Production of Procollagenase by Cultured Human Keratinocytes*

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Using a collagen film assay utilizing 14C-labeled type I collagen, we demonstrated that cultured human keratinocytes produced a procollagenase after treatment with the tumor-promoting phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA). Production of collag enase paralleled alterations in cellular morphology induced by TPA. When procollagenase was immunoprecipitated with antibody to human fibroblast collagenase and analyzed on sodium dodecyl sulfate-polyacrylamide gels, the zymogen was revealed as a 56- and 51-kDa doublet. The keratinocyte-derived collagenase was a neutral metalloprotease, required activation with trypsin for detection in the collagenase assay and produced the characteristic three-quarter and one-quarter length collagen cleavage products when incubated with type I collagen at 25°C. The enzyme was inhibited by serum and cysteine and was largely unaffected by serine, thiol, and carboxyl protease inhibitors. Cycloheximide inhibited the TPA-induced production of collagenase, suggesting that the procollagenase was not stored preformed in the keratinocytes. Keratinocytes treated with a tumor-promoting analogue of TPA also produced collagenase, but cells treated with cytochalasin B, interleukin-1, or two non-tumor-promoting phorbol esters did not. Keratinocyte-derived collagenase may play a role in wound healing and morphogenesis.

Vertebrate collagenase, a neutral metalloendoprotease, cleaves native collagen into specific three-quarter and one-quarter length fragments which are susceptible to further proteolysis (1). In the skin, increased or abnormal collagenase production may contribute to the pathogenesis of several dermatologic diseases including dystrophic epidermolysis bullosa (2), an inherited blistering disorder, and locally invasive basal cell carcinomas (3). Although dermal fibroblasts produce collagenase in vitro (4) and are thought to represent the primary source of collagenase in the skin, the tailfin epidermis of the tadpole was the source of the first vertebrate collagenase described (5). Wound healing studies in humans (6) and animals (7, 8) have suggested the presence of collagenase activity in wound edge epithelium. Eisen (9) also demonstrated increased collagenase activity in the epidermis from patients with epidermolysis bullosa. However, these studies were performed on cutaneous layers (enzymatically separated epidermis and dermis) and therefore could not establish the cellular source of the collagenase. Subsequently, identification of collagenase by indirect immunofluorescence using anticallose antibody demonstrated staining of the papillary dermis but not the epidermis (9). More recently, Woodley et al. (11) demonstrated type I and type IV collagenase activity in the conditioned medium from explants of human epithelium grown on nonviable pig dermis. The explants were grown at an air-fluid interface which promotes keratinocyte and suppresses fibroblast proliferation (12).

Detection of collagenase production in vitro is facilitated under serum-free culture conditions (4,13) and, in fibroblasts, can be augmented or induced by multiple agents including cytochalasin B (14), proteolytic enzymes (15), and interleukin-1 (16). Aggelet al. (17) have shown that production of collagenase by rabbit synovial fibroblasts correlates with changes in cellular morphology characterized by cell rounding and loss of cell to cell contact. The phorbol ester tumor promoter TPA* is a potent inducer of these morphological changes and collagenase production in rabbit synovial fibroblasts (17) and endothelial cells (18). We therefore examined human keratinocyte monolayers for the production of collagenase in the presence or absence of TPA, cytochalasin B, interleukin-1, and extracellular matrix proteins. In this study, we found that human keratinocyte cultures in serum-free medium and in the absence of mesenchymal cells were capable of producing a procollagenase in response to TPA treatment. Furthermore, this new keratinocyte-derived collagenase is similar to that produced by human dermal fibroblasts.

EXPERIMENTAL PROCEDURES

Materials—The L-[^35]S)methionine (800 Ci/mmol) and [1-^14]C)acetic anhydride (20 mCi/mmol) were purchased from American Corp. The [3H]-labeled molecular weight markers used in the SDS-polyacrylamide electrophoresis were purchased from New England Nuclear. Pansorbin (Staphylococcus aureus cells) was obtained from Behring Diagnostics and the Protein A-Sepharose CL-4B was obtained from Pharmacia P-L Biochemicals. Fluoro-Hance was purchased from Research Products International, Mount Prospect, IL and the X-omat XR5 x-ray film was purchased from Eastman Kodak, Rochester, NY. rDNA interleukin-1 was obtained from Cis- tron Technology, Pine Brook, NJ. All other chemical reagents including TPA and the phorbol derivatives, trypsin, soybean trypsin inhibitor, PMSF, NEM, cysteine, bacterial collagenase, cytochalasin B, mersalyl, p-aminophenylmercuric acetate, monesin, and cycloheximide were purchased from Sigma.

The polyclonal rabbit antiserum against human dermal fibroblast collagenase has been previously characterized (19). Two mouse anti-
keratin monoclonal antibodies AE1 and AE3 were kindly provided by Dr. T.-T. Sun (Department of Dermatology, New York University School of Medicine) as undiluted culture supernatants. Fluorescein-conjugated goat anti-mouse IgG was obtained from Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD and rhodamine-conjugated sheep anti-rabbit IgG was obtained from Cappel Laboratories, West Chester, PA.

Materials used to coat the culture dishes included type I collagen purified from rat tail tendons (20) and fibroscinet, purified from human plasma according to Hayashi and Yamada (21).

Cell Cultures—Human keratinocytes obtained from neonatal foreskin fibroblasts (22) were grown to early confluency at 37 °C in 35-mm tissue culture dishes (Corning, Cornning, NY) in serum-free MCDB 153 medium containing 0.1 mM CaCl2 (23) with the following supplements: hydrocortisone, 0.4 μg/ml; insulin, 5 μg/ml; phosphoethanolamine, 0.1 mM; ethanolamine, 0.1 mM; epidermal growth factor, 5 μg/ml; bovine pituitary extract, 150 μg/ml; histidine, 0.24 mM; isoleucine, 0.75 mM; methionine, 0.09 mM; phenylalanine, 0.09 mM; tryptophan, 0.045 mM; tyrosine, 0.075 mM. Cells were used between passages 3 and 7. At confluency, the cells were washed with Hank's balanced salt solution; the medium, as above but without hydrocortisone to avoid suppression of collagenase production (24), was replaced and various agents (see below) added to triplicate keratinocyte cultures (25). The medium was collected after 48-96 h of incubation, clarified and stored at -20 °C until used in the collagenase assay. The following agents were dissolved in Me2SO and added to the keratinocyte cultures to assess their effect on collagenase production: TPA, three phorbol analogues (4a-phorbol 12,13-didecanoate; 4b-phorbol 12,13-didecanoate; phorbol 12,13-didecanoate, and 4-phorbol), cytochalasin B, and cycloheximide. The Me2SO concentration in the medium did not exceed 0.1% and control cultures were treated with 0.1% Me2SO. The interleukin-1 was diluted in culture medium and added to the cultures. Keratinocytes were also cultured on tissue culture plates coated with type I collagen or fibronectin as described (25) and collagenase production with and without TPA stimulation was assessed.

Human fibroblasts harvested from neonatal foreskins were maintained in Dulbecco's minimal essential medium plus 10% fetal bovine serum (FBS). The immunoprecipitation experiments were subcultured into 35-mm tissue culture dishes and  at early confluency production with and without TPA stimulation was assessed.

Precipitation (20) and acetylated with [1-14C]acetic anhydride (1 mCi) were then washed once with distilled water, transferred to new tubes, and recombined with the appropriate sample, heated at 100 °C for 3 min, and 50 μl of each sample (2000 cpm) was analyzed on a 7.5% SDS-polyacrylamide resolving gel run according to Laemmli (29). Gels were lightly stained, impregnated with Fluoro-Hance and exposed to Kodak X-OMAT XAR 5 film for 5 days at -70 °C.

Immunoprecipitation—TPA (20 ng/ml)-treated keratinocytes and fibroblasts in serum-free medium were labeled with [35S]methionine (55 μCi/ml) for 24 h. Before the addition of the labeled methionine, cells were refed with 1 ml of MCDB 153 containing 0.03 mM unlabeled methionine (keratinocytes) or a 1:1 solution of Ham's F-12 medium with Hank's buffered salt solution containing 0.015 mM unlabeled methionine (fibroblasts). The labeled medium was clarified and preabsorbed with pre-immune rabbit serum and Pansorbin as described by Stanley et al. (30). All immunoprecipitation steps were performed at 4 °C, 500 μl of labeled preabsorbed conditioned medium was incubated overnight with 3 μl of rabbit human fibroblast collagenase antiserum or normal rabbit serum. Samples were centrifuged at 12,000 X g for 3 min and transferred to new 1.5-ml polypropylene centrifuge tubes. 50 μl of a 25% slurry of Protein A-Sepharose CL-4B was added to each sample and incubated with agitation for 2 h. Samples were washed three times with 1 ml of buffer containing 0.3% sodium deoxycholate, 0.5% Nonidet P-40, 0.5 mM NaCl, 0.01 M Tris-HCl, pH 7.4, and 0.1% crystalline bovine serum albumin and three times with the same buffer without bovine serum albumin. Samples were then washed once with distilled water, transferred to new tubes, centrifuged, and the water was decanted from the Sepharose beads. Samples above were then dissolved in 1 ml of 1× sample buffer (see above) and added to each sample which was heated for 4 min at 100 °C. Samples were analyzed on a 10% SDS-polyacrylamide gel and fluorography was performed as above.

RESULTS

Since human dermal fibroblasts are known to produce collagenase, it was necessary to establish that the keratinocyte collagenase were free of contaminating fibroblasts and that the collagenase was produced by the keratinocytes. Keratinocytes were grown for at least three passages in low-calcium serum-free MCDB 153 medium, which promotes keratinocyte growth and selects against fibroblast growth (24). Fibroblast proliferation was not noted in subsequent subcultures of these keratinocyte monolayers grown for 14 days in medium which supports fibroblast growth (Dulbecco's minimal essential medium plus 10% fetal calf serum). The cellular source of the collagenase detected in the keratinocyte cultures was verified further by the immunofluorescent studies of TPA- and monensin-treated keratinocytes. Particular intracellular collagenase staining was present in keratin-containing epithelial cells (Fig. 1), confirming the synthesis of collagenase by keratinocytes.

Treatment of pure cultures of neonatal and adult keratinocytes with TPA produced cell rounding and loss of cell to cell contact (Fig. 2) and these morphologic alterations correlated with production of collagenase. No collagenase activity was found in conditioned medium from control cultures treated with Me2SO alone. Mixing experiments using medium from both control and stimulated cells indicated that the lack of enzyme activity in the control medium was not due to the presence of significant amounts of collagenase inhibitor. In stimulated cultures, the morphologic changes and collagenase activity at 48 h occurred after as little as 30 min exposure to TPA. The amount of collagenase activity detected in medium from triplicate cultures of TPA-treated keratinocytes is shown

Electrophoresis and Fluorography of Solubilized Collagen—200 μl of keratinocyte-conditioned medium was activated as above and incubated with the [35S]labeled collagen film for 24, 37, 48, and 63 h at 25 °C. After incubation, the medium containing collagenase and the digested collagen was removed from the collagen film plate and added to 50 μl of × 5 concentrated sample buffer containing 5% SDS, 100 mM glycine, 50 mM Tris-HCl (pH 7.4), 1 mM 2-mercaptoethanol, 0.1% sodium dodecyl sulfate, 5% 2-ME, 0.05% bromphenol blue and diethiothreitol (60 μg/ml). 100 μl of unconditioned medium with medium × concentrated sample buffer was added to each well of the collagen film plate and the plate was floated in a 56 °C water bath for 30 min to solubilize the remaining collagen film. The solubilized collagen was recombined with the appropriate sample, heated at 100 °C for 3 min, and 50 μl of each sample (2000 cpm) was analyzed on a 7.5% SDS-polyacrylamide resolving gel run according to Laemmli (29). Gels were lightly stained, impregnated with Fluoro-Hance and exposed to Kodak X-OMAT XAR 5 film for 5 days at -70 °C.
Collagenase and Human Keratinocytes

FIG. 1. Demonstration of intracellular collagenase and keratin in human keratinocytes by indirect immunofluorescence. Cells were incubated at 37 °C with 10 ng/ml of TPA for 24 h followed by 1 μM of monensin for 3 h, fixed, and permeabilized as described under “Experimental Procedures.” Cells were treated sequentially with mouse monoclonal anti-keratin antibodies or control by hybridoma supernatant and fluoresceinated goat anti-mouse IgG to demonstrate keratin filaments. They were then treated with rabbit anti-collagenase antiserum or normal rabbit serum and rhodamine-conjugated sheep anti-rabbit IgG to demonstrate intracellular collagenase. Cells were examined using epifluorescence optics and photographed using Kodak Tri-X film at an ASA of 1600. Film was developed in Diafine (Acufine Inc., Chicago, Ill.) The first two panels show the same cells stained with the anti-keratin (a) and anti-collagenase (b) antibodies. Diffuse fluorescein-stained keratin filaments are visible to a. Particulate rhodamine-stained intracellular collagenase is seen in these cells in panel b. Rhodamine-stained control cells are shown in c. (Exposure times: a, 30 s; b, 50 s; c, 40 s.)

in Fig. 3A. Maximal collagenase activity (0.11 μg of 14C-labeled collagen solubilized per 10^6 cells/min) was seen at 48 h in response to 10 ng/ml of TPA (1.6 × 10^{-8} M). Collagenase activity was detectable 18 h after TPA exposure (Fig. 3B) and increased little after 24 h. Cells treated with cycloheximide (3 μg/ml) and TPA (100 ng/ml) produced no detectable collagenase activity when conditioned medium was assayed after 48 h, suggesting that the collagenase produced in response to TPA was newly synthesized.

The keratinocytes secreted a latent enzyme which required activation with trypsin for detection in the collagenase assay and was optimally activated by trypsin at concentrations of 5–10 μg/ml of serum-free conditioned medium. Incubation of the conditioned medium with the organomercurial, mersalyl (2.5 mM), resulted in partial activation of the procollagenase: 26.0% ± 3.5 (mean ± S.E., n = 5) of the activity produced by activation by trypsin (10 μg/ml). Similarly, p-aminophenylmercuric acetate (1 mM) activated 18.7% ± 0.9 (n = 5) of the procollagenase activated by trypsin.

Treatment of the keratinocytes for 48 h with 20 ng/ml (3 × 10^{-8} M) of phorbol 12,13-didecanoate, a TPA analogue with
Fig. 3. A, stimulation of collagenase activity by TPA. TPA was added in concentrations shown to triplicate 35-mm culture plates of confluent human keratinocytes containing 2 ml of medium for 6 h after which time the medium was replaced with medium without TPA. The conditioned medium was collected after 48 h of culture. A collagenase film assay was used to quantitate collagenase activity. The type I 14C-labeled collagen had a specific activity of 3.7 x 10^4 cpm/ng. The keratinocyte-derived collagenase was activated with trypsin as described under "Experimental Procedures" prior to assay. Maximum production of collagenase occurred after treatment with TPA at 10 ng/ml; 0.11 ug of 14C-labeled collagen solubilized per 10^6 cells/min. Data are expressed as the mean of triplicate plates ± S.E. B, time course of TPA-stimulated collagenase production. TPA (10 ng/ml) was added to 35-mm culture plates of confluent keratinocytes and conditioned medium from triplicate plates was harvested at the indicated times (closed circles). Control cultures (open circles) were treated with Me2SO alone (0.05%) and the conditioned medium harvested at the times shown. Data are expressed as the mean of triplicate plates ± S.E.

TABLE I

| Cell type       | Interleukin-1 | TPA  | cpm/10^6 cells |
|-----------------|---------------|------|----------------|
|                 | units ng/ml   |      |                |
| Keratinocytes   | 0             | 22 ± 3 |
|                 | 1             | 12 ± 3 |
|                 | 5             | 21 ± 4*|
|                 | 10            | 26 ± 1|
| Fibroblasts     | 0             | 1717*|
|                 | 5             | 2126 ± 117|

* Mean of two cultures.

One culture.

In this study we have demonstrated that cultured human keratinocytes treated with the phorbol ester, TPA, produce a procollagenase similar to that produced by dermal fibroblasts. Conditioned medium from the cultures produced characteristic three-quarter and one-quarter length fragments after incubation with type I collagen. Immunoprecipitation of 35S-labeled TPA-treated cell supernatants with rabbit antiserum to human dermal fibroblast collagenase indicated antigenic similarity of keratinocyte-derived collagenase with the fibroblast enzyme. Fluorograms of SDS-polyacrylamide gels demonstrated 56- and 51-kDa protein bands in the conditioned medium from both the dermal fibroblasts and the keratinocytes (Fig. 4B). These bands correspond in molecular weight with the inactivezymogens produced by human dermal fibroblasts (32).

Experiments with protease inhibitors were designed to characterize the keratinocyte-derived collagenase. Its activity was totally inhibited by EDTA (Table II). Cysteine, which is thought to inhibit collagenase activity by chelation of an intrinsic metal ion of the enzyme (33), decreased the keratinocyte-derived collagenase activity by 93%. Human serum, which contains α2-macroglobulin, a potent collagenase inhibitor (34), reduced collagenase activity by 80%. Little effect on collagenase activity was produced with addition of serine (PMSF) or carboxyl (pepstatin) protease inhibitors. NEM, a thiol protease inhibitor, reproducibly inhibited collagenase activity by approximately 30%, but this effect was independent of the concentration of the inhibitor and therefore was probably nonspecific. A similar effect with NEM was reported in studies of endothelial cell collagenase (18). These data characterize the keratinocyte-derived enzyme as a metalloprotease similar to other vertebrate collagenases (31).

**DISCUSSION**

In this study we have demonstrated that cultured human keratinocytes treated with the phorbol ester, TPA, produce a procollagenase similar to that produced by dermal fibroblasts. Conditioned medium from the cultures produced characteristic three-quarter and one-quarter length fragments after incubation with type I collagen. Immunoprecipitation of the zymogen with antibody raised against dermal fibroblast collagenase revealed a 56- and 51-kDa doublet similar to the tumor-promoting properties, also induced both the morphologic changes and procollagenase production: 0.07 μg of 14C-collagen was solubilized per 10^6 cells/min by activated conditioned medium from these cultures. No collagenase activity nor morphologic changes were seen in keratinocytes treated with 3 x 10^-8 M of 4α-phorbol 12,13-didecanoate or 4α-phorbol, two TPA analogues without tumor-promoting potential. No collagenase activity was detected in medium from keratinocytes treated with 1, 5, or 10 units/ml of interleukin-1; while fibroblasts treated in parallel in the same experiment with 5 units/ml of interleukin-1 produced substantial collagenase activity (Table I). No collagenase production was detected in cells treated with cytochalasin B (1, 5, or 10 μg/ml), an agent shown to induce collagenase production in rabbit synovial fibroblasts (14, 17). Keratinocytes grown on type I collagen or fibronectin did not produce detectable collagenase activity, nor did the presence of these extracellular matrix proteins augment or inhibit the keratinocyte response to TPA (data not shown).

The progressive accumulation of the cleavage products of the 14C-collagen resulting from incubation of the labeled collagen with keratinocyte-conditioned medium is shown in Fig. 4A. These cleavage products, the N-terminal three-quarter fragment (TCα), and a C-terminal one-quarter fragment (TCβ), are specific for vertebrate collagenase and reflect enzyme cleavage at the 775-776 Gly-Ile residues on the α1 chain and the Gly-Leu residues on the α2 chain of type I collagen (31). Immunoprecipitation of 35S-labeled TPA-treated cell supernatants with rabbit antiserum to human dermal fibroblast collagenase indicated antigenic similarity of keratinocyte-derived collagenase with the fibroblast enzyme. Fluorograms of SDS-polyacrylamide gels demonstrated 56- and 51-kDa protein bands in the conditioned medium from both the dermal fibroblasts and the keratinocytes (Fig. 4B). These bands correspond in molecular weight with the inactive zymogens produced by human dermal fibroblasts (32).
Undigested collagen was solubilized with electrophoresis sample buffer. Type I collagen are shown in Lane 1. The progressive degradation of the collagen yielded the characteristic three-quarter size TCα and one-quarter size TCβ fragments of the two α chains. In addition, the three-quarter size cleavage products of the dimers can be seen. B, Immunoprecipitation of procollagenase from the conditioned medium of TPA-treated keratinocytes and fibroblasts. Cells were treated with medium containing 10 ng/ml of TPA for 24 h, then labeled with 35 μCi of [35S]methionine for 24 h. Secreted proteins present in the conditioned culture medium from human fibroblast and keratocyte cultures were immunoprecipitated with pre-immune rabbit serum or rabbit anti-collagenase antiserum (see “Experimental Procedures”), electrophoresed on a 10% SDS-polyacrylamide gel, and fluorographed. Lanes 2 and 4 show the control immunoprecipitations using pre-immune rabbit serum with conditioned medium from fibroblasts and keratinocytes, respectively. Lane 3, fibroblast-conditioned medium with anti-collagenase antiserum. Lane 5, keratinocyte-conditioned medium with anti-collagenase antiserum. The Mγ of the two bands in Lanes 3 and 5 are 56,000 and 51,000, respectively. The migration positions of marker proteins are shown in Lane 1 and include myosin (Mγ × 10^3 = 200), phosphorylase b (97.4), bovine serum albumin (69), IgG β subunit (53), and ovalbumin (46).

Proenzyme produced by human fibroblasts in serum-free medium (32). The enzyme, a metalloprotease, was secreted as an inactive zymogen in serum-free culture. Production of collagenase by the TPA-treated keratinocytes was dependent on new protein synthesis, as evidenced by the inhibitory effect of cycloheximide. This finding and the profile of response of the keratinocyte-derived collagenase to protease inhibitors parallels results obtained with collagenase produced by fibroblasts in vitro (6, 35). Activation of the procollagenase by trypsin and, to a lesser extent, by organomercurials is also a feature shared by human fibroblast collagenase (36). In contrast to fibroblasts (14, 37, 38), keratinocyte collagenase production was not stimulated by cytochalasin B, interleukin-1, or type I collagen. These findings suggest that the intracellular signals resulting in production of collagenase in epidermal cells may differ from those in the fibroblasts. Alternatively, increased production of an inhibitor may mask collagenase activity. Although the regulation of production and secretion of collagenase may differ in fibroblasts and keratinocytes, the limited characteristics of the enzyme described here do not indicate any functional or biochemical differences between the fibroblast and keratinocyte enzymes.

The discovery of keratinocyte-derived collagenase supports the studies by Eisen (6) and Woodley et al. (11) in which collagenolytic activity was detected in explant cultures of wound edge epithelium and keratinocytes grown on a nonviable dermal substrate, respectively. It was not possible to exclude the presence of contaminating fibroblasts producing the observed collagenase activity in the study by Eisen, whereas Woodley et al. excluded this possibility more rigorously. Several methods were used to verify that the collagenase detected in our cultures was produced by the keratinocytes. First, all keratinocytes used in these experiments had been passaged at least three times in serum-free, low-calcium MCDB 153 medium which does not support fibroblast growth (23); keratinocyte cultures refed with medium which supported fibroblast growth did not reveal fibroblasts in subsequent subcultures. Second, no collagenase activity was detected in low passage keratinocyte cultures treated with interleukin-1, a potent stimulator of collagenase production in fibroblasts, while fibroblast cultures treated in parallel in the same experiment produced substantial collagenase activity. Lastly, and most importantly, we demonstrated intracellular collagenase in TPA-treated keratinocytes by indirect immunofluorescence.

The production of collagenase by various cell types including rabbit synovial and human dermal fibroblasts (13, 14), hepatocytes (39), and endothelial cells (18) has been augmented in vitro by multiple agents including cytochalasin B (14), phorbol esters (17), phagocytosis (40), or the calcium ionophore A23187 (41). Aggeler et al. (42) demonstrated that treatment of rabbit synovial fibroblasts with such agents caused a marked alteration in gene expression resulting in induction of procollagenase and that such induction paralleled alterations in cell shape produced by some of these agents. We found that treatment of human keratinocytes with TPA produced similar morphologic changes, such as loss of cellular adhesion and cytoplasmic vacuolization, and that these effects occurred in our TPA-treated keratinocyte cultures in parallel with the production of collagenase. TPA is structurally similar to diacylglycerol and is thought to act at least in part through activation of protein kinase C (43). Protein kinase C has a central role in signal transduction for biologically active substances leading to activation of cellular functions and prolif-
with purification of collagen, R. E. Payne, Jr., for expert technical
lagenolysis has been the production of several cytokines which
losa, and other skin disorders characterized by dermal-epider-
and degradation of the extracellular matrix during the process
epithelial cells may well have an active role in the synthesis
keratinocytes have been reported to synthesize and secrete
production of collagenase may be stimulated in turn by these
arity, which is needed for re-epithelialization of the wound. The
phenotypic alterations are thought to enhance cellular motil-
rectly over the extracellular matrix of the dermis, whose major
fibronectin, fibrin, and type V collagen
and type IV collagen
contribute to the motility of the cells
production of tonofilaments, and loss of desmosomes (45). These
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Previously, the presumptive role of epidermal cells in colla-
genolysis has been the production of several cytokines which
stimulate collagenase production by dermal fibroblasts (51). Our studies suggest that the epidermal cells may play a primary role in collagen degradation through production of collagenase. Production of excessive or unregulated collagenase by keratinocytes could contribute to the pathogenesis of the dermolytic bullous disease, dystrophic epidermolysis bul-
osa, and other skin disorders characterized by dermal-epider-
mal separation. Furthermore, keratinocyte-derived collagenase
may play a role in the mechanism of connective tissue invasion by cutaneous tumors.

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