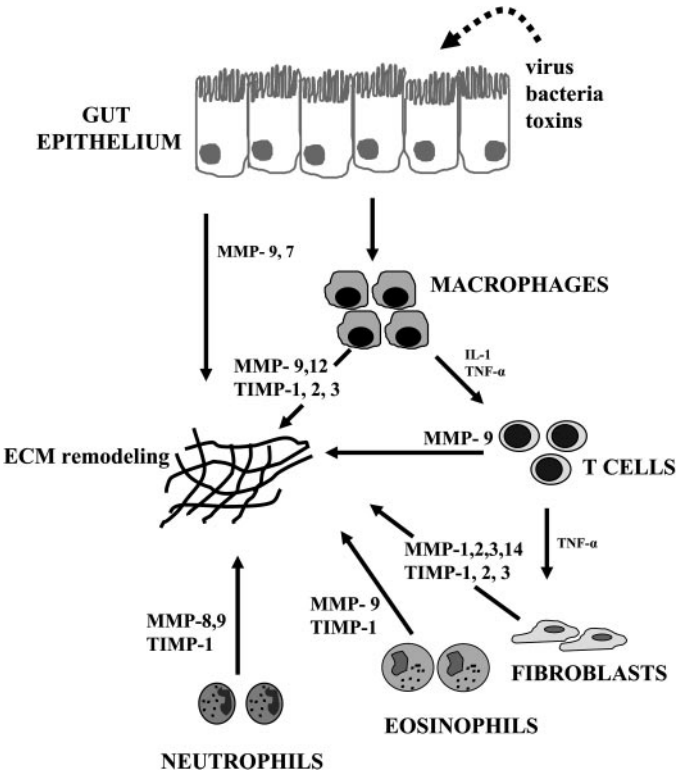

Fig. 1. Summary of different cell types that produce MMPs and TIMPs in inflammatory bowel disease. Luminal virus, bacteria, or toxins can trigger immunological responses with activation of different cells, such as macrophages, fibroblasts, and leukocytes releasing several cytokines, such as TNF-α and IL-1. In addition, activated cells can release both MMPs and TIMPs. Under physiologic conditions, there is a stoichiometric (1:1) MMP/TIMP balance. Inflammatory reactions lead to a relative imbalance between MMPs and TIMPs, and this could lead to disturbed extracellular matrix remodelling (Heuschkel et al., 2000; von Lampe et al., 2000; Kirkegaard et al., 2004).

positive cells localized between the mucosa and submucosa margin plate were found as the most likely cellular source of MMP-3 in this pathological disorder (Pender et al., 2003). Bister et al. (2005) found that other MMPs, such as MMP-7, MMP-26, and MMP-12, are also up-regulated in NEC, suggesting that members of various MMP families may play roles in tissue destruction and remodeling in this disease.

Collagenous colitis is another rare entity whose etiology is unknown, although inflammatory and autoimmune factors, the use of nonsteroidal anti-inflammatory drugs and microorganisms, have been implicated in the pathophysiology of the disease. Clinically, it is characterized by watery diarrhea in the absence of significant endoscopic and radiological findings. However, histologically, ECM deposition is increased in the subepithelial space with a minimal thickness of 10 μm required for diagnosis of the disease. Collagen I and III and fibronectin form a band-like linear deposition below the basement membrane, which does not extend along crypts. The colonic epithelium is often atrophic and may be infiltrated by lymphocytes. The excess deposit of collagens may reflect a local disturbance of ECM turnover, and therefore, MMPs and TIMPs could be implicated in the pathogenesis of the disease. In fact, it has been shown that transcripts of procollagen I, IV, and TIMP-1 are increased in α-actin-positive cells with linear distribution underneath the superficial collagenous layer in colonic samples from patients suffering from collagenous colitis, whereas MMP-1 and -13 transcripts are almost completely absent, resulting in a reduction in ECM degradation and increased ECM synthesis (Gunther et al., 1999).

Diverticular disease is a common disease characterized by mucosal and submucosal herniations through the circular muscle layer, with a marked muscle thickening that could be related to low flexibility and decreased strength of the colonic wall. Therefore, an imbalance between MMPs and TIMPs might be involved in the pathogenesis of diverticular disease through changes in the nature of the ECM components. In fact, it has been shown that collagen synthesis is increased in colonic diverticular disease compared with controls, and MMP-1, -2, -3, and -9 transcripts are almost absent when analyzed using a sensitive quantitative reverse transcription-polymerase chain reaction. By contrast, TIMP-1 and TIMP-2 levels are elevated, suggesting an important role for these enzymes, facilitating the excess deposit of ECM components in this chronic condition (Mimura et al., 2004).

Potential Therapeutic Strategies Involving Modulation of the MMP-TIMP Balance

The data we have now reviewed suggest that the imbalance between MMPs and their endogenous inhibitors plays an important role in the pathophysiology of several intestinal inflammatory disorders. Indeed, when inflammation-induced increase in active MMPs cannot be controlled by TIMPs, such as in IBD, excessive remodelling and ECM degradation lead to intestinal tissue destruction (Fig. 1). In addition, increased MMP activity could amplify and disturb the intestinal immune responses. Therefore, it seems reasonable that MMP inhibitors could be useful in the treatment of this condition, as suggested by diverse studies in several animal models of colitis. Given a large number of selective MMP inhibitors that have been developed and tried successfully in preclinical studies, the list of potential candidates seems to be a long one. However, the pharmacologic development of MMP inhibitors has suffered a serious setback, as the treatment with inhibitors failed to show benefits in patients suffering from advanced cancer (Zucker et al., 2000). Interestingly, MMP inhibitors have been successfully tested in humans in combination with other drugs in periodontitis, a condition characterized by chronic inflammation and remodeling (Lee et al., 2004). It is worth mentioning that there is no cure for IBD. The current pharmacologic strategy in IBD involves the use of aminosalicylates, corticosteroids, and immunosuppressants; however, despite the use of these drugs, IBD patients suffer numerous relapses. Therefore, the need for novel compounds is pressing, and the possibility to use MMP inhibitors in IBD is worth considering. However, given that some MMPs have been implicated in intestinal wound healing, selective MMP inhibitors should be considered.

In some rare forms of colitis, such as collagenous colitis, the MMP-TIMP balance favors TIMPs. The decreased activity of MMPs may result in enhanced deposition of collagen contributing to the disease process. However, in these rare cases, only a few studies have been done, mainly observational; therefore, these data are not as strong as the results obtained in IBD studies. Could TIMP inhibition be a good pharmacological approach in these states? Perhaps, it could be a good strategy to block the TIMP activity in those pathological conditions where ECM deposition is enhanced. However, to date no TIMP inhibitors have been developed, and more

studies need to be done with transgenic or deficient animal experimental models to evaluate the role of TIMPs in the pathophysiology of intestinal inflammation associated with decreased turnover of ECM.

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