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Distinct Expression Profiles of Stromelysin-2 (MMP-10), Collagenase-3 (MMP-13), Macrophage Metalloelastase (MMP-12), and Tissue Inhibitor of Metalloproteinases-3 (TIMP-3) in Intestinal Ulcerations

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Programed expression of matrix metalloproteinases is involved in wound healing in various organs. We have previously demonstrated enhanced expression of collagenase-1, stromelysin-1, matrilysin, and tissue inhibitor of metalloproteinases (TIMP-1) in gastrointestinal ulcerations. To further define the role of matrix-degrading enzymes and their inhibitors in intestinal inflammation and ulcerations, the expression of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (HME, MMP-12), and TIMP-3 mRNAs was studied using *in situ* hybridization and immunohistochemistry in 38 samples representing ulcerative colitis, Crohn's disease, ischemic colitis, and normal intestine. As controls for normally healing intestinal wounds, 12 postoperative samples of rat experimental jejunal anastomoses were also examined. The colitis types studied did not essentially differ in their MMP expression. We found stromelysin-2 mRNA in laminin-5-positive and Ki-67-negative enterocytes bordering the ulcerations. HME was abundantly expressed by macrophages in the vicinity of shedding mucosal epithelium and beneath the necrotic surface of the ulcers. Collagenase-3 and TIMP-3 were expressed by fibroblast-like cells deeper in the remodeling intestinal wall. Expression for stromelysin-2 and collagenase-3 was observed in granulation tissue, but not the epithelium, of the rat anastomoses. Our results suggest a role for stromelysin-2 in epithelial migration and for metalloelastase in macrophage movement and epithelial cell shedding. *(Am J Pathol 1998, 152:1005–1014)*

Epithelial migration and neoangiogenesis as well as matrix degradation and formation are essential features of wound healing. Matrix metalloproteinases (MMPs, matrixins), a group of enzymes that are collectively capable of cleaving all components of the extracellular matrix (ECM), are involved in these events.[1–3] MMPs are divided into four subfamilies, collagenases, gelatinases, stromelysins, and membrane-type MMPs, according to their substrates and structure. Collagenases share the unique ability to degrade fibrillar type I, II, III, and X collagens.[4,5] In addition to the original interstitial (MMP-1) and neutrophil (MMP-8) collagenases, collagenase-3 (MMP-13) was recently cloned from breast cancer tissue.[6] We have previously shown its abundant expression by granulation tissue fibroblasts in chronic venous ulcers, whereas no mRNA is found in normally healing wounds.[7] As MMP-13 is able to degrade gelatin in addition to fibrillar collagens,[8] it may function in degrading collagen types I and III and also their cleavage products during the remodeling of collagenous matrix in chronic wounds.

Stromelysins have a broad selection of substrates, including proteoglycans, type IV, V, IX, and X collagens, laminin, fibronectin, elastin, and globular domains of procollagens I and III.[9] Stromelysin-2 has frequently been associated *in vivo* with cancer invasion.[10,11] It is not expressed in normal skin, but during both human and murine wound healing stromelysin-2 is induced at the migrating epithelial tip, partly co-localizing with collagenase-1.[2,12,13] In cutaneous wounds, stromelysin-2 may be involved in superactivation of secreted procollagenase-1[9] or may facilitate keratinocyte migration by degrading noncollagenous matrix molecules.

Human macrophage metalloelastase (HME, MMP-12), another member of the stromelysin subgroup, was initially

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found in alveolar macrophages of cigarette smokers.[14] It has a broad substrate specificity, being able to degrade elastin, type IV collagen, laminin, fibronectin, vitronectin and heparan, and chondroitin sulfates.[15,16] Thus, in addition to elastin, HME is capable of degrading basement membrane components and macrophages from HME-deficient mice, which are unable to penetrate reconstituted basement membranes *in vivo* and *in vitro*.[17]

Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors of MMPs, which inhibit them by binding to their active site in a 1:1 stoichiometric ratio. The two most recently cloned TIMPs are TIMP-3[18,19] and TIMP-4.[20] By Northern analysis, TIMP-3 was first detected in breast tumors[19] as well as in various normal adult tissues.[18] *In vivo*, it is expressed in developing human embryos and in skin, mammary, and colon cancers.[21] Unlike TIMP-1 and TIMP-2, TIMP-3 protein is tightly bound to surrounding matrix,[22] and in the human eye it forms a component of the Bruch's membrane.[23]

Crohn's disease and ulcerative colitis are chronic, inflammatory disorders of an unknown origin affecting the gastrointestinal tract. Ulcerative colitis is a nongranulomatous disease restricted to the mucosal layers of the colon and rectum and it is histologically characterized by continuous mucosal inflammation with ulcerations and an inflammatory infiltrate of mononuclear cells, neutrophils, eosinophils, and mast cells. Crohn's disease is a granulomatous disease that may affect any portion of the gastrointestinal tract. It presents as patchy inflammation with noncaseating granulomas, neutrophil abscesses, ulceration, and subsequent fibrosis and fistulae formation. Both diseases lead to persistent mucosal damage with high morbidity. Ischemic bowel disease is caused by hypoperfusion of either small or large intestine and results also in ulcerations and mucosal necrosis. The depth of the lesions varies from mucosal to transmural and depends on the extent of hypoperfusion. Chronic ischemia may lead to fibrosis and lesions resembling those of inflammatory bowel disease (IBD).

Our earlier studies on MMP expression in gastrointestinal ulcerations demonstrate that collagenase-1 and stromelysin-1 participate in the remodeling of the intestinal stroma whereas matrilysin associates with epithelial cell migration.[24] The present work was conducted to further characterize the role of stromelysin-2 and two recently cloned MMPs, collagenase-3 and metalloelastase, as well as their inhibitor TIMP-3 in the pathology of IBD. We show that, as in skin wounds, stromelysin-2 is actively expressed by epithelial cells at the ulcer margin. This location, together with the finding that the same cells produce laminin-5, suggests a role for stromelysin-2 in epithelial migration. Collagenase-3 mRNA is detected in stromal cells beneath the ulcer bed, where it may be involved in remodeling of the ulcer stroma. Macrophage metalloelastase is abundantly expressed by macrophages of the inflamed lamina propria and the ulcer base, possibly aiding macrophage migration. TIMP-3 is detected in both normal and inflamed intestine by macrophage- or fibroblast-like cells of the stroma. Our findings indicate that Crohn's, ulcerative, and ischemic colitis do not essentially differ in their MMP expression patterns. Several MMPs are induced during migration and ECM remodeling associated with intestinal ulcerations, and the cell type, its matrix binding receptors, and the composition of the intestinal matrix probably dictate which enzyme is up-regulated.

Materials and Methods

Human Tissue Samples

Formalin-fixed, paraffin-embedded specimens of ulcerative colitis (n = 10), Crohn's disease (colon, n = 8; ileum, n = 6), ischemic colitis (n = 7), and histologically normal colon (n = 3) and ileum (n = 4) were obtained from the Department of Pathology, University of Helsinki. All diseased samples represented an active phase of the disease and were from adult patients. The study was approved by the Ethics Committee of the Department of Dermatology, Helsinki University Central Hospital.

Experimental Model for Intestinal Anastomoses

Animal studies were approved by the Regional Committee for Ethics in Animal Research and Administrative Board at Helsinki University Central Hospital. Adult male Wistar Rats (300 g) were anesthetized with a single intramuscular injection of ketamine (40 mg/kg Vetalar). The jejunum was cut at laparotomy with a steel scalpel approximately 10 cm from the ligamentum of Treitz. Jejunal anastomoses were performed with one layer of interrupted 6–0 polypropylene sutures (Prolene, Ethicon, Norderstedt, Germany). After surgery, the animals had free access to food and water. Animals were sacrificed at 1, 3, 4, 7, 9, and 14 days by an overdose of pentobarbital. To ensure adequate sampling, anastomoses were obtained from two separate animals for each time point. Tissues were embedded in paraffin and cut at 4 μm.

RNA Probes

The production and specificity of the antisense human stromelysin-2, collagenase-3, and TIMP-3 probes have been described.[7,12,21] The HME cDNA used as a template was a kind gift from Steven Shapiro (Pulmonary Department, Washington University, St. Louis, MO). The 650-bp fragment (600 to 1250) was designed with an SP6 RNA polymerase recognition element at the 3′ end and a T7 element at the 5′ end; both antisense and sense probes were transcribed from this polymerase chain reaction product. The murine stromelysin-2 cDNA used as a template was a kind gift from S. Werner (Max-Planck-Institut für Biochemie, Martinsried, Germany).[13] As a control for nonspecific hybridization, sections in each experiment were hybridized with ^{35}S-labeled sense RNA from a bovine tropoelastin cDNA. The validity of this probe as a negative control has been confirmed by Northern[25] and by *in situ* hybridization[26] assays. In addition, collagenase-3 and TIMP-3 sense probes were used as controls in some experiments.

Table 1. Results of Stromelysin-2, Collagenase-3, Macrophage Metalloelastase, and TIMP-3 *In Situ* Hybridization in Inflammatory Bowel Disease and Ischemic Colitis

Histological sample	Number of positive samples/number of hybridized samples			
	Stromelysin-2	Collagenase-3	HME	TIMP-3
Crohn's disease, ileum	4/4	4/6	3/3	4/5
Crohn's disease, colon	7/7	5/8	7/7	7/7
Ulcerative colitis, colon	9/10	5/10	7/7	8/10
Ischemic colitis, colon	7/8	4/8	6/6	8/8
Total	27/29	18/32	23/23	27/30

In Situ *Hybridization*

In situ hybridization was performed on 4-μm sections as described in detail.[25] All samples were treated with proteinase K and were washed in 0.1 mol/L triethanolamine buffer containing 0.25% acetic anhydride. Sections were covered with 50 μl of hybridization buffer containing 2.5×10^4 to 5×10^4 cpm/μl of ^{35}S-labeled antisense or sense RNA probe. After hybridization at 50°C to 55°C for 18 hours in a humidified chamber, the slides were washed under stringent conditions, including treatment with RNAse A to remove unhybridized probe. After 20 to 35 days of autoradiography, the photographic emulsion was developed, and slides were stained with hematoxylin and eosin. The sections chosen for presentation in this report were exposed for 25 to 35 days. Samples previously positive for stromelysin-2 (cutaneous wounds) and for collagenase-3, macrophage metalloelastase, and TIMP-3 (breast and colon carcinomas) were used as positive controls in each experiment.

Antibodies

Tissue macrophages were identified using a monoclonal antibody (KP-1, Dako Corp., Carpinteria, CA, product M814), which reacts with CD68, a specific macrophage marker.[27] Polyclonal antibodies to the amino-terminal end of procollagen I (PC-I, MAb 1912, Chemicon, Temecula, CA) were used to identify activated fibroblasts. The epithelial cells producing laminin-5 were detected with laminin-5 polyclonal antibodies, kindly provided by Karl Tryggvason (Karolinska Institut, Stockholm, Sweden).[28] Monoclonal Mib-1 antibody (0505, Immunotech, Marseille, France) that reacts with the Ki-67 nuclear antigen was used to differentiate proliferating cells from the quiescent intestinal epithelial cells.[29] Matrilysin protein was detected by using affinity-purified antibodies raised in rabbits against a synthetic peptide.[30] Monoclonal antibody that reacts with type IV collagen (M785, Dako, Glostrup, Denmark) was used to stain the intestinal epithelial basement membrane. Endothelial cells were identified by using polyclonal anti-CD31 antibodies (M823, Dako). Polyclonal anti-CD3 antibodies (A452, Dako) were used to identify T lymphocytes, and monoclonal anti-CD20 antibody (U 7021, Dako) was used to identify B lymphocytes.

Immunohistochemistry

Immunohistochemistry was performed on sections serial to those used for *in situ* hybridization. Anti-CD68 antibody was diluted 1:400, PC-I 1:500, laminin-5 1:500, Ki-67 1:500, matrilysin 1:1000, type IV collagen 1:75, CD31 1:100, CD3 1:1000, and CD20 1:500. The peroxidase-anti-peroxidase technique was applied using diaminobenzidine or aminoethylcarbazole (Ki-67, CD3, and CD31) as a chromogenic substrate and Harris hematoxylin as counterstain, as described in detail.[1] Sections were pretreated with 10 mg/ml trypsin (laminin-5, CD68, PC-1, and type IV collagen) or 1 mg/ml Protease type XXVII (P4789, Sigma Chemical Co., St. Louis, MO) (CD3 and CD31) except for matrilysin (no pretreatment) and Ki-67 and CD20, with which microwave oven processing was performed as described.[31]

Results

To assess whether stromelysin-2, collagenase-3, macrophage metalloelastase, or TIMP-3 mRNAs are expressed in the gastrointestinal epithelium in response to injury, 31 samples of ulcerative colitis, Crohn's disease, and ischemic bowel disease were studied using *in situ* hybridization. As normal controls, four samples of histologically normal ileum and three samples of colon were examined using the same probes. All slides were independently analyzed by two investigators (M. Vaalamo and U. Saarialho-Kere) who were unaware of the clinical information, and the samples were processed in at least two different experiments.

Stromelysin-2 Expression Is Associated with Mucosal Erosions

Signal for stromelysin-2 was detected in 20 of 21 samples of IBD and in 7 of 8 samples of ischemic bowel disease (Table 1). The cells expressing stromelysin-2 mRNA were epithelial cells bordering the ulcers or ruptured crypt abscesses (Figure 1, A, D, and F). The cells positive for Ki-67, a marker for cell proliferation, were negative for stromelysin-2 (Figure 1, A and B). Immunostaining of 10 serial sections for laminin-5, an important adhesive ligand for migrating epithelial cells,[32,33] demonstrated co-localization of laminin-5-

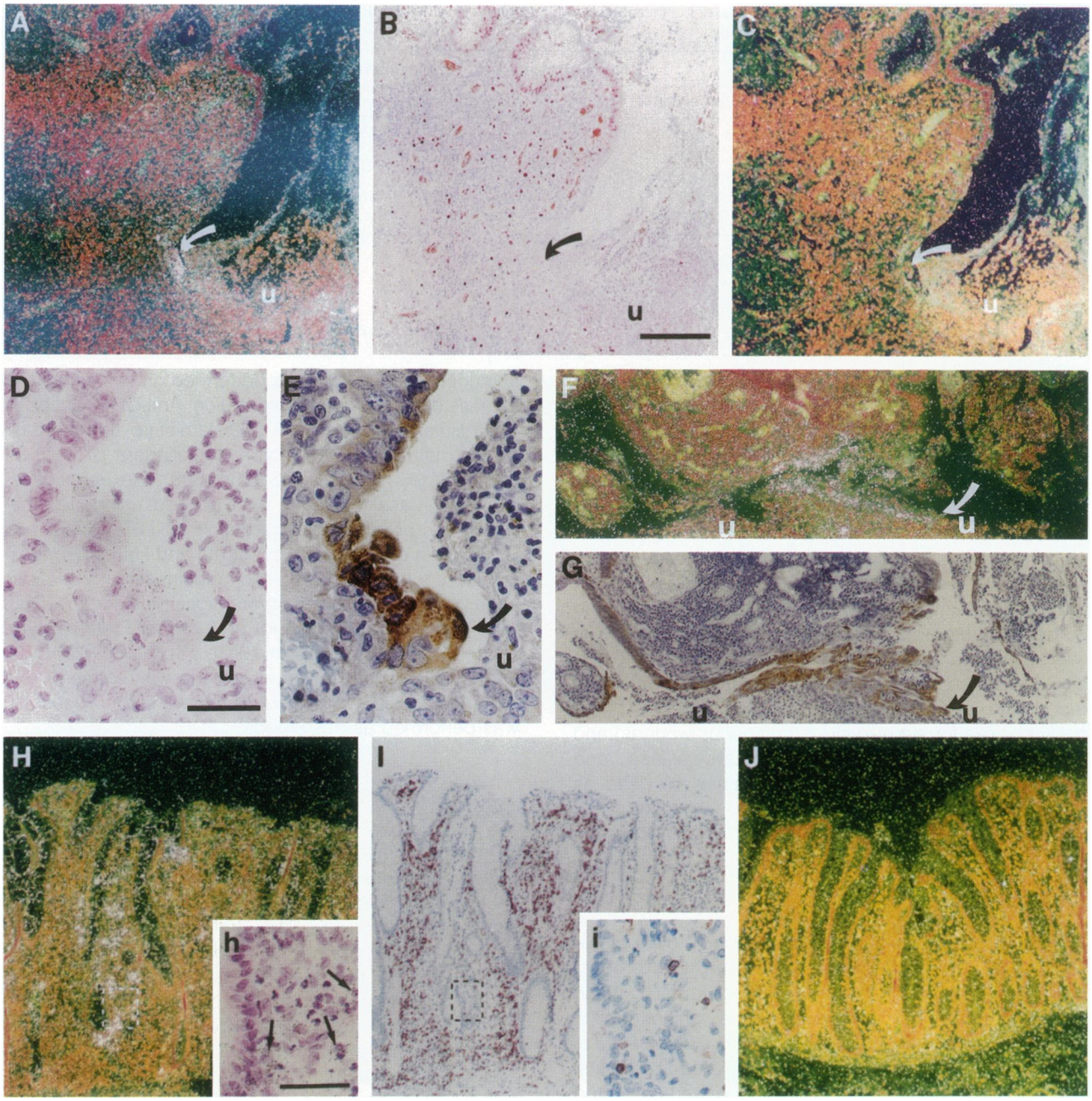

Figure 1. Expression of stromelysin-2 by migrating epithelial cells in Crohn's disease and ulcerative colitis. The **curved arrow** points to the tip of the epithelium; u, the ulcer base. **A:** Dark-field exposure showing signal for stromelysin-2 mRNA in epithelial cells bordering an ulceration in Crohn's disease. **B:** Serial section with immunostaining for Ki-67. **C:** Serial section hybridized with a negative control probe. **D** and **E:** *In situ* hybridization for stromelysin-2 in a sample of ulcerative colitis and a serial section immunostained with laminin-5. **F:** *In situ* hybridization for stromelysin-2 in a sample of ulcerative colitis. **G:** Serial section immunostained for matrilysin. **H:** Dark-field image of *in situ* hybridization for stromelysin-2 in a sample of Crohn's disease. **Inset h:** The **arrows** point to stromelysin-2-positive cells. **I:** Serial section with immunohistochemical staining for T lymphocytes (CD3). **Inset i:** Same area as **inset h**. Note distinct locations of stromelysin-2-expressing cells and T lymphocytes. **J:** Dark-field image of normal intestine with no signal for stromelysin-2. Bars, 50 μm (**A**–C and **F**–**J**), 12 μm (**h** and i), and 6 μm (**D** and **E**).

and stromelysin-2-positive cells (Figure 1, D and E). Immunohistochemical staining of 10 adjacent sections for matrilysin revealed that matrilysin and stromelysin-2 co-localized at the edge of intestinal epithelium bordering the ulcers, whereas matrilysin production continued more distal to the wound edge (Figure 1, F and G). Furthermore, lymphocyte- and macrophage-like cells of the inflamed lamina propria expressed stromelysin-2 mRNA (Figure 1, H and inset h). Staining for CD3 showed that the majority of these cells were not T lymphocytes, even though the expression of stromelysin-2 was occasionally close to T lymphocyte infiltrates (Figure 1, H and I and insets h and i). Staining for B lymphocytes (CD20) demonstrated that areas of stromelysin-2 expression were devoid of these cells (data not shown). All of the samples hybridized with a

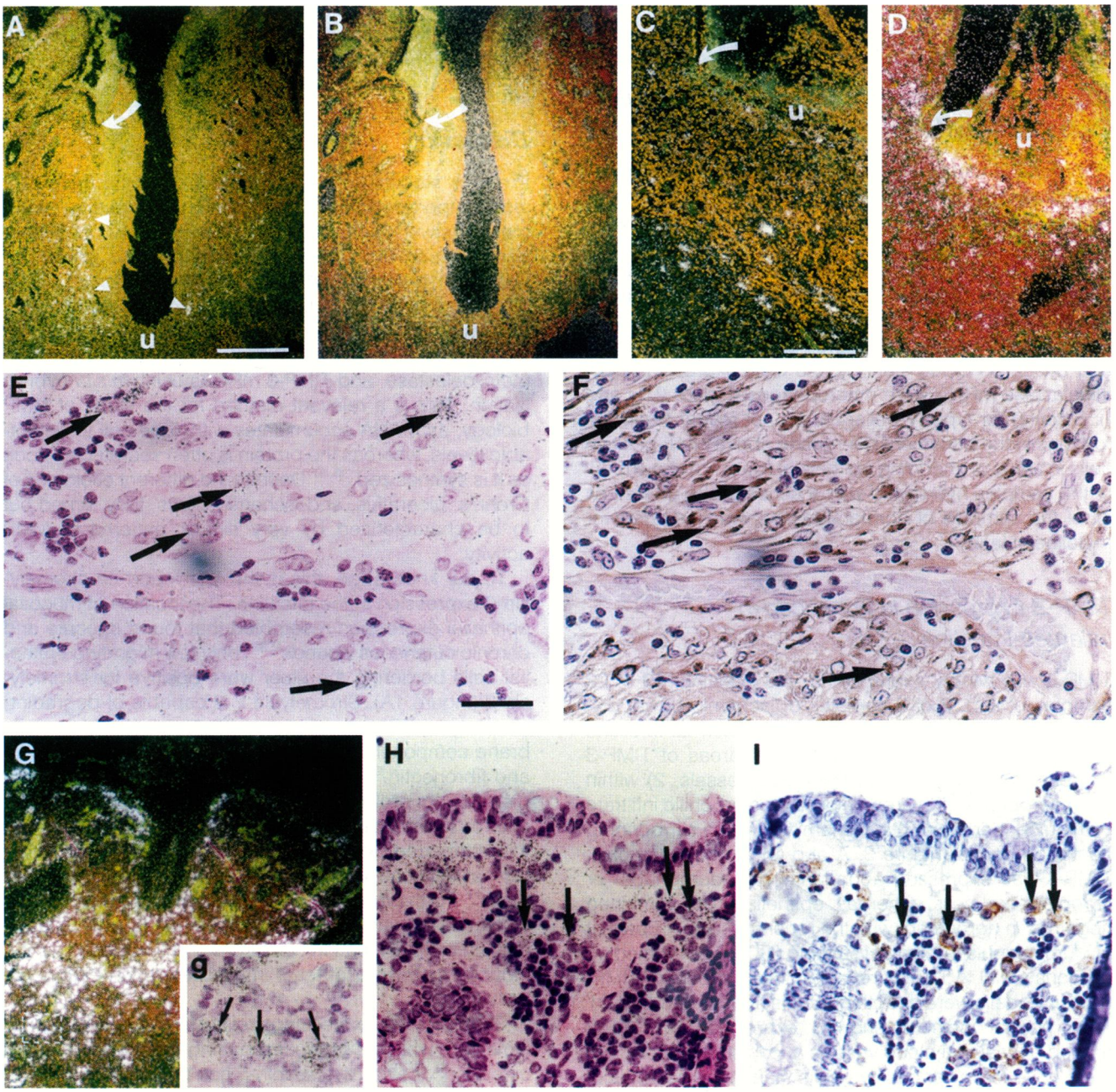

Figure 2. Expression of collagenase-3 mRNA by stromal cells underneath the ulcer bed and HME mRNA in macrophages in inflamed intestine. The **curved arrow** points to the tip of the epithelium; u, the ulcer base. **A:** Dark-field view of an ulceration in a sample of Crohn's disease showing expression for collagenase-3 mRNA beneath the ulcer bed (**arrowheads**). **B:** Same sample hybridized with a sense probe. **C** and **D:** Same sample of Crohn's disease as in Figure 1A hybridized with collagenase-3 (**C**) and metalloelastase (**D**). **E** and **F:** *In situ* hybridization for collagenase-3 and immunostaining for procollagen-I in another sample of Crohn's disease. The **arrows** point to cells that are positive for both collagenase-3 and procollagen-I. **G:** Dark-field image of macrophage metalloelastase mRNA expression in a sample of ulcerative colitis. **Inset g** shows a closer view of positive macrophages (**arrows**). **H:** *In situ* hybridization for metalloelastase in another sample of ulcerative colitis. **G:** Serial section stained with anti-CD68 antibody. The **arrows** point to macrophages that express metalloelastase under the shedding epithelium. Bars, 120 μm (**A**, **B**, and **G**), 25 μm (**C** and **D**), and 12 μm (**E**, **F**, **g**, **H** and **I**).

sense probe were negative (Figure 1C). None of the samples of normal intestine showed expression for stromelysin-2 (Figure 1J).

Fibroblasts of the Wound Bed Express Collagenase-3

Signal for collagenase-3 was detected in 14 of 24 specimens of IBD and in 4 of 8 specimens of ischemic colitis (Table 1). The signal localized in plump macrophage/activated fibroblast-like or spindle fibroblast-like cells in the macrophage-rich zone that lies below the neutrophilic infiltrate of the ulcer bed (Figure 2, A, C, and E). Immunostaining for procollagen I revealed that at least part of the MMP-13-positive cells were fibroblasts (Figure 2, E and F). No signal was found in the intestinal epithelium or nonulcerated lamina propria (Figure 2, A and C). All of the samples hybridized with a sense probe (Figure 2B) as

well as all of the samples of histologically normal intestine were negative.

Macrophages beneath the Shedding Epithelial Cells Express Macrophage Metalloelastase

Macrophage metalloelastase was expressed in 17 of 17 samples of IBD and in 7 of 7 samples of ischemic colitis (Table 1). Particularly in samples of ulcerative and ischemic colitis, abundant expression of HME was detected in plump cells of the lamina propria beneath the shedding epithelial cells (Figure 2, G and H). In all of the sample types, signal for HME in the inflammatory infiltrate was also evident (Figure 2, D, G, and inset g). Morphologically and based on CD68 staining, the cells expressing metalloelastase mRNA were macrophages (Figure 2, H and I). Staining for type IV collagen did not reveal clear disruptions in the basement membrane above HME-positive cells in areas of shedding epithelium (data not shown). All of the samples hybridized with a sense probe as well as all of the samples of normal intestine were negative for metalloelastase.

TIMP-3 Expression Is Enhanced in Inflamed Intestine

TIMP-3 was widely expressed in the intestine; 19 of 22 samples of IBD, 8 of 8 samples of ischemic bowel disease (Table 1), and 4 of 6 samples of normal intestine were positive for TIMP-3. The distinct areas of TIMP-3 expression were 1) around the blood vessels, 2) within the macrophage-rich area below the neutrophilic infiltrate of the ulcer bed, and 3) in the lamina propria surrounding damaged crypts (Figure 3, A and C). In most samples, TIMP-3 was expressed abundantly by macrophage- or fibroblast-like cells, whereas epithelial cells remained negative, in agreement with the recent results of Powe et al.[34] Immunohistochemical staining (CD31) performed on sections serial to TIMP-3 revealed that a subset of TIMP-3-positive cells were endothelial cells (Figure 3, C and D). Also, normal intestine showed expression of TIMP-3 around occasional blood vessels and in stromal cells, but there were far fewer positive cells than in the samples of diseased intestine (data not shown). All of the samples hybridized with a sense probe were negative (Figure 3B).

Stromelysin-2 and Collagenase-3 Are Expressed in Normally Healing Rat Anastomoses

To explore which MMPs are active during normal intestinal wound healing, gut anastomoses of rats were also examined. Expression of stromelysin-2 could be demonstrated from day 4 in stromal cells of the mucosa (data not shown) adjacent to the anastomoses and at least on day 14 in the granulation tissue of the anastomoses (Figure 4, A and inset a). MMP-13 was expressed in plump, macrophage-like cells of the stroma of normally healing experimental anastomoses of the jejunum in 3-, 4-, 7-, and 14-day samples (Figure 4, B and inset b). No signal for either MMP was detected in the epithelium of the samples studied.

Discussion

Previous studies on the expression of MMPs have provided important evidence concerning their role in inflammatory bowel disease. Enhanced expression of collagenase-1, stromelysin-1, matrilysin, and 92-kd gelatinase has been linked to IBD, whereas production of 72-kd gelatinase is not altered.[24,35] The aim of this study was to determine the specific expression patterns and cellular localization of stromelysin-2, collagenase-3, macrophage metalloelastase, and TIMP-3 mRNAs using *in situ* hybridization. This is a relevant method for the study of MMP biology, as mRNA accumulates within the cell to detectable levels, whereas the protein is often secreted as soon as it is synthesized.[36,37] Despite the different etiologies of Crohn's, ulcerative, and ischemic colitis, they turned out to be characterized by very similar patterns of MMP activity.

To our knowledge, this is the first report on stromelysin-2 expression in the intestine. The location of mucosal epithelial expression resembled that found in acute and chronic cutaneous wounds[2,38]; only few migrating epithelial cells bordering the ulcer were positive for stromelysin-2 (Figure 1A). Stromelysin-2 is capable of degrading a variety of ECM substrates, including basement membrane components laminin-1, type IV collagen, entactin, and fibronectin.[9] This ability and the expression pattern distinct from proliferating epithelium suggest a significant role for stromelysin-2 in epithelial migration, which is further supported by the absence of stromelysin-2 mRNA in normal intestine or in colonic adenomas and carcinomas.[39] Furthermore, stromelysin-2 may be involved in activation of matrilysin, another MMP taking part in intestinal epithelial migration, via degrading fibronectin, which induces matrilysin, at least in cultured colon carcinoma cells.[40]

Stromelysin-2-expressing cells stained positively for laminin-5, which is produced by migrating skin keratinocytes.[32,33] Previously, laminin-5 has been linked to intestinal cancer invasion,[28,41] but its production is not enhanced in migrating cells involved in the more superficial process of epithelial restitution[42] nor are we aware of any reports of laminin-5 expression in colonic ulcerations. However, the presence of laminin-5 in the basement membrane of the migrating enterocyte compartment in intact ileum has been reported.[43] Our novel results suggest that intestinal epithelial cells migrating across the wound bed actively produce laminin-5.

Transcription of stromelysin-2 gene is up-regulated by transforming growth factor-β, tumor necrosis factor-α, epidermal growth factor, and keratinocyte growth factor in cultured HaCaT and human keratinocytes.[13,44] Although HaCaT cells are transformed keratinocytes that differ from normal cells in, eg, the regulatory pathways of collagenase-3 and TIMP-3,[21,45] it is tempting to speculate that these cytokines/growth factors regulate the ex-

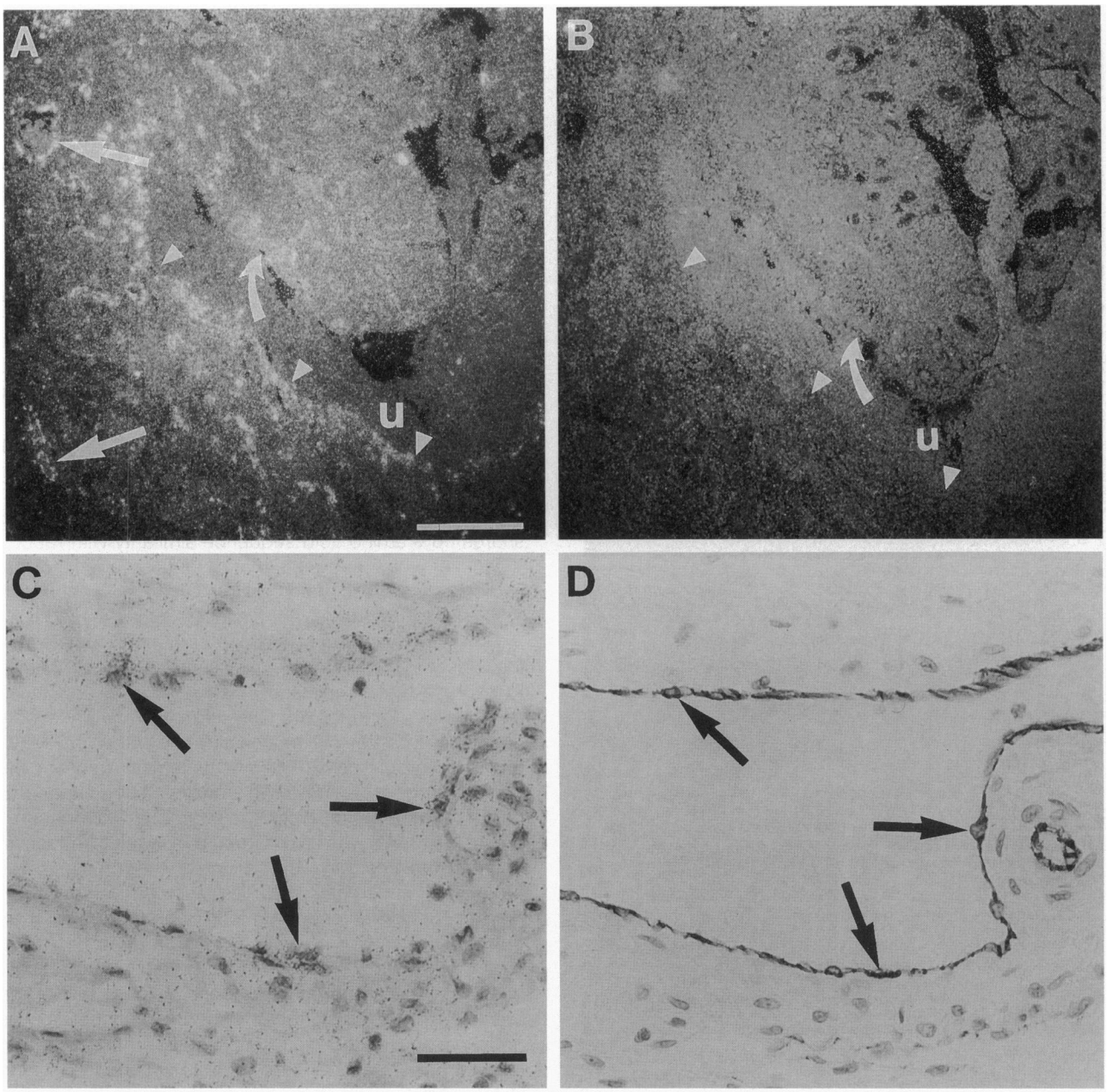

Figure 3. TIMP-3 mRNA is widely expressed in the inflamed intestine. The **curved arrow** points to the tip of the epithelium; u, the ulcer base. **A:** Dark-field exposure showing TIMP-3 mRNA expression in a sample of ulcerative colitis. Note expression beneath the ulcer bed (**arrowheads**) and around blood vessels (**arrows**). **B:** Serial section hybridized with a sense probe. **C:** Bright-field image of a section of ischemic bowel disease with TIMP-3 mRNA expression. **D:** Serial section showing endothelial cells stained for CD31. The **arrows** point to TIMP-3- and CD31-positive cells. Bars, 120 μm (**A** and **B**) and 12 μm (**C** and **D**).

pression of stromelysin-2 also in intestinal epithelium *in vivo*, as their production is enhanced during active IBD.[38,46,47] Furthermore, transforming growth factor-β1 strongly activates expression of stromelysin-2 in HaCaT keratinocytes, and in the gut, transforming growth factor-β1 is expressed mostly by the inflammatory cells closest to the luminal surface.[13,47] Expression of stromelysin-2 was not found in intact epithelium, in agreement with previous data on cutaneous wounds, proposing that its induction may also require contact of epithelial cells with underlying damaged basement membrane or matrix.

Surprisingly, stromelysin-2 was also expressed by macrophage and lymphocyte-like cells in areas of inflammation. This contrasts with our negative findings on stromelysin-2 mRNA in the stromal cells of the healing dermis[2,12] and may reflect differences in the wound repair processes of the gut and skin. Previous *in vitro* work shows that human T lymphocytes are capable of expressing stromelysin-2.[48] Our immunostaining for T lymphocytes indicated, however, that there was often an infiltration of T lymphocytes next to stromelysin-2-expressing cells, yet not co-localizing. This suggests that T lymphocytes do not express stromelysin-2 in the intestine, but could, eg, via production of various cytokines, stimulate other cells of the lamina propria to express stromelysin-2 and in this way participate in the formation

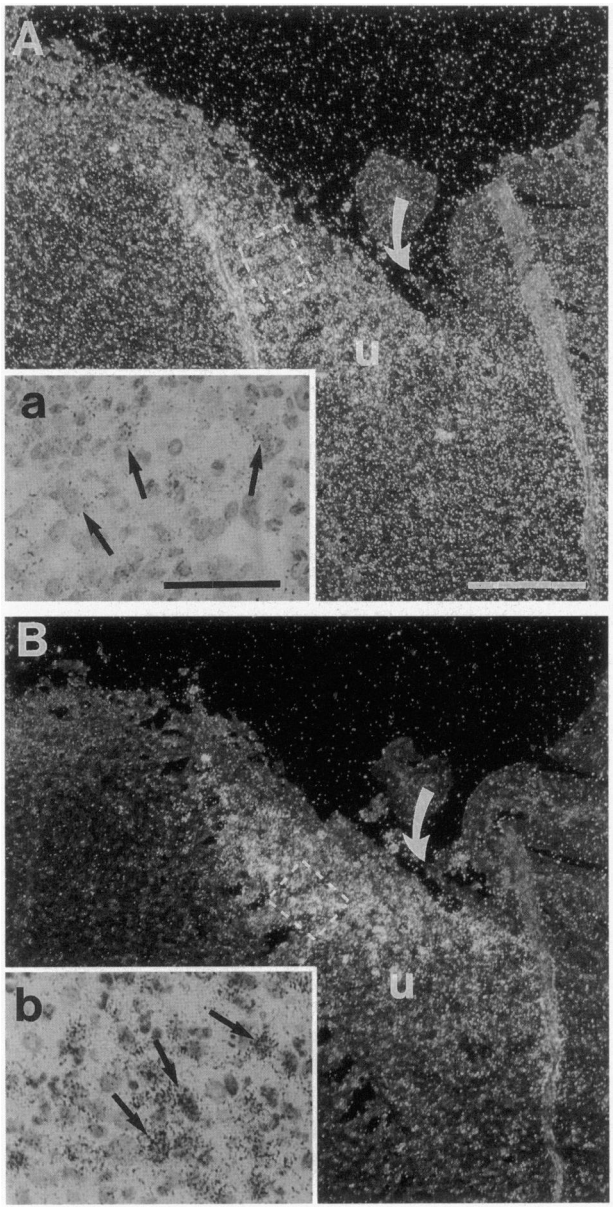

Figure 4. Stromelysin-2 and collagenase-3 are expressed in the granulation tissue of normally healing rat anastomoses. The **curved arrow** points to the tip of the epithelium; u, the ulcer base. **A:** Dark-field image of stromelysin-2 mRNA expression in rat jejunum 14 days after experimental anastomosis. **Inset a** shows stromelysin-2-positive cells (**arrows**) in higher magnification. **B:** Dark-field image of the same sample hybridized with collagenase-3. **Inset b** shows a closer view of collagenase-3-positive cells (**arrows**). Bars, 50 μm (**A** and **B**) and 12 μm (**a** and **b**).

of early lesions of IBD. In fact, during the course of this study, Pender et al[49] provided data on a functional relationship between MMPs and gut injury after T cell activation.

Fibrosis of the submucosa typically develops after inflammation and ulceration during the course of Crohn's disease. Because we found strong expression of collagenase-3 in fibrotic areas of chronic cutaneous ulcers,[7] we assumed the samples of Crohn's disease to express this metalloenzyme abundantly. This was the case, but also the samples of colitis ulcerosa and ischemic bowel disease displayed positive signal. As signal for collagenase-3 was detected only in fibroblasts of the ulcer bed, it could play a role in remodeling of the submucosal matrix. Furthermore, as collagenase-3 was not detected stromally in superficial erosions, nor in all of the samples examined (Table 1), its induction may be a late event during intestinal remodeling in analogy to chronic skin wounds.[2]

Because biopsies of normally healing human intestinal wounds are not feasible to obtain, we examined the role of stromelysin-2 and collagenase-3 in experimental rat anastomoses. Signal for stromelysin-2 was never detected in the epithelium, even though in murine cutaneous wounds it is actively expressed by the migrating epithelium.[13] This may reflect species differences in the intestinal wound repair process.[3,37] Instead we found signal in the mature granulation tissue, indicating a role for stromelysin-2 in the remodeling of the murine intestinal matrix. The induction of collagenase-3 in granulation tissue of rat intestinal anastomoses was detected as early as during the first postoperative day. This is in accordance with recent findings of rat collagenase expression in acetic-acid-induced gastric ulcers.[50] Collagenase-3 shares 86% amino acid sequence similarity with rat collagenase, which suggests that murine collagenase, assumed to be the counterpart of human collagenase-1, really would be an enzyme distinct from it.[6]

This is the first report demonstrating metalloelastase in the intestine. Macrophages are the major cell type known to express metalloelastase in adult tissues, and these cells are crucial in the immune and inflammatory events ongoing in the intestinal mucosa. Strong signal for HME in plump macrophage-like cells in inflamed areas of the intestine suggest a role for this enzyme in tissue degradation required for macrophage migration. Abundant expression of HME was also detected in macrophages just beneath the shedding epithelium of the intestinal wall particularly in samples of colitis ulcerosa. Interestingly, tumor necrosis factor-α production has been shown by the macrophages of corresponding areas in ulcerative colitis, whereas in Crohn's disease it is produced in deeper layers of the lamina propria.[46] Continuity of the basement membrane under the shedding epithelium was not disturbed based on type IV collagen staining. It is tempting to speculate that, whereas collagenase-1 and stromelysin-1 could degrade collagen type III of lamina propria in IBD,[24,50] HME produced by macrophages might cleave fibronectin or laminin, ultimately leading to epithelial cell shedding.

In addition to expression in normal tissues such as placenta, heart, brain, liver, skeletal muscle, kidney, and pancreas,[18] up-regulation of TIMP-3 expression has been linked to carcinomas of epithelial origin[21] and to rat skin wound healing.[3] As has been shown in colon carcinomas,[34] we could demonstrate stromal TIMP-3 expression in round and spindle-shaped cells with fibroblast-like appearance, but the possibility that some of the cells are macrophages cannot be excluded. Furthermore, signal for TIMP-3 was detected in endothelial cells where it may play a role in inhibition of angiogenesis and stabilization of vessel walls in areas of inflammation and repair.[51]

The expression of MMPs is known to be enhanced in chronic ulcers; in chronic cutaneous wounds the number

of epithelial and stromal cells expressing collagenase-1 and stromelysin-1 and -2 is greater than in normally healing wounds.[1,2] Furthermore, collagenase-3 is found exclusively in the chronic wound stroma,[7] whereas human corneas with repair defects overexpress 92-kd gelatinase and collagenase-1.[37] Our current and previous studies show that, as in chronic cutaneous wounds, TIMPs are not detected in the epithelium of chronic gut ulcerations.[2,24] Taken together, these results suggest an imbalance between metalloproteinases and their inhibitors during impaired wound repair.

We conclude that, analogously to cutaneous wounds, stromelysin-2 is up-regulated during migration of mucosal epithelial cells bordering human intestinal ulcers. However, it is not expressed in the epithelium of acute rat intestinal wounds, and thus, we cannot exclude the possibility that stromelysin-2 represents aberrant metalloenzyme expression and actually impairs healing. Stromelysin-2 partly colocalizes with matrilysin, and these MMPs may function in migration in a cascade-like fashion by degrading noncollagenous matrix molecules. Metalloelastase may have a role in inducing epithelial cell shedding whereas collagenase-3 and TIMP-3 are abundantly expressed in the ulcer stroma. Thus, we provide further support to the hypothesis that during intestinal wound healing MMPs and their inhibitors are acting in parallel and/or cascade-like fashion, tightly spatially and temporally expressed to achieve targeted ECM degradation.

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