Enhanced Expression of Interstitial Collagenase, Stromelysin-1, and Urokinase Plasminogen Activator in Lesions of Dermatitis Herpetiformis

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Because dermatitis herpetiformis is characterized by neutrophilic inflammation and destructive changes in the basement membrane zone, we studied the *in situ* expression of interstitial collagenase and stromelysin-1 in 11 lesions. A prominent signal for collagenase mRNA was consistently detected in the basal keratinocytes of rete ridges surrounding the neutrophilic abscesses in 10 of 11 lesions, and the expression was independent of the age of the lesion and the migratory state of the basal keratinocytes. Expression of stromelysin-1 was detected in seven of 11 lesions and co-localized with collagenase. No expression of the 92-kDa gelatinase mRNA or matrilysin protein was found in the vicinity of neutrophilic accumulations or the damaged basement membrane. Urokinase-type plasminogen activator mRNA was found in basal keratinocytes in seven of nine samples. Collagenase, stromelysin-1, and urokinase-type plasminogen activator were not expressed in normal-appearing skin of patients with dermatitis herpetiformis. Our results suggest that in lesions of dermatitis herpetiformis, collagenase and stromelysin-1 may be induced in basal keratinocytes by neutrophil cytokines or by altered cell-matrix interactions through contact of keratinocytes with the matrix due to damaged basement membrane. Stromelysin-1, in particular, may contribute to formation of blisters by degrading basement membrane components. ***Key words: metalloproteinase/keratinocyte/bullous disease. J Invest Dermatol 105:184–189, 1995***

Dermatitis herpetiformis (DH) lesions are characterized histologically by inflammatory changes and destruction of type IV collagen and laminin in the basement membrane zone [1,2], which leads to blister formation above the lamina densa [3]. Among the matrix metalloproteinases, interstitial collagenase degrades fibrillar types I, II, III, and X collagens [4,5], but unlike many other metalloenzymes, it is not able to destroy components of the basement membrane. We have previously demonstrated that collagenase expression is induced by cell-matrix interactions in various types of skin ulcers, where this enzyme is essential for the migration of keratinocytes [6]. In DH, keratinocytes may come into contact with dermal components through discontinuities in the basement membrane [1]. Knowing that inflammatory agents such as cytokines may induce production of metalloproteinases [7,8] and that stromelysins, gelatinases, and matrilysin, in particular, are capable of degrading basement membrane components such as type IV collagen, laminin, and entactin [9–11], we investigated the possible role of these proteases in the pathogenesis of DH lesions. Because plasmin has been implicated in the *in vivo* activation of interstitial collagenase and stromelysin, we also examined the role in this disease of urokinase-type plasminogen activator (uPA), which converts the proenzyme plasminogen to active plasmin.

We report here that collagenase mRNA is expressed by basal keratinocytes in DH lesions. In contrast to findings in dermal wounds [6] and various blistering diseases [12], collagenase expression is not limited to areas that show signs of reepithelialization but also characterizes recent DH blisters. In most lesions, we also found expression of stromelysin-1 and uPA mRNAs, which may contribute to the degradation of basement membrane components or may function as activators of latent collagenase or gelatinases.

MATERIALS AND METHODS

Tissues Formalin-fixed, paraffin-embedded biopsy specimens of DH were obtained from the Department of Dermatology, Helsinki University Hospital, Finland. Biopsies were taken from the lesions in different parts of the body after the diagnosis of DH had been confirmed clinically, histopathologically, and by immunofluorescence showing granular IgA deposits at the basement membrane zone. For comparison, two biopsy specimens from normal-looking skin of DH patients were obtained. Both of the patients had no rash; one of them was treated with a glutein-free diet and dapsone, and the second one with the diet only. Granular deposits of IgA were seen by immunofluorescence in skin specimens of both patients.

***In Situ* Hybridization** *In situ* hybridization was performed on 5-μm sections as described in detail [13]. All samples were treated with proteinase K and were washed in 0.1 M triethanolamine buffer containing 0.25% acetic acid. Sections were covered with 20–40 μl of hybridization buffer containing 2.5–3 $\times$ 10^4 cpm/ml of ^{35}S-labeled antisense or sense RNA probe. Sections were incubated at 55°C for 18 h in a humidified chamber. After hybridization, the slides were washed under stringent conditions, including treatment with RNase A to remove unhybridized probe, and were processed for autoradiography as described [13]. After 10–17 d of autoradio-

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Abbreviations: DH, dermatitis herpetiformis; uPA, urokinase-type plasminogen activator.

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Table I. Histologic Features and Distribution of Collagenase, Stromelysin-1, and uPA mRNAs in the 11 Samples Used in the Study[a]

Sample	Signal for mRNAs in Basal Keratinocytes					Reepithelialization[b]
	Collagenase	Stromelysin	uPA	TIMP-1	92-kDa Gelatinase	
1	+	−	ND	ND	ND	+
2	++	−	−	−	−	+
3	−	−	ND	ND	ND	+
4	+	+	+	ND	−	−
5	++	+	+	−	−	−
6	+	+	+	ND	ND	+
7	+	−	+	−	−	+
8	++	+	+	−	−	−
9	+	+	+	−	−	+
10	+	+	−	ND	−	−
11	+	++	+	ND	−	+

[a] +, intensity of signal; −, no signal detected; ND, not determined.
[b] Samples in which reepithelialization was present also displayed other signs of older DH lesions on light microscopy, such as unilocularity.

graphic exposure, the photographic emulsion was developed, and the slides were stained with hematoxylin and eosin.

RNA Probes The production and specificity of the antisense human interstitial collagenase, stromelysin-1, 92-kDa gelatinase, and tissue inhibitor of metalloproteinases (TIMP-1) probes have been described [14–16]. An 826-base pair fragment, corresponding to positions 835 to 1661 from the 5′ end of the human uPA cDNA (American Type Culture Collection [17]), was generated by polymerase chain reaction and was designed with a T7 RNA polymerase recognition element at the 3′ end and an SP6 polymerase recognition element at the 5′ end. Both sense and antisense probes were transcribed from this polymerase chain reaction product. In addition to sense uPA RNA probe, tissue sections in each experiment were hybridized with ^{35}S-labeled sense RNA transcribed from a bovine tropoelastin cDNA. The validity of this probe as a negative control has been confirmed by Northern [13] and by *in situ* hybridization assays [14]. *In vitro* transcribed RNA was labeled to high specific activity with α[^{35}S-UTP] (more than 1200 Ci/mmol), as described [6].

Assessment of Probe Specificity Expression of uPA mRNA was studied in unstimulated HT-1080 cells, cultured as described [18]. RNA isolation and Northern hybridization were done as described [19], and the stringency conditions were similar to those for the *in situ* hybridization assay. The autoradiogram showed that the uPA probe detects the appropriate 2.4-kb mRNA in HT-1080 cells. A weaker hybridization signal was detected in lipopolysaccharide-stimulated U937 cells [19] that were run simultaneously with HT-1080 mRNA.

Antibodies Antihuman α1(IV) collagen monoclonal antibody was supplied by Dr. John A. McDonald (Mayo Clinic, Scottsdale, AZ). This antibody was raised against the NH_2-terminal domain of the molecule. Specificity of the antibody has been determined by enzyme-linked immunosorbent assay and immunoblotting [6]. Polyclonal affinity-purified rabbit antihuman 92-kDa gelatinase antibodies were supplied by Dr. William C. Parks (Washington University, St. Louis, MO) [6,20]. Affinity-purified polyclonal antiserum against matrilysin was provided by Dr. Howard Welgus (Washington University). The specificity of this antibody was confirmed by Western analysis [21].

Immunohistochemistry On sections serial to those used for *in situ* hybridization, immunostaining for type IV collagen and 92-kDa gelatinase was done by the peroxidase-antiperoxidase technique using diaminobenzidine as chromogenic substrate, as described in detail [6,20]. The matrilysin antibody was diluted 1:1000 and reacted at 4°C overnight. Controls were performed with rabbit preimmune serum or preimmune mouse ascites fluid.

RESULTS

Tissues To assess whether collagenase and stromelysin-1 are expressed in DH, 11 samples were studied using *in situ* hybridization. In addition, a subset of samples was examined for the expression of 92-kDa gelatinase and uPA mRNA **(Table I)**. Four of 11 samples were histologically considered early multilocular blisters displaying multiple neutrophilic papillary microabscesses. Seven were older specimens showing unilocular blisters with epidermal regeneration at the edges or aligned flattened cells in the roof **(Fig 2)**, although three of these still had characteristic papillary microabscesses at the periphery. Serial sections of all samples were stained with antibodies for type IV collagen to reveal any disruption in the basement membrane. The localization of 92-kDa gelatinase and matrilysin protein was examined by immunohistochemistry in a subset of samples (nine of 11 and five of 11, respectively).

Collagenase, Stromelysin-1, and uPA mRNA Are Expressed by Basal Keratinocytes A prominent signal for collagenase was detected in basal keratinocytes in 10 of 11 samples, regardless of the age of the blister **(Figs 1*A*, 2*A*)**. The only negative sample was an older unilocular blister, which showed reepithelialization at the bottom of the blister (data not shown). Collagenase expression was not limited to regenerating epidermis, but was also detected in keratinocytes having no light-microscopic evidence of migration **(Figs 1*A*,*F*, 2*C*)**. Type IV collagen staining was either absent or faint under keratinocytes expressing collagenase mRNA **(Fig 1*F*)**. A moderate signal for collagenase was also found in stromal cells in six of 11 samples (data not shown). Lymphocytes and polymorphonuclear leukocytes were always negative.

Expression of stromelysin-1 mRNA was found in basal keratinocytes in seven of 11 samples, regardless of the age of the blister. Unlike in dermal wounds [15], stromelysin-1 was produced by the same populations of basal keratinocytes as was collagenase **(Figs 1*B*, 2*B*)**, whereas no stromelysin-1 mRNA was detected in the dermal stroma of these lesions.

An epidermal signal for uPA mRNA was found in basal keratinocytes in six of seven samples **(Figs 1*C*, 2*H*)**. The same samples also expressed stromelysin-1, in agreement with the *in vitro* results that both enzymes are induced by the same cytokines and growth factors [22]. Stromal signal for uPA was detected in two of seven samples in macrophage-like cells of perivascular inflammatory infiltrates. Sections hybridized with sense uPA RNA probe had only background signal **(Fig 1*D*)**.

In the specimens of normal-looking skin from two DH patients, which both displayed IgA deposits by immunofluorescence, no signal for collagenase, stromelysin, or uPA was found. No signal was apparent on sections hybridized with sense RNA transcribed from bovine tropoelastin DNA **(Fig 2*I*)**. As reported earlier, collagenase, stromelysin-1, and uPA are not expressed in the epidermis of healthy human skin [6,15,23].

Expression of 92-kDa Gelatinase and TIMP-1 Expression of 92-kDa gelatinase mRNA was not found in any of the eight samples analyzed, which agrees with previous results showing that this enzyme is actively expressed by eosinophils but that neutrophils contain the protein [24]. Immunohistochemistry revealed faint

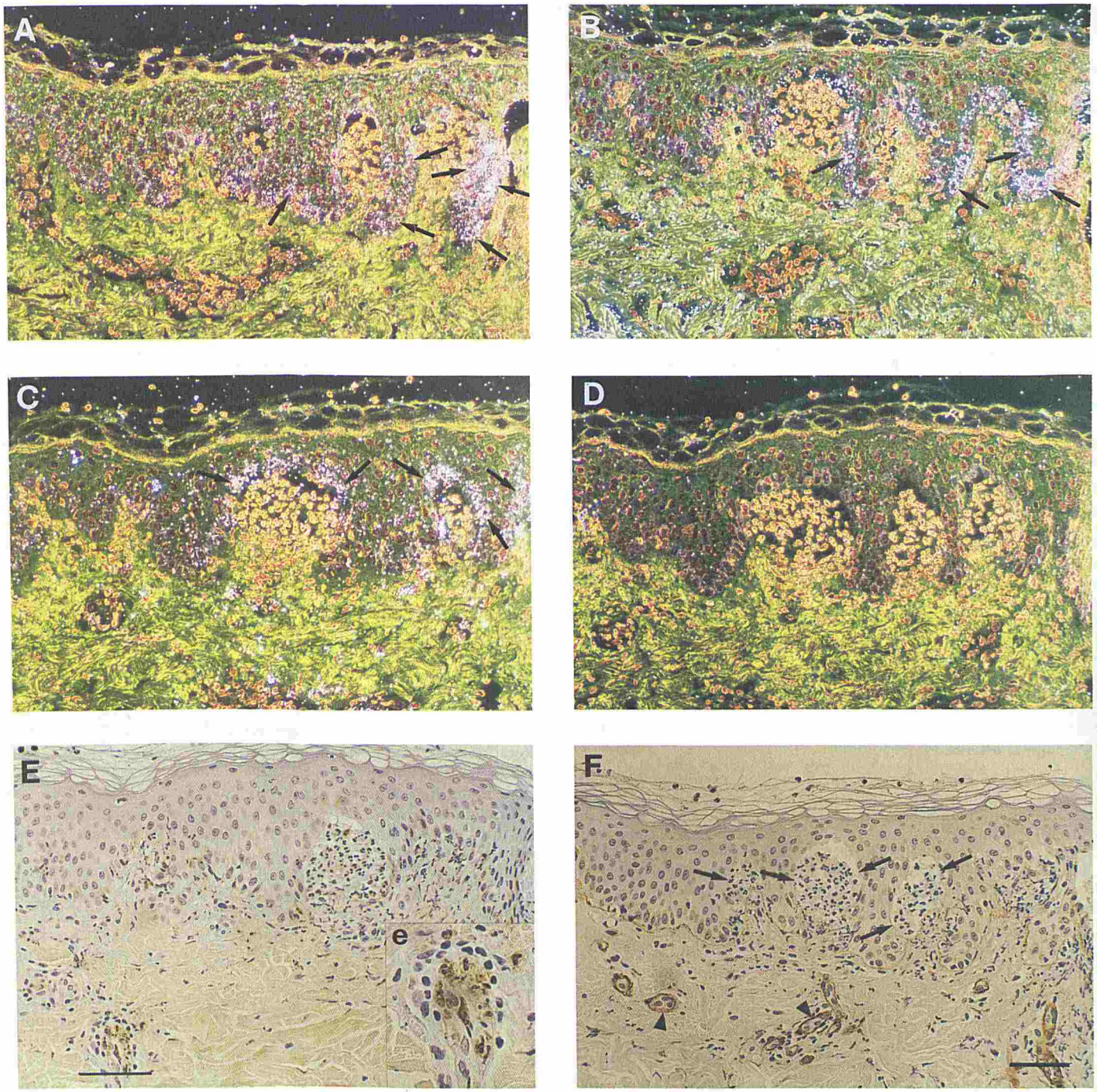

Figure 1. Collagenase, stromelysin-1, and uPA mRNAs are expressed by basal keratinocytes in an early DH lesion. Serial sections were hybridized with collagenase (*A*), stromelysin-1 (*B*), uPA antisense (*C*) and sense (*D*) probes, or processed for immunohistochemistry with 92-kDa gelatinase (*E*) and type IV collagen antibodies (*F*), as described in *Materials and Methods*. *A*, collagenase (*arrows*) and *B*, stromelysin-1 (*arrows*) mRNA co-localize to basal keratinocytes in rete ridges in the vicinity of neutrophil abscesses. *C*, uPA mRNA is evident in basal keratinocytes (*arrows*). *D*, a section hybridized with sense uPA mRNA is negative. *E*, immunostaining for 92-kDa gelatinase reveals positive neutrophils (*inset e*) in a perivascular infiltrate from the *underlined area*, whereas most neutrophils in the microabscess are negative. *F*, parallel section to *B* is stained for type IV collagen antibody. Disruption of the basement membrane is evident around neutrophil accumulations (*arrows*), and staining is also fainter around the tips of rete ridges than in normal-looking skin. A strong normal immunostaining reaction for type IV collagen is detected around multiple blood vessels (*arrowheads*). *A-D*, dark-field images; time for autoradiography was 13–17 d. *Bar*: *A-F*, 16 μm.

staining for 92-kDa gelatinase in three of seven samples in neutrophils belonging to dermal perivascular infiltrates, but not in neutrophilic microabscesses near the basement membrane **(Fig 1*E*)**. However, we cannot exclude the possibility that the 92-kDa–gelatinase-negative neutrophils had emptied their contents before forming the microabscess. Squamous cell carcinomas were used as positive controls in the immunostaining experiments, and they displayed numerous 92-kDa–gelatinase-positive neutrophils and eosinophils, as described previously [20].

Five samples were analyzed for the expression of TIMP-1

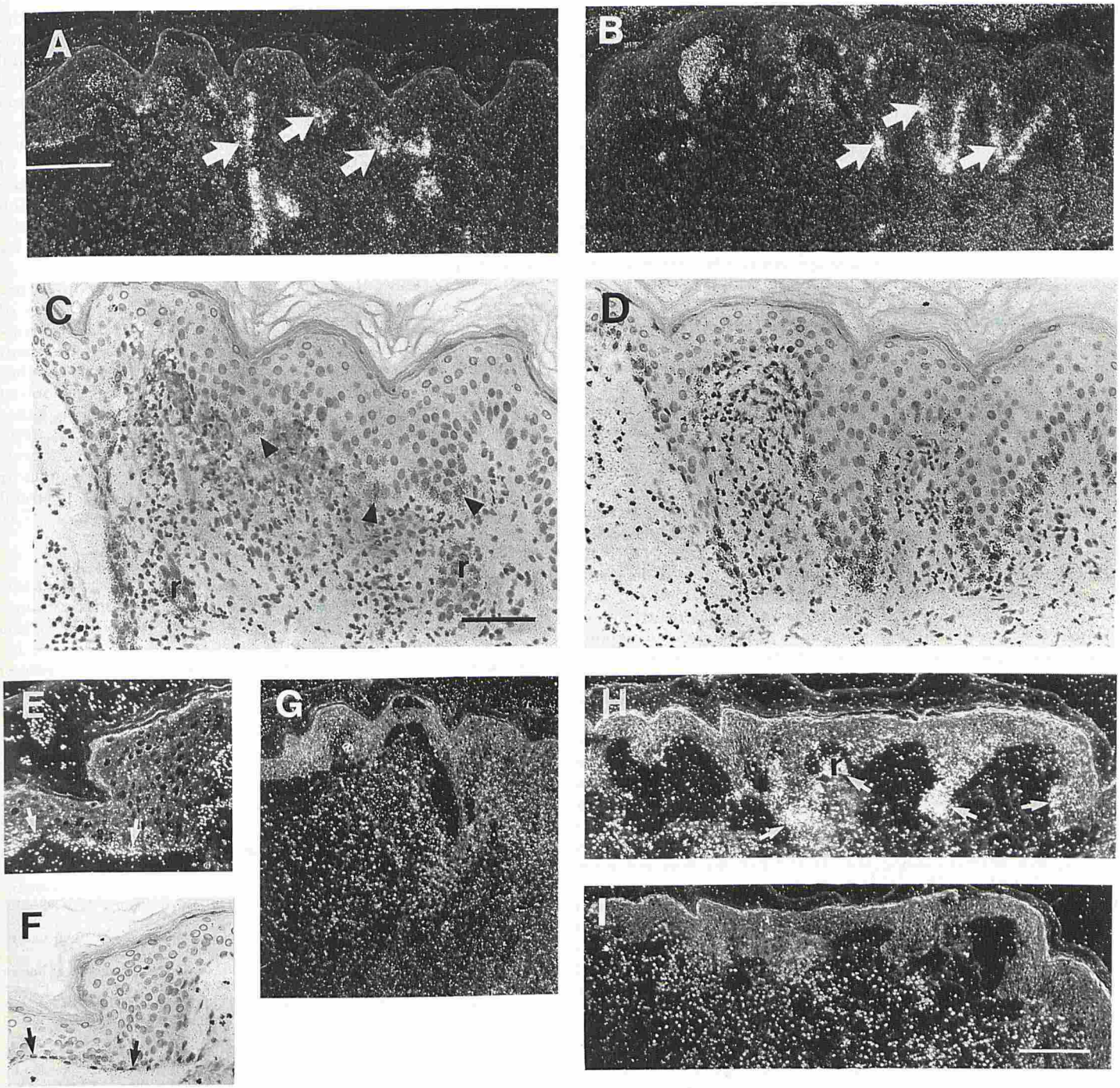

Figure 2. Collagenase and stromelysin-1 mRNA co-localize in an older DH lesion. Serial sections were hybridized with collagenase (*A*), stromelysin-1 (*B*), 92-kDa gelatinase (*G*), and uPA (*H*) antisense and sense bovine tropoelastin (*I*) probes, as described in *Materials and Methods*. *A*, collagenase and *B*, stromelysin-1 mRNAs are spatially correlated and expressed in basal keratinocytes of rete ridges; the *arrows* mark the corresponding spots in each figure. *C*, in addition to basal keratinocytes with no light-microscopic evidence of migration or regeneration (*arrowheads*), regenerating, aligned, flattened keratinocytes (*arrows*) at the roof of a blister express collagenase (*E*). The *underlined area* in *A* is shown in a higher magnification in a dark-field (*E*) and bright-field (*F*) image. *D*, bright-field image of the area shown in *B*. *G*, no signal for 92-kDa gelatinase is seen in neutrophils. *H*, uPA mRNA is detected in basal and suprabasal keratinocytes (*arrows*) and in a detached rete ridge (r). *I*, a slide hybridized with the sense bovine tropoelastin probe is negative. *Bars*: *A,B,G-I*, 47 μm; *C-F*, 23 μm.

mRNA. All samples showed abundant stromal signal, but TIMP-1 mRNA never co-localized with collagenase mRNA in the basal keratinocytes (data not shown).

Immunohistochemistry Matrilysin has a broad substrate specificity, and it can degrade components of the basement membrane such as entactin [11] and possibly type IV collagen. Because it can function as a collagenase activator [25], we carried out immunostaining analysis with rabbit polyclonal antibodies against matrilysin. All five samples examined, however, were negative (data not shown). Samples of normal skin having abundant sweat glands were used as positive controls.‡

‡ Saarialho-Kere U, Welgus HG, Parks WC: Prominent and constitutive expression of matrilysin is characteristic of exocrine epithelium (abstr). *J Invest Dermatol* 102:612, 1994.

In agreement with previous studies [1,2], immunostaining for type IV collagen revealed disruption of the basement membrane in all samples at the sites of neutrophilic accumulations or fully developed DH blisters **(Fig 1*F*)**. The type IV collagen immunostaining was always localized at the floor of the blister, and was either fragmented or completely absent under collagenase-positive keratinocytes.

DISCUSSION

We report here that interstitial collagenase is expressed by basal keratinocytes in both early and older DH lesions and that the expression does not seem to require epidermal regeneration to take place **(Table I)**. Our novel results contrast with those obtained in chronic ulcers [6], acute wounds [26], and a variety of blistering diseases including subtypes of epidermolysis bullosa, porphyria, and pemphigus [12], in which keratinocyte migration in particular seems to be associated with collagenase expression. Furthermore, collagenase mRNA is encountered much more frequently in DH than in other bullous disorders [12,24]. However, also in DH, signal for collagenase mRNA was restricted to basal keratinocytes, confirming our previous data that only these cells, and not the more differentiated keratinocytes, are competent to respond to stimulators of collagenase production.

Recent studies have suggested that cytokines may be involved in the development of skin lesions in DH, where interleukin-8 is expressed in the epidermal basal layer of lesional skin [27]. Although the pathogenesis of DH blisters is poorly understood, accumulation of neutrophils with subsequent inflammation may lead to degradation of extracellular matrix components and to formation of vesicles through stimulation of local protease expression by cytokines. Neutrophils, for example, secrete interleukin-1, but this stimulates fibroblasts rather than keratinocytes to produce collagenase [28]. We detected collagenase mRNA in half of the samples in plump stromal macrophage-like cells, but based on hematoxylin and eosin staining only, we cannot exclude the presence of activated fibroblasts among the mRNA-containing cells. It is interesting that no collagenase is expressed in bullous pemphigoid samples [24], which resemble DH lesions histologically except that the cells filling the blister are eosinophils. The importance of the accumulation of neutrophils is substantiated by the finding that normal-looking skin of DH patients does not show metalloenzyme or uPA mRNA expression.

Increased amounts of collagenase and elastase activity have been demonstrated in blister fluids of DH patients by enzyme assays [29,30]. In addition to keratinocyte collagenase, polymorphonuclear neutrophil collagenase may contribute to this activity. Most of the elastase activity may be derived from neutrophils, because although 92-kDa gelatinase and matrilysin are elastolytic, we were not able to detect any expression of these enzymes in the neutrophilic microabscesses.

Alternatively, contact of keratinocytes with extracellular matrix *via* integrin receptors may lead to collagenase production, as has been suggested for dermal wounds [6]. Keratinocyte expression of collagenase is inhibited by laminin but stimulated by collagen types I and IV [31]. Destruction of laminin, which occurs in DH blisters [2], might thus be sufficient to induce collagenase expression. As suggested previously [6,32], reconstitution of the basement membrane zone may terminate collagenase expression.

Stromelysin-1, a potent degrading enzyme of the basement membrane and an activator of tissue procollagenase [33], was expressed in basal keratinocytes in the majority of DH samples. It is interesting that localization of the signal seemed to correlate with that for collagenase. Although cell culture studies have suggested that these enzymes are co-regulated, our previous *in vivo* data in chronic ulcers and in several blistering diseases have not shown this type of co-localization.

In addition to malignant neoplasms, uPA mRNA has been detected in migrating keratinocytes during wound healing [34] and in basal cells of psoriatic epidermis [23]. Apart from its capacity of activating prostromelysin and procollagenase, it also leads to the formation of plasmin, which is capable of degrading matrix components such as fibrin and fibronectin deposited at the dermal papillae in the early lesions of DH [35]. Thus, uPA may be needed to remodel the underlying basement membrane and dermal stroma or to recruit metalloenzymes.

Disruption of both type IV collagen and laminin has been reported in DH [1,2]. Among the metalloenzymes capable of degrading basement membrane components, we found no specific signal for 92-kDa gelatinase expression or matrilysin protein, which suggests that either stromelysin-1 or uPA may contribute to the basement membrane degradation or remodeling after attack by neutrophilic proteases.

Our studies show that collagenase, stromelysin-1, and uPA are expressed in basal keratinocytes of DH lesions in the vicinity of neutrophilic accumulations. Stromelysin-1, in particular, may contribute to the formation of DH blisters by degrading basement membrane components. Furthermore, it may function together with uPA as an activator of latent collagenase. The up-regulation of metalloenzymes may be produced by neutrophil cytokines either directly or by cytokine-induced regulation through the uPA-plasmin system. Alternatively, altered contact of basal keratinocytes with the matrix components, through discontinuities in laminin or type IV collagen, may contribute to up-regulation of epidermal collagenase in DH.

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ANNOUNCEMENT

The Foundation Rene Touraine Scientific Meeting 1995 will be held Friday, November 17, 1995 in Paris at Ministère de l'Enseignement Supérieur et de la Recherche, Amphithéâtre Poincaré, 1, rue Descartes, 75 005 Paris.

The meeting, "Keratinocytes in Proliferation," will feature the following: Christian Brechot, Cell Cycle and Its Control; Caroline Dive, The Apoptose; Stuart Yuspa, Epidermal Carcinogesis; Fiona Watt, Control of Epidermal Cell Proliferation and Differentiation; Bernard Coulomb, Dermal Control of Epidermal Growth; Thomas Luger, Cytokines and Neurohormones: Regulatory Role in Inflammation and Keratonocyte Proliferation; Gerald Krueger, Is Psoriasis a Keratinocyte Disease?; and Marc Fergusson, Wound Healing: The Control of Epithelialisation and Scarring.