Arch Dermatol Res (1998) 290 (Suppl): S47–S54

U. K. Saarialho-Kere

Patterns of matrix metalloproteinase and TIMP expression in chronic ulcers

Abstract **Controlled proteolysis is needed for cell migration, angiogenesis, and matrix remodeling during normal wound repair. Our objective has been to investigate how chronic leg ulcers differ from normally healing wounds (pinch graft donor sites) with respect to their metalloproteinase expression patterns. Using in situ hybridization and immunohistochemistry, we found that collagenase-1 (MMP-1), stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) were expressed in keratinocytes bordering both acute and chronic wounds. Unlike MMP-1, signal for collagenase-3 (MMP-13) was not detected in keratinocytes but exclusively in fibroblasts deep in the ulcer bed of chronic wounds, suggesting that while MMP-1 production is important for migration, MMP-13 plays a role in matrix remodeling. Tissue inhibitor of metalloproteinase (TIMP)-1 was not detected in the epidermis of any chronic wound sample while it was expressed in keratinocytes bordering normally healing wounds. TIMP-3 was abundantly expressed in stromal fibroblast- and macrophage-like cells surrounding vessels and sweat glands in both types of wounds. Our results suggest that there are no qualitative differences in the expression of MMPs-1, -3 and -10 in the epidermis of chronic vs normally healing wounds. However, the number of stromal cells expressing MMP-1 and MMP-3 was greater in chronic vs acute wounds, whereas MMP-10 was never detected in the dermis. TIMP-1 expression near the basement membrane in acute, but not in chronic, wounds suggests that the balance between MMPs and their inhibitors may be altered in poorly healing wounds. Analogous to chronic cutaneous wounds, MMP-1 and -3 are abundantly expressed in chronic small and large bowel ulcers, while the migrating surface epithelium is negative for TIMP-1 expression.**

Work presented at the 7th Annual Meeting of the European Tissue Repair Society, Special Symposium "Proteolysis and Tissue Repair", 23–26 August 1997, Cologne, Germany

U. K. Saarialho-Kere
Department of Dermatology,
Helsinki University Central Hospital, Meilahdentie 2,
FN-00250 Helsinki, Finland
Tel.: +358-9-4716254, Fax: +358-9-471-6561
e-mail: ulpu.saarialho-kere@helsinki.fi

Introduction

Proteolytic degradation of extracellular matrix (ECM) is an essential feature of repair and remodeling during cutaneous wound healing. Members of the matrix metalloproteinase (MMP) enzyme family may theoretically be involved in various tasks during wound repair: (1) removal of devitalized tissue, (2) epidermal-mesenchymal interactions during keratinocyte migration, (3) angiogenesis, (4) remodeling of newly synthesized connective tissue during maturation, and (5) regulation of activities of certain growth factors [1].

The MMPs are zinc-dependent endopeptidases collectively capable of degrading essentially all components of ECM (see [2]). Controlled breakdown of ECM by MMPs plays an important role in detachment and migration of cells, as well as in tissue remodeling in several physiological situations, e.g. developmental tissue morphogenesis, tissue repair, and angiogenesis. It is likely that in these situations distinct MMPs cooperate in parallel and/or cascade-like fashion to achieve effective and targeted ECM degradation, which is also controlled by specific tissue inhibitors of metalloproteinases (TIMPs). In skin, several different types of cells are capable of producing MMPs: keratinocytes, fibroblasts, macrophages, endothelial cells, mast cells, eosinophils, and neutrophils. The basal expression of most MMPs in cultured cells is low, and these enzymes are induced at transcriptional level by a variety of cytokines and growth factors, relevant also during wound repair, such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), and TGF-β depending on cell type [3]. With some exceptions [4–6], MMPs are not constitutively ex-

pressed in the skin in vivo but are induced temporarily in response to exogenous signals, such as various cytokines or growth factors, cell-matrix interactions [7, 8], and altered cell-cell contacts [9].

At present, the MMP gene family consists of 15 structurally related members, which can be divided into subgroups of collagenases, gelatinases, stromelysins, and membrane type MMPs (MT-MMPs) according to their substrate specificity and primary structure. The collagenase subgroup currently consists of three members, collagenase-1 or fibroblast collagenase (MMP-1), collagenase-2 or neutrophil collagenase (MMP-8), and the recently discovered collagenase-3 (MMP-13); these are the principal secreted neutral proteinases capable of initiating degradation of native fibrillar collagen types I, II, III, and V. MMP-1 degrades type III collagen most efficiently while MMP-8 is more potent in degrading type I collagen. Collagenase-3 (MMP-13), in turn, degrades type II collagen sixfold more effectively than type I and III collagens and displays almost 50-fold stronger gelatinolytic activity than MMP-1 and MMP-8 [10, 11]. In addition, MMP-13 also degrades type IV, IX, X, and XIV collagens, large tenascin C, and fibronectin [12]. Rat and murine interstitial collagenases are homologues of human MMP-13 instead of MMP-1 [13].

Stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) are able to degrade a wide range of substrates including fibronectin, type IV, V, IX, and X collagens, elastin, laminins, gelatin, and proteoglycan core proteins. Stromelysin-3 (MMP-11) is structurally less closely related to MMP-3 and MMP-10 and to date, MMP-11 has not been shown to degrade any ECM component. In contrast to other MMPs, except MT-MMPs, MMP-11 is activated prior to secretion by a Golgi-associated proteinase, furin [14].

The gelatinase subgroup of MMPs contains two members, 72 kDa gelatinase (gelatinase-A, MMP-2) and 92 kDa gelatinase (gelatinase-B, MMP-9), also called 72 kDa and 92 kDa type IV collagenases, respectively. MMP-2 and MMP-9 are thought to play an important role in the final degradation of fibrillar collagens after initial cleavage by collagenases. It is therefore possible that due to their ability to initiate and continue degradation of fibrillar collagen of type I [15, 16], MMP-2 and MMP-9 play a more important role in the remodeling of collagenous ECM than has been previously thought.

Matrilysin (MMP-7), the smallest member of MMP gene family, has a broad substrate specificity, being able to degrade elastin, fibronectin, laminin, entactin, type IV collagen, and proteoglycan core proteins. Macrophage metalloelastase (MMP-12) degrades elastin, type IV collagen, laminin, fibronectin, vitronectin and heparan sulphates [19]. Recently, a new member of the MMP gene family was cloned independently by two groups and assigned numbers MMP-18 [20] and MMP-19 [21], but its ECM substrate specificity has not been determined yet.

The MT-MMP subgroup contains four members, all of which have a transmembrane sequence in the COOH-terminal. MT-MMPs contain a cleavage site for furin proteinases between the propeptide and the catalytic domain providing the basis for furin-dependent activation of latent MT-MMPs prior to secretion. The substrate specificity of MT1-MMP (MMP-14) is best known, and it was initially shown to cleave and activate latent MMP-2 [17]. However, recent observations show that MT1-MMP also degrades ECM components, e.g. native type I, II and III collagen, gelatin, fibronectin, laminin-1, and cartilage proteoglycan core protein [18]. The substrate specificities of other MT-MMPs are not known.

Methods

Tissue specimens

Informed consent was obtained from individual subjects for all procedures. Human skin samples were collected from a total of 17 patients with chronic leg ulcers who underwent excision and grafting procedures at the Department of Plastic Surgery, Helsinki University Central Hospital. They had all been treated with conservative measures without reduction in the ulcer surface area. The tissue material was formalin-fixed and paraffin-embedded.

As controls for acute wounds, biopsies of normally healing donor areas on the anterior thigh were obtained from 15 patients of the Department of Dermatology, University of Helsinki, undergoing pinch grafting procedure [22, 23]. On day 0, pinch grafts approximately 1.5 cm in diameter and extending to full-thickness at their centers, had been cut off from the donor site and transferred to the ulcer area in the ankle region. Thereafter, 1, 2, 3, 5, 6 and 7 days post-wounding, these small wound areas in the upper thigh were biopsied. All patients were women ranging in age from 62 to 94 years.

Formalin-fixed, paraffin-embedded archival specimens of gastric ulcers (n = 5), duodenal ulcers (n = 3), samples demonstrating idiopathic inflammatory bowel disease (IBD) (ulcerative colitis (n = 5), Crohn's colitis (n = 7), and enteritis (n = 6), as well as specimens representing histologically normal duodenum (n = 2), ileum (n = 3), and colon (n = 3) were obtained from the Department of Pathology, University of Helsinki. All IBDs were in an active, ulcerative phase.

In situ hybridization. In situ hybridization was performed on 5-μm sections as described in detail [24]. All samples were treated with proteinase K and were washed in 0.1 M triethanolamine buffer containing 0.25% acetic anhydride. The sections were hybridized overnight with 1×10^6 cpm of ^{35}S-labeled RNA probes at 55 °C. After hybridization, the slides were washed under stringent conditions, including treatment with RNase A, and were processed for autoradiography as described [24]. After 10–30 days of autoradiographic exposure, the photographic emulsion was developed, and slides were stained with hematoxylin and eosin.

Immunohistochemistry

On sections parallel to those used for in situ hybridizations, tissue macrophages were identified by a monoclonal anti-macrophage antibody (KP-1; M814, DAKO Corp, Carpinteria, CA) which reacts with CD-68, a specific macrophage marker. Activated fibroblasts were stained with a monoclonal antibody to the NH_2-terminals of the type I procollagen (PC-I) molecule (MAB 1912; Chemicon, Temecula, CA). Monoclonal α-smooth muscle actin (6582; Bio-Makor, Rehovot, Israel) antibody was used to identify myofibroblasts. Monoclonal anti-laminin antibody (LAM-89, Sigma, product no. L-8271) and polyclonal rabbit anti-human fibronectin (Dako, product no A245) antiserum were used to stain these matrix proteins. Monoclonal MIB-1 antibody (Immunotech, Marseille, France, product no. 0505), which reacts with the Ki-67 nuclear antigen, was used to detect proliferating cells from resting intestinal epithelial cells. Matrilysin protein was detected using an

affinity-purified antibody raised in rabbits against a synthetic peptide [6]. Sections were processed for immunohistochemistry as described [7] using 3,3'-diaminobenzidine tetrahydrochloride or aminoethylcarbazole (α-smooth muscle actin, Ki-67) as chromogenic substrate. Controls were performed with rabbit preimmune serum or preimmune mouse ascites fluid.

Cell Cultures and Northern Analysis. Normal human skin fibroblasts were maintained in Dulbecco's modification of Eagle's medium (DMEM, Flow Laboratories), 2 mM glutamine, 100 IU/ml penicillin-G, and 100 mg/ml of streptomycin. Collagen gels were prepared using bovine dermal collagen, Cellon (Strassen, France) containing 95% type I collagen and 5% type III collagen. The fibroblasts were cultured in collagen gels as described [8]. As a control, fibroblasts were plated as monolayer and cultured in DMEM supplemented with 10% FCS for 48 h. Promonocytic U937 cells, THP-1 cells and human alveolar macrophages were cultured as previously described [25].

To isolate total RNA from the dermal fibroblasts inside collagen gels, the gels were briefly treated with 0.5 mg/ml collagenase

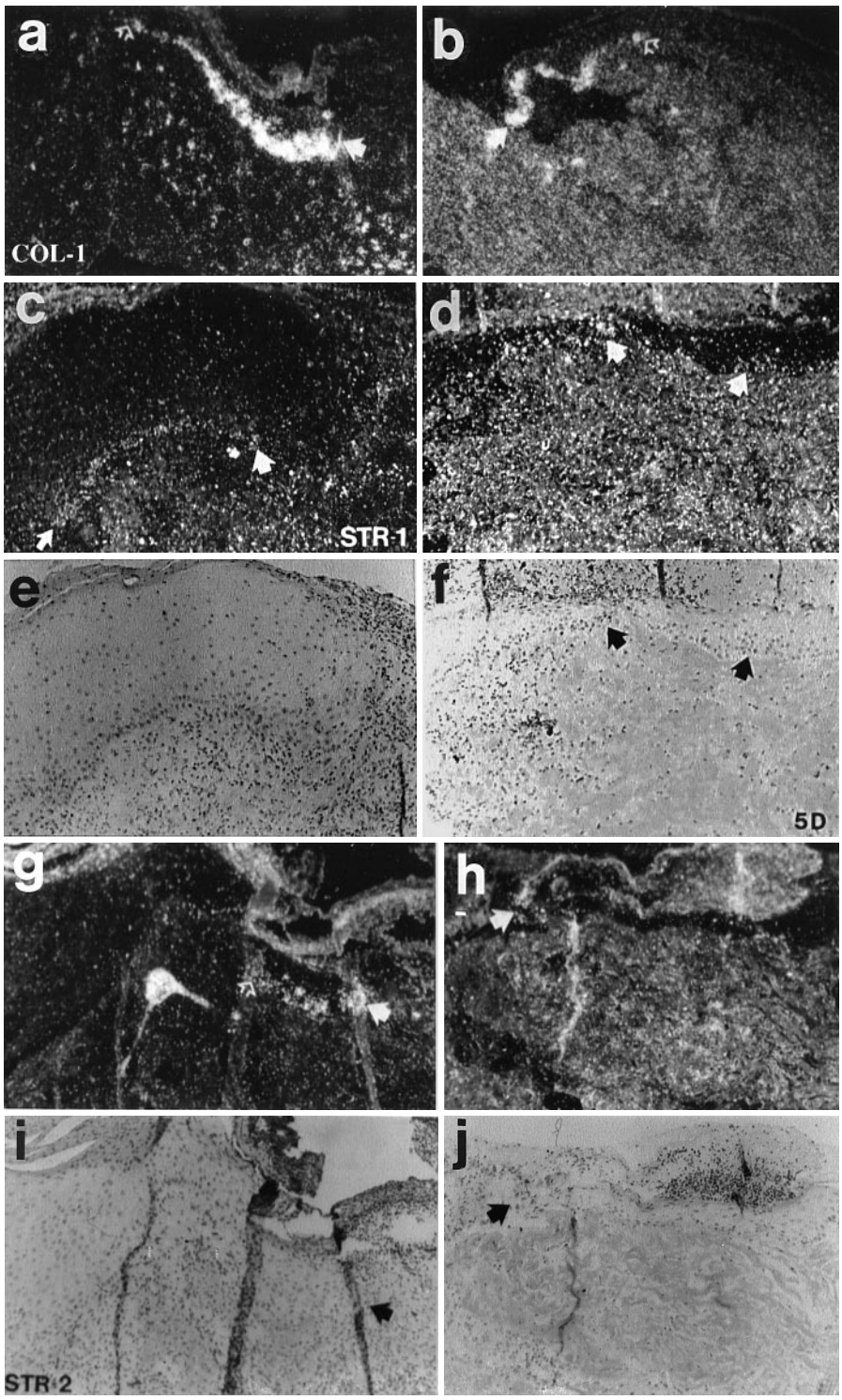

Fig. 1 Collagenase-1 expression in a venous stasis ulcer and **b** in a 2 day normally healing wound. **c** Stromelysin-1 expression in a venous stasis ulcer and **d** in a 5-day nearly reepithelialized normally healing wound. **e, f** Corresponding bright-field images. **g** Stromelysin-2 expression in a venous stasis ulcer and **h** in a 4 day acute wound. **i, j** Corresponding bright-field images. Signal for each matrix metalloproteinase (MMP) mRNA is depicted by *arrows*

(type II, Sigma) in phosphate-buffered saline (PBS, pH 7.4) with 1 mM $CaCl_2$. Total cellular RNA was isolated from all the cell types studied using the single step method [26]. Aliquots of total RNA (10–17 μg) were fractionated on 0.8% agarose gel, the filter was hybridized with ^{32}P-labeled cDNAs for MMP-1 and MMP-13 [8], and the ^{32}P-cDNA-mRNA hybrids were visualized with autoradiography.

Results

Chronic cutaneous wounds

Spatially and temporally controlled expression of several distinct MMPs appears to be associated with ulcer repair. MMP-1 is consistently expressed by migrating keratinocytes that have moved off the basement membrane in different types of wounds; these include acute normally healing wounds (Fig. 1 b) as well as chronic ulcers such as venous stasis ulcers (Fig. 1 a). In agreement with our results, data on acute experimental [7, 27] and burn wounds [28] as well as wounds representing aberrant healing, such as ulcerated pyogenic granulomas [38], demonstrate MMP-1 expression bordering ulcers. Furthermore, MMP-1 is also expressed in keratinocytes when the basement membrane remains partly intact such as during the repair of suction blisters and bullas produced by a variety of blistering skin diseases [29]. The production of MMP-1 by migrating keratinocytes may aid in dissociating the cell from the collagenous dermal matrix and promote efficient locomotion over dermal and provisional matrices. In fact, keratinocytes use MMP-1 to cleave collagen to gelatin, thereby providing a substrate that is more conducive to migration [30]. Several studies have demonstrated a key role for altered cell-matrix interactions, particularly contact with type I collagen, in initiating keratinocyte MMP-1 synthesis [31]. When reepithelialization is complete, MMP-1 expression is shut off in basal keratinocytes. Modulation of intracellular calcium can block secretion of MMP-1 by keratinocytes which have moved away from the stratum basalis and from their natural substrate [32].

Both MMP-3 and MMP-10 are expressed by basal keratinocytes in acute and chronic wounds (Fig. 1 c–j) [33, 34], yet by distinct populations of these cells. MMP-3 is expressed further away from the wound edge and is probably not needed for reepithelialization but for restructuring the newly formed basement membrane (Fig. 1 c–f). Since MMP-3-positive proliferating keratinocytes reside on an intact basement membrane the primary stimulus for its production may be a soluble factor such as TNF-α, IL-1, EGF, PDGF or TGF-β [34]. MMP-10 expression colocalizes with that of MMP-1 at the epithelial tip bordering both acute and chronic ulcers (Fig. 1 g–j). This enzyme may be involved in the superactivation of cosecreted collagenase or may facilitate keratinocyte migration by degrading noncollagenous matrix molecules.

During wound repair both MMP-1 (Fig. 1 a) and MMP-3, but not MMP-10, are also expressed in dermal fibroblasts and participate in the formation and removal of granulation tissue and resolution of scar tissue [28, 34]. The number of cells expressing MMP-1 and -3 in the stroma of chronic wounds is greater than the number of positive cells in normally healing wounds.

In contrast to MMP-1, collagenase-3 (MMP-13) is not expressed by keratinocytes bordering normally healing wounds (Fig. 2 b). However, MMP-13 is abundantly expressed by stromal cells in chronic wounds distinct from areas of stromal MMP-1 expression [8]. By using PC-1, α-smooth muscle actin and CD-68 immunostaining we were able to show that many of these MMP-13 positive cells are activated fibroblasts. Furthermore, by northern analysis we were not able to detect MMP-13 in three types of macrophage-like cells (U937, THP-1, alveolar macrophages) [8]. Interestingly, human skin fibroblasts cultured in three-dimensional collagen gel also express MMP-13, whereas dermal fibroblasts cultured on tissue culture plastic do not [8], indicating an important role for cell-matrix interactions in the control of fibroblast MMP-13 expression. Furthermore, our results clearly show that while MMP-1 is critical for reepithelialization, MMP-13 is involved in the degradation of type I and III collagen and their cleavage products in the chronic ulcer bed and may play a role in the pathogenesis of chronic ulcers.

Matrilysin is a constitutive product of the epithelium of eccrine and apocrine sweat glands [6]. However, it is not

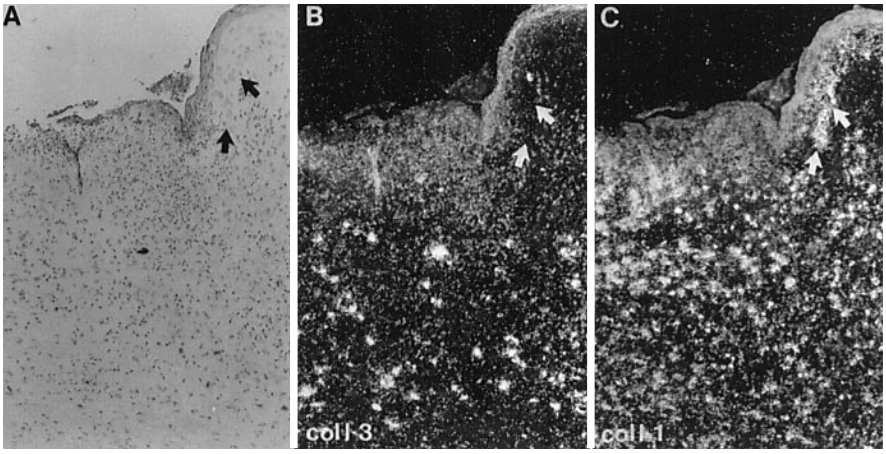

Fig. 2 A–C **A** Bright field image of a chronic venous ulcer. **B** Signal for collagenase-3 mRNA in the stroma. **C** Signal for collagenase-1 in the epidermis and stroma. *Arrows* show the wound edge epithelium

up-regulated in vivo in healing cutaneous wounds [7]. Human macrophage metalloelastase can be expressed by stromal macrophage-like cells of the chronic wounds (Vaalamo and Saarialho-Kere, unpublished).

We are not aware of studies on the expression of gelatinases mRNAS in chronic cutaneous wounds. However, other groups have shown in normally healing wounds that MMP-2 is important in the prolonged remodeling phase [39] and its expression remains stable during the days immediately after creation of the wound [40]. MMP-9 can be found both in blister fluid and in situ in tissues during reepithelialization of suction blisters [41]. It could be involved in several early steps of wound repair: detachment of keratinocytes from the basement membrane, promoting cell locomotion in wound matrix, and remodeling of the fibrin-fibronectin matrix [40].

It is likely that the balance between proteinase and inhibitor levels plays a crucial part in successful wound healing process. TIMP-1 is temporally and spatially regulated during cutaneous wound repair [28, 33]. We have not detected TIMP-1 in the epidermis of chronic wounds (Fig. 3 a, c). However, in agreement with previous data [28], it was detected in the epidermis of acute human wounds (Fig. 3 b, d) until their reepithelialization and stromally until day 7. Particularly in 3-day and 5-day wounds, TIMP-1 was expressed in basal keratinocytes further away from the wound [33]. Furthermore, compared to acute wounds, fluid from nonhealing venous and pressure ulcers contains high levels of activated gelatinases and low levels of TIMP-1 [35–37]. Lack of TIMP-1 expression in keratinocytes of chronic ulcers vs positive expression in the epithelium of normally healing wounds suggests that excessive proteolysis retards the healing of venous ulcers. TIMP-1 is often expressed perivascularly in both chronic and acute wounds [28, 38], suggesting that it may be protecting these areas from proteolytic degradation. Analogously, epidermal TIMP-1 may be inhibiting metalloenzymes from degrading the epidermal basement membrane.

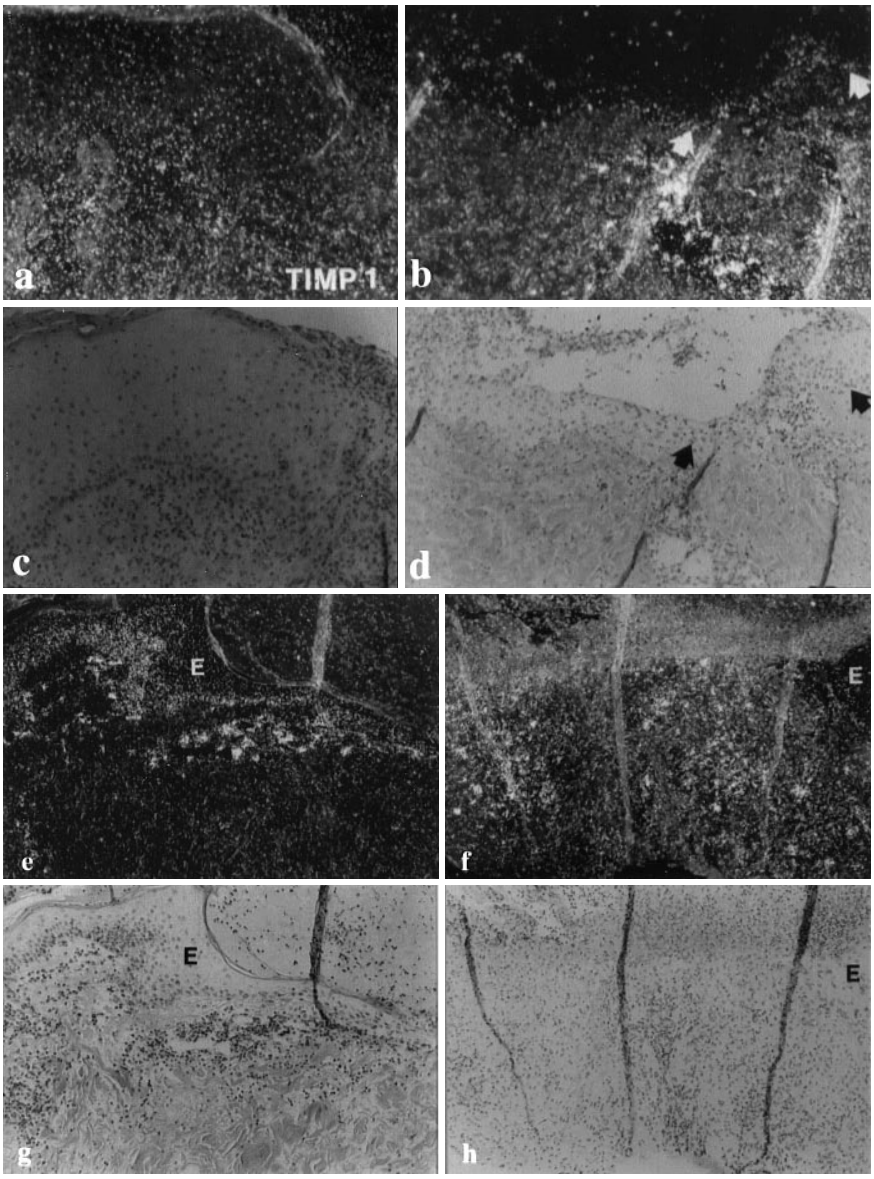

Fig. 3 a–h **a** Expression of tissue inhibitor of metalloproteinase (TIMP)-1 in a chronic venous ulcer and **b** in a 3-day normally healing wound. **c, d** Corresponding bright field images. **e** TIMP-3 expression in an acute 5-day wound and **f** in a chronic venous ulcer. **g, h** Corresponding bright-field images. *Arrows* depict the TIMP-1 mRNA positive area in b. *E*, epidermis

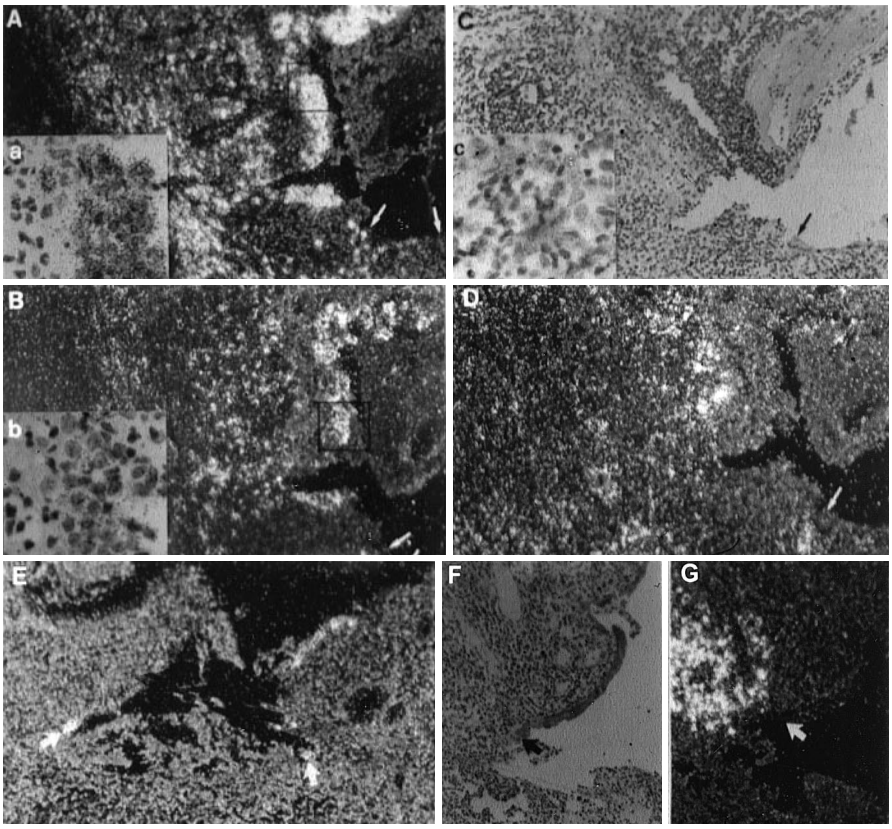

Fig. 4 A–G **A** Stromal expression of collagenase-1, **B** stromelysin-1 and **D** tissue inhibitor of metalloproteinase (TIMP)-1 in an ulcer caused by Crohn's disease. *Arrows* mark the mucosal epithelium. *Inset a*, Collagenase mRNA in large round cells in the base and margins of the ulcer; *inset b*, stromelysin-1 mRNA in the ulcer stroma in plump macrophage-like cells; *inset c*, collagenase mRNA does not colocalize with darker CD-68-positive cells. **E** Expression of matrilysin mRNA in the epithelium of an ulcer caused by ulcerative colitis. **F** Serial sections demonstrating matrilysin protein and **G** collagenase-1 mRNA. *Arrows* mark epithelial tips bordering the ulcers

TIMP-3 was produced in stromal fibroblast-like cells in chronic ulcers (Fig. 3 f, h) and in acute wounds (Fig. 3 e, g). However, TIMP-3 was expressed in keratinocytes in normally healing wounds (Fig. 3 e, g), while keratinocytes bordering chronic venous ulcers were negative (Vaalamo and Saarialho-Kere, unpublished) (Fig. 3 f, h).

Gastrointestinal ulcers

Healing of gastrointestinal ulcers is known to bear several similarities to repair processes seen in skin wounds. Therefore, we also investigated whether gastrointestinal ulcers involve a similar expression of MMPs by the injured surface epithelium [42]. Unlike in skin lesions, MMP-1 is not produced by the surface epithelium bordering gastrointestinal ulcers but is prominently expressed in activated fibroblasts in the gut stroma in ulcers of Crohn's disease and ulcerative colitis (Fig. 4 A). The intensity of signal for collagenase mRNA was very high in IBD specimens whereas peptic ulcers of the stomach and duodenum displayed fewer positive stromal cells. As in chronic cutaneous wounds, the mucosal epithelium of chronic IBD lesions is devoid of MMP-13 mRNA (Vaalamo and Saarialho-Kere, unpublished).

In IBD samples, MMP-3 is partly expressed in the same areas as MMP-1 in stromal round, activated fibroblast/macrophage-like cells (Fig. 4 B). Unlike in skin wounds, MMP-3 was never detected in the epidermis.

Surprisingly, matrilysin (MMP-7) mRNA and protein were detected in the epithelium bordering gastrointestinal ulcers regardless of the type of ulcer (Fig. 4 E, F). No MMP-7 was seen in normal gastrointestinal mucosa, suggesting that matrilysin is not involved in the regular epithelial renewal. Immunostaining for laminin-1 and fibronectin was abnormally weak at the site of basement membrane under MMP-7-positive epithelial cells and by doing the Ki-67 immunostaining we were able to show that matrilysin production is associated with migration in intestinal ulcers [42]. Alternatively, MMP-7 may be needed to remodel the gastrointestinal basement membrane due to its ability to cleave entactin, fibronectin and type IV collagen.

Analogously to chronic skin wounds, abundant TIMP-1 mRNA was detected in the granulation tissue at the base of the ulcers, but never in the mucosal epithelium (Fig. 4 D). Furthermore, we have not detected TIMP-3 in the mucosal epithelium of IBD lesions (Vaalamo and Saarialho-Kere, unpublished). However, it is abundantly expressed in the vicinity of the blood vessels and damaged crypts of the inflamed intestine, generally in deeper layers than TIMP-1.

Conclusions

Our results in both cutaneous and gastrointestinal wounds suggest that a variety of MMPs are up-regulated during wound repair (Table 1). While there are no qualitative differences in the expression of MMPs-1, -3, and -10 in chronic and normally healing cutaneous wounds, a greater

Table 1 Expression of metalloproteinases in chronic ulcers

Enzyme	Epithelium		Stroma	
	Skin	Gut	Skin	Gut
Collagenase-1	+	–	+	+
Collagenase-3	–	–	+	+
Stromelysin-1	+	–	+	+
Stromelysin-2	+	+	–	+
Matrilysin	–	+	–	–
Metalloelastase	–	–	+	+
TIMP-1	–	–	+	+
TIMP-3	–	–	+	+

number of stromal and epithelial cells produce MMP-1 and -3 in chronic vs acute wounds (Fig. 1). MMP-13 is not involved in the normal repair of dermal wounds but plays an important role in the stromal remodeling of chronic ulcers, possibly decelerating the repair process. As in chronic gastrointestinal ulcers, TIMP-1 and -3 are not expressed by epithelial cells in abnormally healing cutaneous wounds. However, the spatially and temporally regulated epithelial expression of TIMP-1 and -3 in acute wounds suggests that there may be an imbalance between proteinases and their inhibitors contributing to the chronicity of, e.g. venous leg ulcers. Although in both skin and gut the ultrastructure of the basement membrane is assumed to be similar, the intestinal wounds heal more rapidly. Differences in the composition of the intestinal matrix and the matrix-binding receptors on an epithelial cell may dictate its pattern of MMP expression. Thus, MMP-1 would be needed to facilitate migration of keratinocytes over the collagen-rich matrix in healing skin whereas MMP-7 would serve the same role in the repair of gut epithelium.

Acknowledgements I would like to thank Dr. William C. Parks for continuing collaboration and discussions; Drs. Maarit Vaalamo and Kristiina Airola for their contributions on the role and regulation of MMPs and their inhibitors in normal vs chronic wounds; and Dr. Veli-Matti Kähäri and his group, collaborators in studying the role and regulation of MMP-13 and TIMP-3 in skin. Our work has been supported by grants from the Paulo Foundation and Helsinki University Central Hospital Research Fund.

References

1. Gearing AJH, Beckett P, Christodoulou M et al. (1994) Processing of tumor necrosis factor alpha precursor by metalloproteinase. Nature 4:370:555–557
2. Woessner JF (1994) The family of matrix metalloproteinases. Ann NY Acad Sci 732:11–30
3. Mauviel AJ (1993) Cytokine regulation of metalloproteinase gene expression. Cell Biochem 53:288–295
4. Hasty KA, Pourmotabbed TF, Goldberg GI et al. (1990) Human neutrophil collagenase. A distinct gene product with homology to other matrix metalloproteinases. J Biol Chem 265:11421–11424
5. Ståhle-Bäckdahl M, Parks WC (1993) 92 kDa gelatinase is actively expressed by eosinophils and secreted by neutrophils in squamous cell carcinoma. Am J Pathol 142:1–6
6. Saarialho-Kere UK, Crouch EC, Parks WC(1995) Matrix metalloproteinase matrilysin is constitutively expressed in adult human exocrine epithelium. J Invest Dermatol 105:190–196
7. Saarialho-Kere UK, Kovacs SO, Pentland AP, Olerud JE, Welgus HG, Parks WC (1993) Cell-matrix interactions modulate interstitial collagenase expression by human keratinocytes actively involved in wound healing. J Clin Invest 92: 2858–2866
8. Vaalamo M, Mattila L, Johansson N et al. (1997) Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. J Invest Dermatol 109:96–101
9. Lacraz S, Isler P, Welgus HG, Dayer J-M (1994) Direct contact between T-lymphocytes and monocytes is a major pathway for the induction of metalloproteinase expression. J Biol Chem 269: 22027–22033
10. Knäuper V, Lopez-Otin C, Smith B, Knight G, Murphy G (1996) Biochemical characterization of human collagenase-3. J Biol Chem 271:1544–1550
11. Mitchell PG, Magna HA, Reeves LM et al. (1996) Cloning, expression and type II collagenolytic activity of matrix metalloproteinase. J Clin Invest 97:761–768
12. Knäuper V, Cowell S, Smith B et al. (1997) The role of the C-terminal domain of human collagenase-3 (MMP-13) in the activation of procollagenase-3, substrate specificity, and tissue inhibitor of metalloproteinase interaction. J Biol Chem 272: 7608–7616
13. Freije J MP, Diez-Itza I, Balbin M et al. (1994) Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. J Biol Chem 269:16766–16773
14. Pei D, Weiss SJ (1995) Furin-dependent intracellular activation of the human stromelysin-3 zymogen. Nature 375:244–247
15. Aimes RT, Quigley JP (1995) Matrix metalloproteinase-2 is an interstitial collagenase. J Biol Chem 270:5872–5876
16. Okada Y, Nara K, Kawamura K et al. (1995) Localization of matrix metalloproteinase 9 (92 kDa gelatinase/type IV collagenase = gelatinase B) in osteoclasts; implications for bone resorption. Lab Invest 72:311–322
17. Sato H, Takino T, Okada Y et al. (1994) A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 370:61–65
18. Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y (1997) Membrane type I matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. J Biol Chem 272:2446–2451
19. Chandler S, Cossins J, Lury J, Wells G (1996) Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. Biochem Biophys Res Commun 228:421–429
20. Cossins J, Dudgeon TJ, Catlin G, Gearing AJH, Clements JM (1996) Identification of MMP-18, a putative novel matrix metalloproteinase. Biochem Biophys Res Commun 228:494–498
21. Pendas AM, Knäuper V, Puente XS et al. (1997) Identification and characterization of a novel human matrix metalloproteinase with unique structural characteristics, chromosomal location and tissue distribution. J Biol Chem 272:4281–4286
22. Ceilley RI, Rinek MA, Zuehlke RL (1977) Pinch grafting for chronic ulcers on lower extremities. J Dermatol Surg Oncol 3: 303–309
23. Poskitt KR, James AH, Lloyd-Davies ERV et al. (1987) Pinch skin grafting of porcine dermis in venous ulcers: a randomised clinical trial. Br Med J 294:674–676
24. Prosser IW, Stenmark KR, Suthar M et al. (1989) Regional heterogeneity of elastin and collagen gene expression in intralobar arteries in response to hypoxic pulmonary hypertension as demonstrated by in situ hybridization. Am J Pathol 135:1073–1088
25. Saarialho-Kere UK, Welgus HG, Parks WC (1993) Distinct mechanisms regulate interstitial collagenase and 92 kDa gelatinase expression in human monocytic-like cells exposed to bacterial endotoxin. J Biol Chem 268:17354–17361

26. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid gunaidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162 : 156–159
27. Inoue M, Kratz G, Haegerstrand A, Ståhle-Bäckdahl M (1995) Collagenase expression is rapidly induced in wound-edge keratinocytes after acute injury in human skin, persists during healing, and stops at re-epithelialization. J Invest Dermatol 104 : 479–483
28. Stricklin GP, Liying L, Jancic V, Wenczak BA, Nanney LB (1993) Localization of mRNAs representing collagenase and TIMP in sections of healing human burn wounds. Am J Pathol 143 : 1657–1666
29. Saarialho-Kere UK, Vaalamo M, Airola K, Niemi K-M, Oikarinen AI, Parks WC (1995) Interstitial collagenase is expressed by keratinocytes which are actively involved in re-epithelialization in blistering skin diseases. J Invest Dermatol 104 : 982–988
30. Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC (1997) The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. Cell Biol 137 : 1445–1457
31. Sudbeck BS, Parks WC, Welgus HG, Pentland AP (1994) Collagen-stimulated induction of keratinocyte collagenase is mediated via tyrosine kinase and protein kinase C activities. J Biol Chem 269 : 30022–30029
32. Sudbeck BD, Pilcher BK, Pentland AP, Parks WC (1997) Modulation of intracellular calcium levels inhibits secretion of collagenase-1 by migrating keratinocytes. Molec Biol Cell 8 : 811–824
33. Vaalamo M, Weckroth M, Puolakkainen P et al. (1996) Patterns of matrix metalloproteinase and TIMP-1 expression in chronic and normally healing human cutaneous wounds. Br J Dermatol 135 : 52–59
34. Saarialho-Kere UK, Pentland AP, Birkedal-Hansen H, Parks WC, Welgus HG (1994) Distinct populations of basal keratinocytes express stromelysin-1 and stromelysin-2 in chronic wounds. Clin Invest 94 : 79–88
35. Wysocki AB, Staiano-Coico L, Grinnell F (1993) Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. J Invest Dermatol 101 : 64–68
36. Bullen EC, Longmaker MT, Updike DL et al. (1995) Tissue inhibitor of metalloproteinases-1 is decreased and activated gelatinases increased in chronic wounds. J Invest Dermatol 104 : 236–240
37. Weckroth M, Vaheri A, Lauharanta J, Sorsa T, Konttinen YT (1996) Matrix metalloproteinases, gelatinases, and collagenase in chronic leg ulcers. J Invest Dermatol 106 : 1119–1124
38. Saarialho-Kere UK, Chang E S, Welgus HG, Parks WC (1992) Expression of interstitial collagenase, 92 kDa gelatinase and TIMP-1 in granuloma annulare and necrobiosis lipoidica diabeticorum. J Clin Invest 90 : 1952–1957
39. Agren GS (1994) Gelatinase activity during wound healing. Br J Dermatol 131 : 634–640
40. Salo T, Mäkelä M, Kylmäniemi M, Autio-Harmainen H, Larjava H (1994) Expression of matrix metalloproteinase-2 and -9 during early human wound healing. Lab Invest 70 : 176–182
41. Oikarinen A, Kylmäniemi M, Autio-Harmainen H, Autio P, Salo T (1993) Demonstration of 72-kDa and 92-kDa forms of type IV collagenase inhuman skin: variable expression in various blistering diseases, induction during re-epithelialization, and decreases by topical glucocorticoids. J Invest Dermatol 101 : 205–210
42. Saarialho-Kere UK, Vaalamo M, Puolakkainen P, Airola K, Parks WC, Karjalainen-Lindsberg M-L (1996) Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. Am J Pathol 148 : 519–526