Demonstration of 72-kDa and 92-kDa Forms of Type IV Collagenase in Human Skin: Variable Expression in Various Blistering Diseases, Induction During Re-Epithelialization, and Decrease by Topical Glucocorticoids

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Type IV collagenases have been shown to play an important role in tumor metastasis and wound healing. In the present study, we have demonstrated the presence of 72-kDa and 92-kDa forms of type IV collagenase in human skin by biochemical and *in situ* hybridization techniques. *In situ* hybridization allowed us to localize the 72-kDa form mostly to fibroblasts and the 92-kDa form to the epidermis and endothelial cells. The presence of type IV collagenase was confirmed by Western blotting. Enzyme activity was assayed in spontaneous blisters (18 subjects) and suction-induced blisters (29 subjects) by the zymography method, and by using type IV collagen as the substrate. Thus, it was possible to detect both the 92-kDa and 72-kDa forms in spontaneous and induced blisters. An especially high level of the 92-kDa enzyme was found in a bullous pemphigoid patient.

Type IV collagenases were studied during re-epithelialization of the blister, using the suction-blister model. There was a marked induction of the 92-kDa type that was confirmed to be in the regenerating, migratory, epithelium by *in situ* hybridization studies. These results indicate that 92-kDa type IV collagenase may play an essential role in the normal physiology and integrity of the skin and may be an important regulator of re-epithelialization. It was also shown that potent topical glucocorticoid down-regulated the 92-kDa type collagenase, suggesting that glucocorticoids may have a beneficial role in some skin diseases by decreasing type IV collagenase activity and, thus, reducing tissue destruction. *J Invest Dermatol 101:205–210, 1993*

Type IV collagenases, which belong to the matrix metalloproteinases family [1], form a group of enzymes that have the ability to degrade basement membrane collagen [2] and some other components such as elastin [3] and other collagen types V, VII, and X [4–7]. At least two distinct forms of type IV collagenase of 72-kDa (MMP-2) and 92-kDa (MMP-9) have been found [1]. These enzymes are synthesized by various cells such as fibroblasts, keratinocytes, and inflammatory cells [2]. Especially 72-kDa collagenase is produced by fibroblastic cells, and 92-kDa form mainly by inflammatory cells and keratinocytes [2,8]. Type IV collagenases have been suggested to have an important role in many biologic and pathologic processes, such as wound healing, angiogenesis, embryonic development, inflammation, and tumor metastasis [2,8–11].

The role and localization of type IV collagenase in human skin has not been thoroughly studied. In this paper, we have studied type IV collagenases in various blistering diseases and in healthy skin, and their regulation by topical glucocorticoids. The results demonstrate that both 72-kDa and 92-kDa forms of type IV collagenase are expressed in human skin and are induced during re-epithelialization and decreased by topical glucocorticoids. By *in situ* hybridization studies it was possible to localize these collagenase types to distinct compartments of the skin.

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Abbreviation: DEPC, diethyl pyrocarbonate.

PATIENTS AND METHODS

In the first part of the study, blister-fluid samples were collected from spontaneous blisters of 18 patients. The clinical data from these patients is presented in Table I. The samples were taken before any specific treatment. In one patient (number 7), blister-fluid samples were also taken after 5 d treatment with dapsone. From some patients biopsies were obtained for routine histologic and immunohistochemical analyses.

In the second part of the study, suction blisters were induced on the abdominal skin of 29 voluntary male subjects (ages 19–22) as described previously [12]. The diagnoses included atopic eczema (n = 20), psoriasis vulgaris (n = 2), condyloma accuminata (n = 2), erysipelas cruris (n = 1), herpes simplex (n = 1), atheromata scrotalis (n = 1), eczema nummulare (n = 1), and hyperhidrosis palmoplantaris (n = 1). The blisters were induced on the clinically healthy skin. To study the effect of re-epithelialization on type IV collagenase, blister-fluid samples were taken by a Mantoux syringe immediately after blister formation and at 24 or 72 h post-blistering. For each sample, 2–3 intact blisters were emptied and pooled and the remaining blisters were protected by a cap until used. Pooled samples were kept frozen at −20°C until assayed.

To study the effect of topical glucocorticoid, or all-trans retinoic acid, on type IV collagenase, glucocorticoid creams (hydrocortisone (Uniderm), hydrocortisone-17-butyrate (Locoid), clobetasol-17-propionate (Dermovate) all-trans retinoic acid (Avitcid 0.1%), or vehicle were applied three times a day on the abdominal skin for 1 to 4 d, until blisters were induced, as indicated in *Results* and in the figure legends.

For *in situ* hybridization studies, whole suction blisters were removed

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Table I. Clinical Data of Patients with Bullous Diseases and Type IV Collagenase Activity in Blister Fluids

Patient Number	Diagnosis[a]	Age (years)/sex	Localization of blister	Dpm[b]	Type IV Collagenase Activity Densitometric Units[c] 72 kDa	92 kDa	Ratio 92 kDa/72 kDa
1	Ery	73/M	—	1270	97.4	272.0	2.78
2	Ery	58/F	Lower leg	572	217.3	264.2	1.22
3	Ery	73/F	Arm	711	186.3	150.4	0.81
4	Ery	22/M	—	1406	ND[e]	ND	ND
5	BP	69/M	—	ND	153.9	555.1	3.57
6	BP	73/F	—	ND	201.4	423.0	2.08
7	BP	74/M	Lower leg	ND	113.5 (46.4)[d]	2215.0 (412.6)[d]	20.0
8	BP	70/F	Lower leg	260	99.9	224.0	2.22
9	BP	64/F	—	771	ND	ND	ND
10	Pompholyx	32/M	Arm	2002	36.2	158.9	4.35
11	Pompholyx	46/M	Arm	ND	119.6	519.6	4.35
12	EM	26/M	Arm	ND	62.6	363.4	5.88
13	EM	38/M	Arm	1060	ND	ND	ND
14	RA	52/F	Lower leg	ND	237.5	590.3	2.50
15	RA	63/F	—	ND	340.2	667.2	1.96
16	RA	75/F	Lower leg	ND	94.1	174.7	1.85
17	CB	26/M	—	ND	117.6	415.2	3.57
18	CB	25/M	Genital	ND	67.4	331.7	5.00

[a] Ery, erysipelas; BP, bullous pemphigoid; EM, erythema multiforme; RA, rheumatoides arthritis (friction bulla); CB, cryo blisters induced by liquid nitrogen.
[b] Type IV collagenase was assayed as described in *Patients and Methods,* using (^{3}H)proline-labeled type IV collagen as substrate. The activity is expressed as dpm per 50 μl blister sample.
[c] 72-kDa and 92-kDa type IV collagenases were assayed by zymography. The activities are expressed as densitometric units (5 μl aliquots were used for assay).
[d] In patient 7, blister fluids were also collected after treatment with dapsone for 5 d.
[e] ND, not determined.

under local anesthesia immediately after blister formation and 24, 48, and 72 h later. The samples were then processed as described below. All the samples were taken according to the rules of the Helsinki declaration.

Assay of Type IV Collagenase Activity The reaction was carried out as previously described [13]. Briefly, the enzyme was activated by treatment with trypsin (10 μg/ml) for 10 min at 35°C, followed by the addition of soybean trypsin inhibitor (40 μg/ml) and N-ethylmaleimide (4 mM). (^{3}H)proline-labeled type IV collagen (4000 cpm/reaction) was added as substrate and the reaction was allowed to proceed for 18 h at 35°C, in a final volume of 650 μl. The reaction was terminated by the addition of 20 μl of 1 mg/ml bovine serum albumin and 100 μl of a solution containing 10% trichloroacetic acid and 5% tannic acid. After a 30-min incubation on ice, the mixture was centrifuged at 10,000 × *g* for 15 min and the radioactivity of the supernatant was measured in a scintillation counter.

Zymography: For zymography, the method of Heussen and Dowdle [14] was used, except that the substrate was gelatin labeled with 2-methoxy-2,4-diphenyl-3(^{2}H)-furanone (Fluka, Ronkonkoma, NY), according to the method of O'Grady *et al* [15]. The advantage of this method is that the lysis can be monitored visually, or by photographic inspection under long-wave ultraviolet light, and the capacity of proteins in the samples to bind Coomassie brilliant blue does not affect the pattern of lysis. Prior to electrophoresis, samples for zymography were pre-incubated at 22°C for 1 h with 1% sodium dodecylsulfate (SDS) and electrophoretic sample buffer, and pre-stained molecular weight standard proteins were reduced with 5% 2-mercaptoethanol. Zymography was performed in 1.5 mm 10% polyacrylamide slab gels containing 2 mg/ml gelatin. After electrophoresis, type IV collagenase was activated by incubating at 37°C. In each experiment the cleavage rates were monitored under long-wave ultraviolet illumination, and activation was continued until visually clear bands could be observed. In these experiments activation time was usually overnight. After the activation was finished the gels were photographed under long-wave ultraviolet illumination. Cleavage rate estimates were made by determining the rates of disappearance of the gelatin by densitometric scanning at the negatives of the photographed gels using a Computing Densitometer model 300A (Molecular Dynamics). To establish the conditions for assay of type IV collagenase, a standard curve was performed by using various amounts of blister fluid. The cleavage rate was linear at least between 1 to 10 μl of blister fluid (not shown). In some patients blisters were induced repeatedly and zymograms were compared from these. The variability in enzyme activity between different blisters induced on the same subjects was relatively small, usually less than ten percent. In one series of experiments, zymograms were incubated for 4 h at 37°C with control buffer, 25 mM ethylenediaminetetraacetic acid, 1 mM 1,10 phenanthroline, or 2 μg/ml of recombinant human inhibitor of metalloproteinase, a gift from Dr. David Carmichael (Synergen, Boulder, Co.). In some experiments blister roofs (blister roofs of six blisters were pooled) were immediately frozen after blister induction and kept at −70°C. Samples were then homogenized in 50 μl of sample buffer and 25 μl was used for zymography assay.

Western Immunoblotting Aliquots of the blister fluids were electrophoresed in a 10% SDS-polyacrylamide gel and blotted onto nitrocellulose. The nitrocellulose membrane was blocked with 5% nonfat milk/phosphate-buffered saline for 45 min, and incubated overnight, at room temperature, with rabbit anti-72-kDa type IV collagenase IgG, diluted 1:500 (kindly provided by Dr. Birkedal-Hansen) and goat anti-92-kDa type IV collagenase (kindly provided by Dr. Murphy). Biotinylated anti-rabbit and anti-goat immunoglobulins (1:500 and 1:100, and avidin-biotin complex, horseradish peroxidase (ABC/HPP, Dako)/ 3,3-diaminobenzidine tetrahydrochloride were used to develop the blots.

RNA Probes and *In Situ* Hybridization Skin biopsies from eight cases of suction blisters were routinely fixed in 10% formalin and embedded in paraffin. Four-micrometer sections were used for *in situ* hybridization. A detailed description of the preparation of paraffin sections for *in situ* hybridization has been described previously [16]. A 635-base pair (bp) Sca I Sac I fragment of the K-191 human 70-kDa type IV collagenase cDNA clone [17] was cloned into the M13 polylinker site of pSP64 and pSP65 vectors (Promega). A 634-bp Xba I-Hind III fragment of the K-174.1 human 92-kDa type IV collagenase cDNA clone [18] was cloned into the pGEM 4Z vector (Promega, Wisconsin). For transcription, a riboprobe transcription kit (Promega) was used and the transcripts were labeled with either ^{35}S-rCTP or ^{35}S-rUTP to specific activities of 3 × 10^6 cpm/40 μl. All the solutions used with the RNA probes were treated with 0.1% diethylpyrocarbonate (DEPC, Fluka).

In situ hybridization with anti-sense and sense RNA probes has been described previously [16]. The hybridizations were performed at 50°C, followed by washing, autoradiography for 7-15 d, and staining of the sections with hemotoxylin and eosin.

In the analysis the hybridization signal was regarded as positive only when there were 10 or more grains clearly concentrating within the cytoplasm of a cell. Diffusely distributed grains over the section were regarded as unspecific background labeling.

Other Assays For statistical analyses, Wilcoxon signed rank test and linear regression were used.

RESULTS

Demonstration of 72-kDa and 92-kDa Type IV Collagenases in Blistering Diseases and Suction Blisters In the first set of experiments, type IV collagenase was assayed using ^{3}H-labeled type

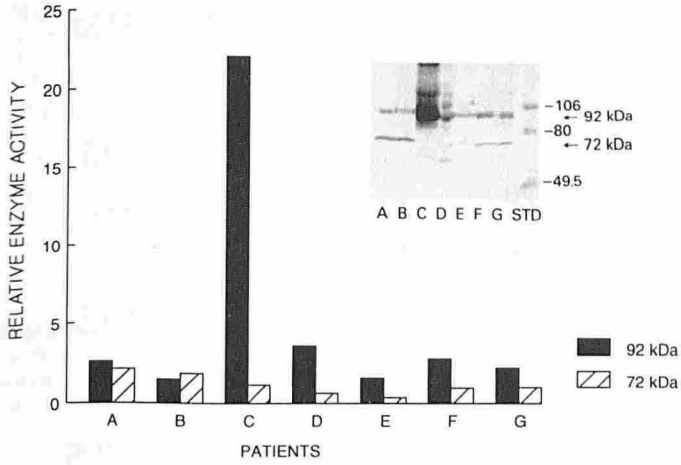

Figure 1. Demonstration of type IV collagenase in various blistering diseases. Blister-fluid samples were collected from spontaneous blisters of various blistering diseases and aliquots were analyzed by zymography. The zymography gel is shown and the positions of the 92-kDa and 72-kDa enzymes are indicated. The relative enzyme activities are expressed as densitometric units ($\times 10^{-2}$). The 92-kDa enzyme is represented by the *black columns* and the 72-kDa enzyme by *lined columns*. Samples in *A* and *B* were from erysipelas patients (patients 2 and 3 in Table I); *C, F,* and *G* were from bullous pemphigoid patients (patients 7, 6, and 8 in Table I); *D* was from a pompholyx patient (patient 10 in Table I); and *E* was from a erythema multiforme patient (patient 12 in Table I).

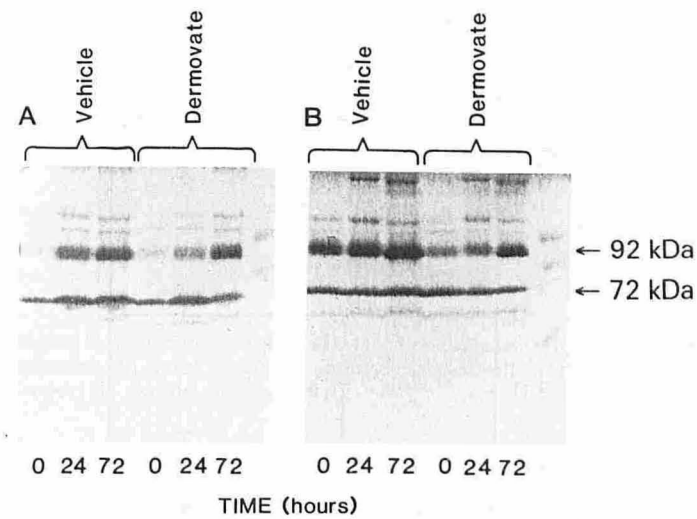

Figure 2. Effect of re-epithelialization and topical glucocorticoid on type IV collagenase in blister fluids. The abdominal skin of two patients was treated with either vehicle, or Dermovate, three times a day for 4 d until blisters were induced. Samples were collected 0, 24, and 72 h post-blistering and analyzed by zymography. The positions of the 92-kDa and 72-kDa enzymes are shown.

IV collagen as the substrate. Type IV collagenase-like activity could be detected in all blister samples studied (see Table I). Because this method is relatively non-specific (other proteases such as stromelysin are also able to degrade type IV collagen) [7], a zymography method was utilized in further experiments. As shown in Fig 1, clearly detectable type IV collagenase activity could be found in blister fluids collected from patients with various blistering diseases. Especially high activity (demonstrated several times) was observed in one patient with bullous pemphigoid. Type IV collagenase was detected as two forms of 92-kDa and 72-kDa. The ratio between the 92-kDa and 72-kDa collagenases varied in different blistering diseases (Fig 1, Table I). The ratio was higher in blister fluids obtained from bullous pemphigoid and pompholyx patients, and was lowest in blister fluids obtained from erysipelas patients (Fig 1 and Table I). Type IV collagenase was detected by zymography in all the blister fluids analyzed (Table I). In one patient, blister fluids were also collected after treatment with dapsone for 5 d (patient 7), and the level of type IV collagenase was found to be decreased when compared to pre-treatment levels. The 92-kDa activity was 413 densitometric units after the 5 d treatment with dapsone, compared to the pre-treatment value of 2215.

To correlate enzyme activity and histologic findings, biopsy samples were obtained from some patients for routine histologic analysis. These samples were obtained from three bullous pemphigoid patients (numbers 5, 7, and 8) and from one erythema multiforme patient (number 13). In all these samples eosinophilic granulocytes could be observed and in some samples also some mononuclear lymphocytes. However, due to the small number of samples analyzed by histology, no definite correlation between inflammation and type IV collagenase could be observed.

Because type IV collagenase could be detected in blister fluids collected from patients with blistering diseases of different pathogenesis, the question was raised as to whether type IV collagenase would also be present in suction blisters induced on the healthy skin of volunteers. The suction blisters were induced on the healthy looking abdominal skin of volunteers. A total of 29 volunteers were studied and type IV collagenase (both 92-kDa and 72-kDa forms) could be demonstrated in suction-blister fluids in all of them. There was a positive correlation between the expression of 92-kDa and 72-kDa enzymes in suction blister fluids ($r = 0.58$, $p = 0.036$).

In some experiments blister roofs after blister induction were homogenized and used for zymography. Both 92-kDa and 72-kDa enzymes could be detected in these samples (not shown).

Effect of Re-Epithelialization on 72-kDa and 92-kDa Type IV Collagenases The suction-blister model can be used to study the effect of re-epithelialization of the blister base on type IV collagenase activity. As shown in Figs 2 and 3 by zymography, there was a more than twofold induction of the 92-kDa type IV collagenase during re-epithelialization ($p = 0.03$). The changes in the 72-kDa form were not as marked and, in fact, the 92-kDa/72-kDa ratio increased significantly during re-epithelialization ($p = 0.045$) (Fig 3).

Effect of Topical Glucocorticoid and All-Trans Retinoic Acid on 72-kDa and 92-kDa Type IV Collagenases In some patients, the effects of topical clobetasol-17-propionate and all-trans retinoic acid were studied. Topically applied clobetasol-17-propionate decreased the 92-kDa type IV collagenase and, interestingly, the enzyme activity remained lower during re-epithelialization when compared to non-treated skin (Figs 2 and 3). The other steroids tested did not inhibit the 92-kDa enzyme as markedly as clobetasol-17-propionate (not shown).

All-trans retinoic acid, which is extensively used for treatment of acne and photo-aging, had essentially no effect on the 92-kDa and 72-kDa activities of type IV collagenase when applied for 4 d before blister induction (not shown).

Demonstration of Type IV Collagenases in Blister Fluid and Keratinocytes by Western Blotting Type IV collagenase was studied in blister fluids by Western blotting. By employing antibodies against the 72-kDa and 92-kDa forms, it was possible to confirm the presence of type IV collagenases in blister fluid (Fig 4).

Detection of 72-kDa and 92-kDa Type IV Collagenases by *in situ* Hybridization The 92-kDa type IV collagenase mRNA was strongly expressed in the epidermis, the signal being concentrated mainly in the basal and middle layers of the stratified epithelium (Fig 5). The signal was especially strong in the regenerating epithelium. Although strong, the signal seemed to be somewhat lower in the normal epidermis. The endothelial cells in the proliferated capillaries beneath the blister area contained a clear signal for the presence of the 92-kDa type IV collagenase mRNA (Fig 5).

The 72-kDa type IV collagenase mRNA was mainly, and

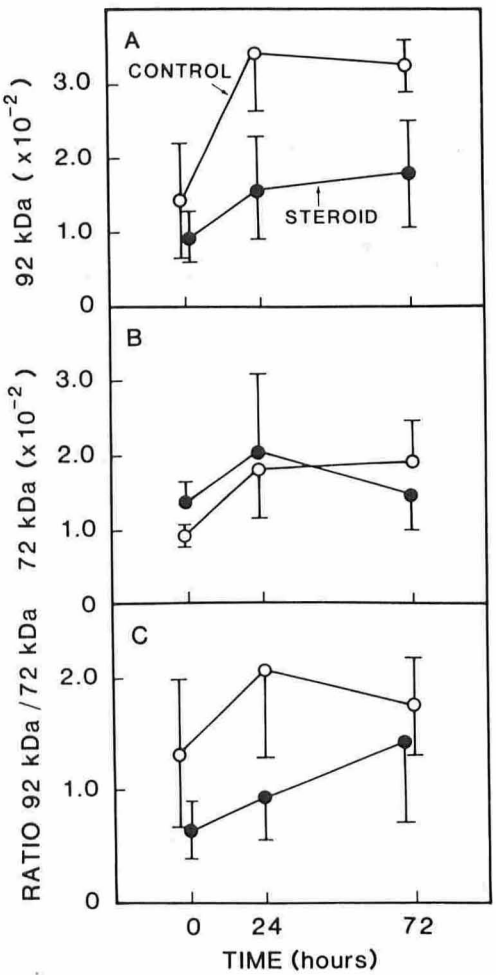

Figure 3. Effect of re-epithelialization and topical glucocorticoid on the 92-kDa and 72-kDa enzymes and on the 92-kDa/72-kDa ratio in blister fluids. A total of four patients were treated with vehicle, or Dermovate, three times a day for 4 d (the zymography of two of these patients is shown in Fig 2). Samples were collected at 0, 24, and 72 h post-blistering and analyzed by zymography. The values are the mean ± SD. Statistical significances: changes in the 92-kDa and 72-kDa enzyme levels and in the 92-kDa/72-kDa ratios of controls were statistically significant from 0 to 24 h ($p = 0.03$, 0.045, and 0.045 respectively). In Dermovate-treated samples, 92-kDa activity was significantly lower at all time points ($p = 0.045$, 0.05, and 0.061). The 92-kDa/72-kDa ratio was significantly lower in Dermovate-treated samples at 0 ($p = 0.045$) and 24 ($p = 0.061$) h.

strongly, expressed in the fibroblastic dermal cells beneath the blisters (Fig 6). A low signal was also seen in the endothelial cells, but its level was much lower than that observed for the 92-kDa type IV collagenase mRNA. Expression of the 72-kDa mRNA could also be detected in the normal dermal fibroblasts. Only occasional cells in the basal epidermis expressed mRNA for the 72-kDa type IV collagenase (Fig 7). This expression was detected more clearly in the normal epidermis than in the epithelium of the blister area.

DISCUSSION

In the present study we have demonstrated the presence of type IV collagenase in human skin by assaying its activity, by Western blotting, and by *in situ* hybridization. We have shown that two forms of type IV collagenase, of 72-kDa and 92-kDa, are expressed in different cells. The 92-kDa form was mainly found in epidermis and endothelial cells, whereas the 72-kDa enzyme was mostly expressed in fibroblasts. This indicates that these enzymes may have different roles in skin physiology and in pathologic conditions. The results

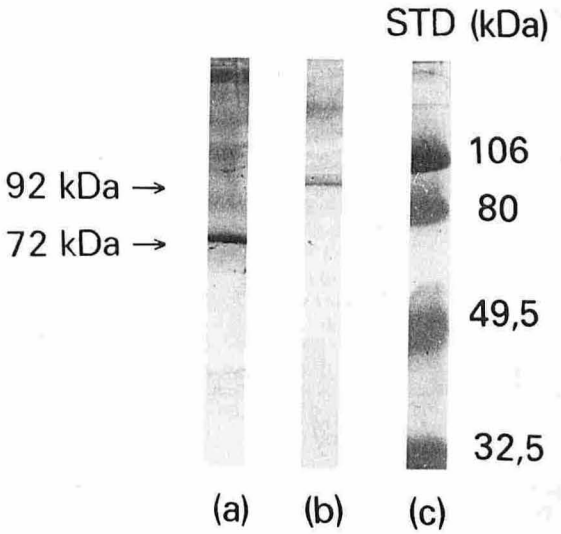

Figure 4. Western blot analysis of blister fluid using polyclonal 72-kDa type IV collagenase *(a)* and monoclonal 92-kDa type IV collagenase antibodies *(b)*. For Western analysis 5 μl aliquots of blister fluid (collected from patient number 7) were run, non-reduced, on a 10% SDS-polyacrylamide gel electrophoretogram, blotted onto nitrocellulose, reacted with anti-72-kDa type IV collagenase and anti-92-kDa type IV collagenase antibodies and visualized with a second antibody conjugated to biotin-avidin complex horseradish peroxidase. The position of the pre-stained molecular weight standards are indicated on the right. Bands corresponding to the 72-kDa and 92-kDa enzymes were detected in blister fluid.

further indicated that detectable activities of the 92-kDa and 72-kDa enzymes are found in various blistering diseases and even in normal skin. The ratio of the 92-kDa to 72-kDa enzymes varied in various blistering diseases perhaps due to the various levels of blister formation, various degree of inflammation, and the variable degrees of injury to the epidermis and dermis induced by blister formation. Even though high levels of the 92-kDa enzyme were found in some patients with bullous pemphigoid, it is too early to speculate the role of this enzyme in blister formation in this disease. Because it is known that type IV collagenase is capable of degrading different proteins, such as type IV and VII collagens (see *Introduction*), which have a central role in dermoepidermal junction, it may participate in, or enhance, blister formation in several blistering diseases.

The origin of 92-kDa type IV collagenase in the various blistering diseases studied here is currently unknown. It is possible that some of 92-kDa enzyme found in blister fluids is derived from inflammatory cells that are abundantly present in several blistering diseases [19]. For example, polymorphonuclear leukocytes and eosinophils may be present in erythema multiforme and bullous pemphigoid [19] as also found in the present study. Erysipelas is also characterized by a heavy leukocyte infiltration [20]. Previously, it

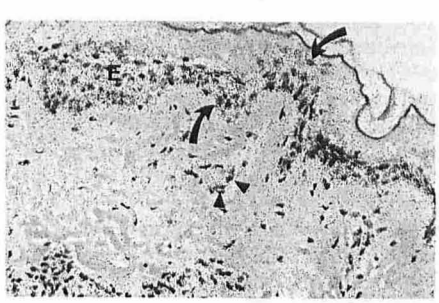

Figure 5. The expression of 92-kDa type IV collagenase mRNA in the regenerated epidermal cells (E) *(arrows)* in a 48-h blister. The signal in the endothelial cells is marked with arrowheads. *Scale bar,* 50 μ.

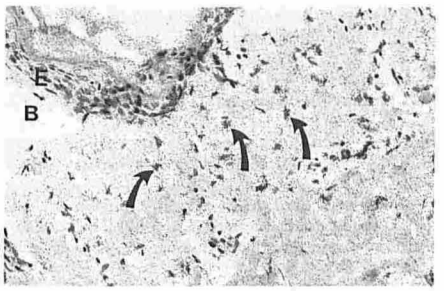

Figure 6. The expression of the 72-kDa type collagenase mRNA is present in the dermal fibroblasts *(arrows)* beneath a 48-h blister (B). Note that at this location blister floor has not yet been covered by new epidermis. The cells in the detached epidermis (E) are negative. *Scale bar,* 50 μ.

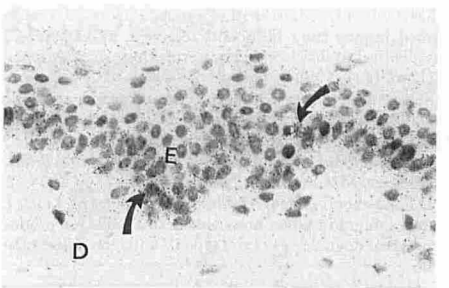

Figure 7. The expression of 72-kDa type IV collagenase mRNA in normal epidermis (E) shows occasional positively labeled cells in the basal layer *(arrows)* (D, dermis). *Scale bar,* 50 μ.

has been shown that various inflammatory cells, such as polymorphonuclear leukocytes and macrophages, contain 92-kDa type IV collagenase activity [2]. Thus, it is possible that, in diseased skin, some of the 92-kDa enzyme could be derived from injured epidermis and inflammatory cells.

Type IV collagenase may have a particularly important role in the re-epithelialization of wounds. Here, we have devised a suction-blister model that can be used *in vivo* to study the regeneration of the skin in humans. We were able to detect a significant induction of the 92-kDa enzyme during re-epithelialization, both by zymography and by *in situ* hybridization. This is in agreement with previous studies in which type IV collagenase has been shown to have an important role in tumor invasion and wound healing (see *Introduction;* [21]). It is possible that, during the migration of epithelial cells, cells abundantly produce type IV collagenase, which may facilitate cell movement. Transforming growth factor beta 1, which enhances wound healing, has been shown to induce the 92-kDa activity in keratinocytes, further supporting the role of 92-kDa collagenase in re-epithelialization [8]. Interestingly, the fibroblasts also contained high levels of mRNA of the 72-kDa enzyme during regeneration, suggesting that re-epithelialization process also involves dermal elements. This was supported by the fact that, during regeneration, mRNA of the 92-kDa enzyme in proliferating endothelial cells of blood vessels was abundantly present. This may indicate that angiogenesis during re-epithelialization involves the activation of the 92-kDa enzyme facilitating blood-vessel formation. Indeed, previous studies have shown that, in the experimental neovascularization model, migrating endothelial cells produce type IV collagenolytic activity [22].

The suction blister model was used to study the effects of topical glucocorticoids and all-trans-retinoic acid on type IV collagenase. Glucocorticoids constitute the main treatment modality for various inflammatory skin diseases and bullous diseases. Ninety-two-kilodalton type IV collagenase activity was down-regulated following a relatively short (2 d) treatment with topical glucocorticoid. The lack of suppression of 72-kDa form with topical steroids could be due to diminished bioavailability of the topical steroid to the dermal fibroblasts. Previous studies have shown that glucocorticoids decrease interstitial collagenase activity [23]. *In vitro* studies have also revealed that type IV collagenolytic activity was reduced in HT-1080 cells and human skin fibroblasts in response to glucocorticoids *in vitro* [24,25]. This is the first demonstration that glucocorticoids also inhibit type IV collagenase in human skin *in vivo*. This finding may have a great clinical significance for the treatment of blistering diseases. It should also be noted that glucocorticoids increase the synthesis of [26] plasminogen activator inhibitor-1, which has an important role in the plasminogen-plasmin cascade [27]. In addition it is known that plasmin can increase type IV collagenolytic activity through activation of the pro-enzyme [27]. Thus, it is possible that glucocorticoids have at least a dual inhibitory effect on type IV collagenase in blistering diseases, firstly by directly inhibiting type IV collagenase synthesis and, secondly, by inhibiting its activation. Topical all-trans-retinoic acid did not exhibit such marked actions on type IV collagenase as glucocorticoids. It is possible that longer treatment would give different results.

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ANNOUNCEMENT

The workshop Mouse Mutations as Animal Models and Biomedical Tools: Skin and Hair Mutations will be held at The Jackson Laboratory, Bar Harbor, Maine, September 29 to October 2, 1993.

This workshop will present spontaneous mouse mutations with skin and hair abnormalities that are useful for a variety of biomedical studies. Live mice and study sets of histologic materials will be available for examination. Seminars will be held on current research in dermatology with emphasis on mouse-human homologies.

The American Academy of Dermatology certifies that this educational activity has been recognized for 17 hours of AAD Category I credit and may be used toward the AAD's CME Award.

For further information, contact Suzanne Serreze, The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609-1500. Tel. 207/288-3371; Fax 207/288-8254.