Induction and Repression of Collagenase-1 by Keratinocytes Is Controlled by Distinct Components of Different Extracellular Matrix Compartments*

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In all forms of cutaneous wounds, collagenase-1 (matrix metalloproteinase-1 (MMP-1)) is invariably expressed by basal keratinocytes migrating over the dermal matrix. We report that native type I collagen mediates induction of MMP-1 by primary human keratinocytes. Collagen-mediated induction of MMP-1 was rapid, being detected 2 h after plating, and was transcriptionally regulated. As demonstrated by in situ hybridization, only migrating keratinocytes expressed MMP-1, suggesting that contact with collagen is not sufficient to induce MMP-1 expression in keratinocytes; the cells must also be migrating. Upon denaturation, type I collagen lost its ability to induce MMP-1 expression but still supported cell adhesion. Other dermal or wound matrix proteins, such as type III collagen, fibrin, and fibronectin, and a mixture of basement membrane proteins did not induce MMP-1 production. In the presence of collagen, laminin-1 inhibited induction of MMP-1 but laminin-5 did not. Taken together, these observations suggest that as basal keratinocytes migrate from the basal lamina onto the dermal matrix contact with native type I collagen induces MMP-1 expression. In addition, our findings suggest that re-establishment of the basement membrane and, in particular, contact with laminin-1 provides a potent signal to down-regulate MMP-1 production as the epithelium is repaired.

Normal cutaneous wound healing, as well as healing in essentially all tissues, involves an orderly progression of events to re-establish the integrity of the injured tissue. The initial injury starts a programmed series of interdependent yet functionally separate responses, such as re-epithelialization and epithelial proliferation, inflammation, angiogenesis, fibroplasia, matrix accumulation, and eventually resolution. During each stage in this process, proteinases are needed to remove or remodel extracellular matrix components in both the epithelial and interstitial compartments to accommodate cell migration and tissue repair (1).

In a thorough examination of normally healing wounds and of a variety of chronic ulcers, we found that collagenase-1, a member of the matrix metalloproteinase (MMP) family with the ability to cleave fibrillar collagens type I, II, and III at a specific locus in their triple helical domain, is invariably expressed by basal keratinocytes at the edge of repairing tissue (2–4). In all, we have examined >100 different human skin specimens, representing a variety of chronic ulcers, blisters, and normally healing wounds, and in each sample with injury that breached the basement membrane, collagenase-1 was prominently and invariantly expressed by basal keratinocytes migrating over the dermal wound bed. The invariable expression of collagenase-1 in all forms of wounds and the confinement of its expression to periods of active re-epithelialization suggest that this enzyme plays a critical role in keratinocyte function during healing. Indeed, we recently demonstrated that keratinocyte migration on a native collagen matrix requires the catalytic activity of collagenase-1 (5). Thus, identifying the factors and events in a wound environment which control collagenase-1 expression will aid our understanding of fundamental processes of tissue repair.

The precise, spatially confined expression of collagenase-1 by migrating keratinocytes in vivo suggests that altered cell:matrix interactions, which are established as cells migrate off the basement membrane, induce enzyme expression. Indeed, collagenase-1 is only expressed by keratinocytes that are not in contact with an intact basement membrane (3, 4, 6, 7). Furthermore, expression of collagenase-1 is shut off once re-epithelialization is complete and the basement membrane is restored (3, 6). These in vivo findings suggest that altered cell:matrix interactions, which form as cells move from the basement membrane onto the dermal and provisional matrices and back onto the basement membrane at the completion of re-epithelialization, regulate the site-specific expression of collagenase-1 in keratinocytes.

In this report, we show that native fibrillar type I collagen is a principal, if not the main, stimulus initiating collagenase-1 production. Many other molecules that migrating keratinocytes could encounter in a wound setting, such as fibrin, fibronectin, or type III collagen, did not affect collagenase-1 production. In addition, we show that laminin-1 effectively inhibits collagen-mediated collagenase-1 expression, suggesting that renewed contact with this basement membrane component provides a signal to cease collagenase-1 expression and halt keratinocyte migration. Our findings support the idea that distinct cell:matrix interactions regulate keratinocyte behavior at different stages of repair.

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The abbreviations used are: MMP, matrix metalloproteinase; ELISA, enzyme-linked immunosorbent assay; PMA, 12-0-tetradecanoylphorbol 13-acetate; EGF, epidermal growth factor; RT, reverse transcription; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; DMEM, Dulbecco’s modified Eagle’s medium.
EXPERIMENTAL PROCEDURES

Cell Culture—Primary keratinocytes were isolated from full thickness skin obtained from reductive mammoplasty and lateral abdominoplasty and grown in high Ca\(^{2+}\) (1.8 mM) medium as described previously (8). Cell suspensions were plated on 3.8-cm\(^2\) tissue culture dishes precoated with a 100 \(\mu\)g/ml solution of bovine type I monomeric collagen (Vitrogen; Collagen Corp., Palo Alto, CA). Primary keratinocytes were also cultured on dishes precoated with 20 \(\mu\)g of fibronectin (Sigma) or presaturated with a 100 \(\mu\)g/ml solution of laminin-1 (Upstate Biotechnology, Inc, Lake Placid, NY), laminin-5 (supplied by Dr. Robert E. Burgese, Harvard University), Matrigel® (Becton Dickinson, Bedford, MA), type III collagen (R&D Systems), type IV collagen, vitronectin, rat or mouse type I collagen, or gelatin. Rat tail collagen was prepared by differential salt precipitation, and gelatin was generated by heating native type I collagen (bovine, rat, or mouse) for 10 min at 45 or 80 °C or by cleavage for 1 h at 37 °C with 14.5 \(\mu\)g/ml Clostridium collagenase (Worthington) that had been further purified by Sephadryl S-200 gel filtration (supplied by Dr. Edmond C. Crouch, Washington University, St. Louis, MO). The bacterial enzyme was inactivated by addition of serum. In experiments with two matrices, various amounts of laminin-1 or laminin-5 were combined with 100 \(\mu\)g/ml type I collagen, and dishes were coated with the resultant mixtures. Collagenase-1 accumulation in the medium was assessed 48 h later by competitive enzyme-linked immunosorbent assay (ELISA) and normalized to total cell protein (9).

Keratinocyte cell lines were grown under recommended conditions. The HaCaT human keratinocyte cell line, provided by Dr. Norbert Fusenig, German Cancer Research Center, was grown in DMEM containing heat-inactivated fetal calf serum at 1% (Hepes, pH 7.4, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin). The JS.1 and JS.6 cell lines were grown in DMEM containing 10% fetal calf serum, 2 \(\mu\)M glutamine, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin. The SCC-4 and SCC-25 cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD; CRL-17, CRL-1624, and CRL-1628, respectively). KB cells were grown in DMEM containing 10% fetal calf serum, 2 \(\mu\)M glutamine, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin. The SSC-4 cell line was grown in a 1:1 DMEM:Ham’s F-12 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin. The ISC-1 cell lines were provided by Dr. John C. Ansel (Oregon Health Sciences University) and grown in 3:1 keratinocyte growth medium (Clonetics, San Diego, CA). DMEM medium supplemented with 10% fetal calf serum, 2.5 \(\mu\)g Hepes, pH 7.4, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin. To determine if collagenase-1 was inducible in these cell lines, they were plated on plastic or type I collagen and treated with 30 \(\mu\)g/ml epidermal growth factor (EGF; Sigma) or 5 \(\times\) 10^{-9} M 12-O-tetradecanoylphorbol 13-acetate (TPA) for 48 h.

In Situ Hybridization—Collagenase-1 mRNA was detected in formalin-fixed tissue samples and cultured cells by hybridization with \(^{35}S\)-labeled antisense RNA described previously (10, 11). Keratinocytes were grown on collagen- or gelatin-coated Lab-Tek chamber slides (Nunc, Naperville, IL) for 48 h and were hybridized with 2.5 \(\times\) 10^{5} cpm \(^{35}S\)-labeled antisense RNA overnight at 57 °C. For hybridization, slides were washed under stringent conditions, including RNase A treatment, and were processed for autoradiography. After development of the photographic emulsion, slides were stained with hematoxylin-eosin. The specificity of the antisense RNA probe for collagenase-1 and the complete lack of reactivity with the sense probe has been demonstrated in previous studies (2, 3).

Protein Synthesis—Total protein synthesis was determined in cultured keratinocytes as described elsewhere (9). Cells were cultured under various conditions for 24 h and were then incubated for 1 h in leucine-free medium to deplete the intracellular pool of leucine, followed by a 3-h pulse in leucine-free medium containing 1 \(\mu\)Ci/ml ^{14}C-leucine (42 Ci/mmol). The medium and cell layer were collected, and proteins were precipitated with trichloroacetic acid and processed for scintillation spectrophotometry.

RNA Analysis—Collagenase-1 mRNA was detected by a reverse transcription-polymerase chain reaction (RT-PCR) assay modified from an established quantitative procedure (12). Total RNA was isolated by phenol-chloroform extraction. To remove any contaminating DNA, RNA was treated with RQI RNase-free DNase and RNasin (both from Promega, Madison, WI) as described previously (12). DNase-treated RNA (10 ng) was reverse transcribed with random hexamers using kit reagents and under the manufacturer’s recommended conditions (GeneAmp RNA PCR kit, Perkin Elmer Corp.). For each sample, a parallel reaction was run with no reverse transcriptase. The 3′-antisense primer was complementary to bases 1496–1518 in the 3′-untranslated region (5′-GGTCAGGACAGAGATGTGTCC′-3′), and the 5′ sense primer was defined by bases 1114–1135 of the hemopexin-like domain (5′-GGGCT-
the confinement of collagenase-1 expression by migrating basal keratinocytes seen in vivo. Primary keratinocytes grown in high calcium medium and plated on denatured type I collagen (gelatin) formed these same subpopulations but, as stated, did not express collagenase-1 (Fig. 1, Gel).

To assess if other interstitial proteins or if components of the provisional matrix or basement membrane induce or stimulate collagenase-1 expression, we plated primary keratinocytes on various purified connective tissue proteins and assessed the ability of the cells to attach, spread, and produce collagenase-1 (Table I). Attachment was scored as zero if less than 5% of the keratinocytes adhered to a substratum after the dishes were rinsed and refed 18 h after plating. Spreading was scored as zero if less than 5% of attached cells formed a cytoplasmic apron typical of primary keratinocytes. In all cases, either most keratinocytes (>80%) attached to or spread on a matrix protein or, as for fibronectin and fibrin (Table I), they did not.

Primary keratinocytes did not attach to uncoated tissue culture plastic or to dishes coated with fibrin or fibronectin, components of the provisional wound matrix (Table I). In contrast, keratinocytes adhered to and spread on the basement membrane proteins, laminin-1 and laminin-5, and on Engelbreth-Holm-Swarm tumor matrix (Matrigel®), which contains laminin-1, type IV collagen, entactin/nidogen, and heparin sulfate proteoglycan, and on type III collagen, a dermal matrix component. None of these matrices, however, induced or stimulated collagenase-1 production as assessed by ELISA (Table I).

Consistent with the in situ hybridization data (Fig. 1), gelatin supported cell adhesion and spreading but did not induce collagenase-1 production. In contrast, native type I collagen supported keratinocyte attachment and spreading and mediated induction of collagenase-1 expression (Table I) in a dose-dependent manner (Fig. 2A). The number of cells attached was equivalent at all concentrations of type I collagen (data not shown), indicating that this dose response was not an artifact of cell adhesion. The apparent inhibitory effect with high coating concentrations of collagen (500 and 1000 μg/ml) may be due to sequestering of some enzyme in the matrix or to negative cooperativity. This decrease in collagenase-1 was not a consequence of the collagen contributing to the quantification of total cell protein used to normalize the ELISA data. Stimulation of collagenase-1 expression in keratinocytes on native type I coll-

**Fig. 1.** Collagenase-1 mRNA is expressed by migrating keratinocytes in vivo and in vitro. Upper panels, sections of pyogenic granuloma were hybridized with an 35S-labeled antisense RNA probe specific for collagenase-1 mRNA. Under dark-field illumination (left panel), prominent autoradiographic signal for collagenase-1 mRNA was seen in basal keratinocytes (small arrows) at the margin of a small ulceration (U). Collagenase-1-positive cells were present at the wound edge (large arrow) and extended along the basal layer away from the ulcerative area. Signal strength decreased progressively and eventually was not detectable (open arrow) at some distance from the ulcer margin. As is seen in the bright-field, higher magnification photomicrograph, autoradiographic signal for collagenase-1 mRNA was confined to basal keratinocytes and was not seen in any cells within the suprabasal epidermis (E). Autoradiographic exposure was for 14 days, and sections were stained with hematoxylin and eosin. Bar = 100 μm (left panel) or 50 μm (right panel). Bottom panels, primary human keratinocytes were plated onto tissue culture slides coated with denatured type I collagen (gelatin (Gel)) or native type I collagen (Col) and 48 h later were processed for in situ hybridization for collagenase-1 mRNA. On both substrata, keratinocytes differentiate, forming blurred foci (*) of stratified cells surrounded by a monolayer of tightly opposed cells. Bordering these cell islands, and often detached from them, are migrating keratinocytes (arrows). Reflecting the phenotype of basal cells involved in re-epithelialization in vivo, collagenase-1 mRNA was expressed only in keratinocytes migrating from the colonies of proliferating and differentiated cells (arrows). Autoradiographic exposure was for 21 days, and sections were stained with hematoxylin and eosin. Bar = 50 μm.
ligen was specific relative to total protein synthesis, which did not differ among cells plated on collagen, gelatin, or Matrigel (data not shown).

Purified type IV collagen and vitronectin also induced colla-
genase-1 production (Table I) equal to that induced by native type I collagen. As we discussed, we propose that the effect with type IV collagen represents an in vitro artifact. In contrast, vitronectin, similar to type I collagen, may provide a wound-specific factor that maintains or stimulates collagenase-1 expression during re-epithelialization (see “Discussion”).

We performed various controls to assure that collagen-mediated induction of collagenase-1 expression was due to contact with the matrix protein and not to a contaminating factor. The same induction of collagenase-1 was detected in keratinocytes plated on type I collagen isolated from rat, mouse, and bovine tissue (Table I). Because these proteins were isolated by different methods, collagenase-1 induction was likely mediated by a specific interaction with collagen. In addition, keratinocytes were plated on gelatin generated by heat denaturation of native type I collagen at 45 °C, a temperature which would be permissive to maintaining the activity of a contaminating inductive factor, or at 80 °C, which would likely inactivate most protein factors. Keratinocytes were also grown on type I collagen that had been digested with highly purified bacterial collagenase, which specifically cleaves Gly-X-Y domains and does not degrade noncollagenous proteins. Collagenase-1 production was induced only in cells cultured on intact, native type I collagen, and only background levels were detected in the cultures grown on the denatured or digested collagen substrata (Fig. 2B). These results indicate that contact with native type I collagen, and not a contaminating factor, induces collagenase-1 expression by keratinocytes.

Contact with Type I Collagen Rapidly Initiates Collagenase-1 Expression—Because only a defined subpopulation of keratinocytes expressed collagenase-1 in response to collagen (Fig. 1), we used RT-PCR to assess the temporal expression of collagenase-1 mRNA upon contact with native type I collagen. Signal strength for collagenase-1 RT-PCR cDNA product increased exponentially between 19 and 27 cycles using 10 ng of RNA and increased linearly between 1 and 25 ng of RNA at 25 cycles

![Fig. 2. Collagenase-1 production is induced by native type I collagen. A, primary human keratinocytes were grown on dishes pre-coated with the indicated concentrations of bovine native type I collagen. Medium was collected at 48 h later, and collagenase-1 levels were determined by ELISA. B, keratinocytes were grown on dishes coated with 100 μg/ml native type I collagen or gelatin generated by heating at 45 or 80 °C or by degradation with bacterial collagenase. Conditioned medium was collected at 48 h, and collagenase-1 accumulation was determined by ELISA. Data in both graphs are the mean ± SD of triplicate determinations of triplicate samples and are normalized to the cellular protein content.](https://example.com/fig2)

![Fig. 3. Type I collagen rapidly induces collagenase-1 expression. Normal human skin was processed for keratinocyte isolation as described under “Experimental Procedures.” As the trypsin-dispersed cell suspension was prepared, some cells were collected for RNA isolation (0 h). The remaining keratinocytes were plated on type I collagen-coated dishes. Total RNA was isolated at the indicated times, collagenase-1 and GAPDH mRNAs were reverse transcribed, and the cDNA were amplified and detected by Southern hybridization. Duplicate control samples were processed without reverse transcriptase (−RT). Autoradiographic band density was quantified, and the signal strength for collagenase-1 mRNA is expressed relative to that for GAPDH mRNA. Autoradiography was for 2 h for both blots.](https://example.com/fig3)
on native type I collagen at a high density, and expression increased markedly and progressively over the next 10 h. Collagenase-1 mRNA dropped to low levels between 24 to 48 h post-plating (Fig. 3) coincident with the keratinocytes reaching confluency.

Collagen Induces Collagenase-1 Promoter Activity in Transiently Transfected Keratinocytes—To determine if contact with native type I collagen induces collagenase-1 gene transcription, primary keratinocytes plated on collagen or gelatin were transiently transfected with a CAT expression construct containing 2.2 kilobase pair of the human collagenase-1 promoter. As we demonstrated in other studies (16), changes in the activity of this promoter construct parallel changes in endogenous transcriptional activity of the collagenase-1 gene. In addition, we demonstrated that both downstream (−72 to −66) and upstream (−2010 to −1954) elements are required for full and appropriately regulated activity of the collagenase-1 promoter (16). Consistent with secreted enzyme and steady-state mRNA levels, relative CAT activity was about 5-fold greater in cells plated on native collagen than in keratinocytes on gelatin (Fig. 4). No CAT activity was detected in cells transfected with a promoterless construct (data not shown), indicating that the collagen-responsive elements lie within the collagenase-1 promoter. Luciferase activity, which was expressed from a cotransfected plasmid under control of a thymidine kinase promoter, did not differ significantly among samples (Fig. 4). Transfection with a pCMV-β-galactosidase construct revealed that greater than 80% of the primary keratinocytes were transfected (data not shown). Because CAT activity conferred from this region of the collagenase-1 promoter reflects endogenous production, these results indicate that collagen-stimulated expression of collagenase-1 is controlled at the level of transcription.

Collagen Does Not Induce Collagenase-1 in Keratinocyte Cell Lines—Six human keratinocyte cell lines derived from squamous cell carcinomas were screened for their ability to express collagenase-1 production in response to native type I collagen. EGF and PMA were used as positive controls because these agents potentiate stimulate collagenase-1 production in keratinocytes (15). All keratinocyte cell lines had low to undetectable basal levels of collagenase-1 production, and only a subset of lines increased enzyme expression in response to PMA and/or EGF. None of the cell lines tested responded to collagen (Table II). Furthermore, stimulation of collagenase-1 by either PMA or EGF was not modulated by either substrate. These results suggest that transformed keratinocytes respond differently to signals from the extracellular matrix than do primary cells, suggesting that properly regulated wound healing responses may be lost in oncogenically transformed cells.

Inhibition of Collagen-mediated Induction of Collagenase-1—In vivo observations by Inoue et al. (6) and our group (3) demonstrate that collagenase-1 expression in wounded skin is down-regulated upon the completion of re-epithelialization. At this stage, cell:cell contacts and keratinocyte interactions with the basement membrane are restored, and we assessed which or if both of these events regulate the turn-off of collagenase-1. As shown by in situ hybridization of cultured cells (Fig. 1), collagenase-1 is expressed only by keratinocytes that are at the edge of monolayer foci or separate from other cells. Keratinocytes with obvious, well formed cell:cell contacts did not express collagenase-1. Thus, because keratinocytes have the capacity to deposit their own basement membrane matrix, either cell:cell interactions or contact with basement membrane proteins may down-regulate collagenase-1 expression in these stationary keratinocytes.

To assess the role of cell:cell contacts, we plated primary keratinocytes on collagen-coated dishes or glass slides at different densities and assessed collagenase-1 expression by in situ hybridization at 1 day post-plating and enzyme accumulation in the medium by ELISA at 72 h post-plating. Cells plated at 3 × 10^6 cells per dish were nearly confluent when plated, whereas the other groups were at variable stages of confluency proportion to their initial plating density. At 24 h post-plating, subconfluent cultures showed strong mRNA expression, whereas only occasional cells in confluent cultures had a weak signal for collagenase-1 mRNA (Fig. 5, B and C). Similarly, collagenase-1 mRNA, as detected by RT-PCR, was expressed briefly and transiently in keratinocytes plated at confluent density (Fig. 4). At 72 h, when the medium was collected for ELISA of collagenase-1, cells plated at 2 or 3 × 10^6 cells/dish were confluent; those plated at 1 × 10^6 or 1.5 × 10^6 cells/dish still had areas of unoccupied surface (data not shown). Collagenase-1 levels were inversely proportional to the initial plating density (Fig. 5A). Thus, barring cells from migrating (by plating at a high density), and possibly the formation of cell:cell contacts, blocks collagen-mediated induction of collagenase-1.

In wounded or intact skin, keratinocytes on basement membrane do not express collagenase-1 (17). Similarly, primary

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**Table II**

Production and regulation of collagenase-1 by primary keratinocytes and keratinocytes cell lines

| Cell line | Matrigel® | Type I collagen |
|-----------|-----------|----------------|
|           | Control + EGF | + PMA | Control + EGF | + PMA |
| Primary keratinocytes | 0.17 | 10.85 | 0.68 | 6.98 |
| HaCaT | 0.05 | 3.32 | 0.40 | 0.06 | 2.56 | 0.20 |
| KB | 0.00 | 0.01 | 2.58 | 0.00 | 0.10 | 1.40 |
| JS-1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| JS-6 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SSC-4 | 0.09 | 0.11 | 0.24 | 0.08 | 0.10 | 0.29 |
| SSC-25 | 0.21 | 1.36 | 1.48 | 0.19 | 1.36 | 1.43 |

Primary keratinocytes or keratinocyte cell lines were plated on Matrigel®- or collagen-coated dishes, and some cultures were exposed to 30 ng/ml EGF or to 5 × 10⁻⁸ M PMA. Control cells were on matrix only. Media samples were collected 48 h later, and collagenase-1 levels were determined by ELISA and normalized to total protein in the cell layer. Primary human keratinocytes were not exposed to EGF. Collagenase-1 levels shown as 0.00 were equal to or less than the background values for the ELISA. The data shown are the numerical mean of media samples from duplicate wells assayed in triplicate. Variance was less than 10% for all samples.
keratinocytes on a complex mix of basement proteins (Matrigel®) do not express this MMP (Table I). Because collagenase-1 expression is induced by type IV collagen but not by laminin-1 (Table I), contact with laminin-1 within the newly formed basement membrane may participate in down-regulating collagenase-1 production. To assess if laminin-1 inhibits collagen-mediated stimulation of keratinocytes, we plated cells on dishes coated with 100 μg/ml type I collagen, the lowest concentration that mediated maximal stimulation of collagenase-1 production (Fig. 2A), mixed with various concentrations of purified laminin-1. Collagen-mediated induction of collagenase-1 was inhibited about 50% with relatively low concentrations (between 0.01 and 0.1 μg/ml) of laminin-1, and enzyme levels were reduced to near basal amounts with 100 μg/ml laminin-1 (Fig. 6A). Migrating keratinocytes produce and deposit laminin-5 (18), but this component of the epidermal basement membrane did not inhibit or significantly augment collagen-mediated induction of collagenase-1 (Fig. 6B). These data support the idea that reformation of the basement membrane and contact with specific components, particularly laminin-1, provides a signal to repress collagen-mediated collagenase-1 expression. Taken together, our data suggest that both the restoration of cell:cell contacts and interactions with the basement membrane contribute to the regulation of collagenase-1 during wound repair.

DISCUSSION

In this report, we demonstrate that contact with native type I collagen induces collagenase-1 expression in migrating keratinocytes. These findings suggest that altered cell:matrix interactions regulate the invariable and prominent expression of collagenase-1 by basal keratinocytes seen during active stages of re-epithelialization in all human wounds with a breached basement membrane. In addition, we show that matrix-mediated regulation of collagenase-1 is dependent on the native, triple helical conformation of type I collagen. Although collagen that had been denatured, solubilized, or enzymatically degraded supported cell attachment, these non-triple helical substrates did not induce or stimulate collagenase-1 expression in keratinocytes. In addition, type III collagen, which is structurally similar to type I collagen, did not induce collagenase-1 expression (Table I). Together, these findings suggest that cell contact with type I collagen, the most abundant protein in the dermis, comprising greater than 70% of its dry weight (19), provides a critical and specific determinant regulating collagenase-1 production by keratinocytes. Furthermore, our findings suggest that re-establishment of the basement membrane and cell:cell contacts at the completion of wound closure mediates down-regulation of collagenase-1 expression. Thus, contact with specific components of different matrix compartments mediate functionally distinct cellular activities.

Three key in vivo observations support the idea that altered cell:matrix interactions regulate the spatially precise pattern of collagenase-1 production in migrating keratinocytes during wound repair. One, as we (3, 4) and others reported (6), immunostaining for type IV collagen or laminin showed that collagenase-1-positive keratinocytes are not in contact with a basement membrane but rather are moving over the dermal or wound bed matrix. Two, collagenase-1 is not expressed by basal keratinocytes in nonulcerative pyogenic granuloma or in blisters, such as bullous pemphigoid, which separate above basement membrane, but this MMP is expressed by migrating keratinocytes in blisters that form below the basement membrane, such as in recessive dystrophic epidermolysis bullosa (3, 4). Three, in acute wounds (3, 6) and in cultured skin equivalent models (20), collagenase-1 expression ceases once re-epithelialization is completed and a basement membrane has re-formed. Together, these findings support the hypothesis that keratinocytes acquire a collagenolytic phenotype upon contact with the dermal matrix, and the data we provide here indicate that native type I collagen is the inductive molecule in the dermis that controls this response. Furthermore, this induction is specific for collagenase-1. Although keratinocytes also pro-

FIG. 5. Collagenase-1 is not induced in confluent keratinocytes. Various concentrations of primary keratinocytes (cells/ml) were plated on collagen-coated dishes or slides. A, at 3 d post-plating, the media and cell layers were collected, and collagenase-1 accumulation was assessed by ELISA and normalized to total protein. Data represent the mean ± S.D. of triplicate samples. At 1 d post-plating, the slides were processed for in situ hybridization for collagenase-1 mRNA. Shown are slides of cells plated at 1 × 10⁶ cells/ml (B) or 3 × 10⁶ cells/ml (C).

FIG. 6. Laminin-1 but not laminin-5 blocks collagen-mediated induction of collagenase-1. A, primary keratinocytes were plated on dishes coated with 100 μg/ml type I collagen containing the indicated concentrations of laminin-1. B, keratinocytes were plated on dishes precoated with the indicated concentration of type I collagen and/or laminin-5. For both experiments, media and cell layers were collected 3 days later, and collagenase-1 accumulation was assessed by ELISA and normalized to total protein. Data represent the mean ± S.D. of triplicate samples.
duce 92-kDa gelatinase,stromelysin-1, andstromelysin-2, we
have not detected any changes in expression of these MMPs
mediated solely by matrix (21).

The type I collagen-binding receptor, α2β1, is constitutively
expressed on keratinocytes (22, 23), and as we reported re-
cently, this integrin transduces collagen-mediated induction
of collagenase-1 (5). Similarly, collagen stimulates collagenase-1
expression in fibroblasts via ligand binding to α2β1 (24, 25).
Although basal keratinocytes also express α1β1 and α3β1, ke-
ratinocytes preferentially use α2β1 to bind to type I collagen
(26). The interaction of keratinocytes with the dermal matrix,
and in particular with type I collagen, may provide an early
and critical signal to initiate the epithelial response to wound-
ing. Because the epidermis is not normally in contact with type
I collagen, it is tempting to speculate that the basal production
of α2β1 keeps keratinocytes primed and ready to respond to
injury.

Earlier studies by Woodley and associates showed that ke-
ratinocytes on nonviable dermis produce collagenolytic en-
zymes (27) and that contact with type I collagen enhances basal
production of this activity (28). Although seemingly similar,
there are notable differences between their and our experimen-
tal conditions that impact on our respective findings. Whereas
we grew keratinocytes in high Ca2+ medium, which promotes
differentiation and duplicates the subpopulations of keratino-
cytes seen in vivo (Fig. 1), Petersen et al. (29) used low Ca2+-
medium which maintains the keratinocytes in a basal pheno-
type (30). Thus, this change in culture conditions may lead to
changes in cell behavior and responsiveness. Indeed, Petersen
et al. (31) demonstrated that keratinocytes grown in low Ca2+-
medium constitutively expressed collagenase-1, whereas, as we
show here, collagenase-1 is induced in keratinocytes in high
Ca2+ medium only if plated on the appropriate substratum.

In addition to native type I collagen, contact with vitronectin
and type IV collagen also mediated induction of collagenase-1
in keratinocytes (Table I). The wound bed is rich in vitronectin
(32), and contact with this serum glycoprotein may provide an
additional matrix-derived signal that can regulate keratinocyte
behavior during wound healing. However, the vitronectin re-
ceptor, αvβ5, is not present in intact skin but is expressed in
migrating basal cells in response to injury (33) but is appar-
etly activated in isolated keratinocytes (34). Thus, this matrix
protein is likely not involved in rapid induction of collagenase-1
after wounding but may play a role in maintaining the collag-
enolytic phenotype of migrating keratinocytes. The wound en-
vironment is also rich in fibrin and fibronectin, but primary
keratinocytes did not attach to these substrates (Table I).

These findings were not surprising. Grinnell and co-workers
demonstrated that keratinocytes freshly isolated from skin do
not adhere to fibronectin (35), and keratinocytes in intact skin
do not express α5β1 or the αv subunit (33, 36), the integrins
that bind fibronectin and fibrin, respectively (34). As for αvβ5,
α5β1 is induced in response to wounding and is expressed on
the basal surface of the same population of keratinocytes pro-
ducing collagenase-1 (3). The lack of α5β1 in intact skin sup-
ports the conclusion that binding to fibronectin does not induce
collagenase-1 expression in keratinocytes. In contrast, fi-
bronectin fragments binding α5β1 stimulate collagenase-1 ex-
pression in fibroblasts (37), indicating that fundamentally dis-
tinct mechanisms control MMP expression in different cell
types.

We believe that type IV collagen-mediated induction of col-
lagenase-1 by keratinocytes, which has also been reported by
others (28), is an in vitro artifact. Because they constitutively
express β1 integrins, keratinocytes in intact skin can interact
with type IV collagen in the basement membrane. However,
our in vivo observations (3) and our RT-PCR data (Fig. 4)
demonstrate that keratinocytes in intact skin do not express
collagenase-1. In addition, primary keratinocytes grown on
Matrigel®, which contains type IV collagen, do not make col-
lagenase-1 (Table I), presumably because laminin-1 over-
whelms any potential inductive effect of type IV collagen. Be-
cause type IV collagen in the context of a basement membrane
does not induce collagenase-1 expression in keratinocytes, the
relevance of its ability to do so in a pure form is questionable.

After all, other than that in anchoring plaques, keratinocytes
in vivo would likely not interact with “pure” type IV collagen.

The observation that cultured keratinocytes on basement
membrane or cells in intact skin do not express collagenase-1
suggests that a basal lamina protein blocks enzyme production.
We show here that laminin-1, but not laminin-5, effectively
represses type I collagen-mediated induction of collagenase-1
(Fig. 6). Analogously, laminin-1 and type I collagen mediate
opposite effects on breast epithelial cell differentiation (38, 39).
During wound healing in vivo, laminin-5 and collagenase-1 are
expressed by migrating keratinocytes (18). Zhang and Kramer
(40) reported that laminin-5 promotes keratinocyte migration,
and we recently reported that keratinocyte migration on a
collagen matrix requires the activity of collagenase-1 (5). In-
cidentally, laminin-5, as well as laminin-1 and type IV colla-
gen, are not substrates for collagenase-1. Thus, this basement
membrane glycoprotein and this MMP serve distinct yet re-
quired functions during re-epithelialization.

Laminin-1 is deposited in the newly formed basement mem-
brane just behind the migrating front of epidermis (33). As
re-epithelialization progresses, the mass of laminin-1 deposited
under the previously migrating keratinocytes would accumu-
late, providing a site-specific mechanism to down-regulate col-
lagenase-1 expression. Consistent with this idea, our data (Fig.
6) demonstrate that relatively small concentrations of lami-
in-1 can block the inductive effect of native type I collagen.

The molecular mass of a triple helical type I collagen monomer
is about 300 kDa and is about 900 kDa for a fully processed
laminin-1 heterotrimer. Thus, assuming one binding event per
molecule, one laminin-1 molecule effectively blocked the induc-
tion of collagenase-1 mediated by about 3,000 collagen mole-
cules. Because type I collagen is so abundant in the dermis, it
is not surprising that the inhibitory effect of laminin-1 is so
potent.

In addition to cell:matrix interactions, our data suggest that
cell:cell contacts influence the turn-off of collagenase-1 expres-
sion at the completion of re-epithelialization. Plating keratino-
cyes at a confluent density prevented induction of collagena-
se-1, even though the cells were in contact with type I collagen
(Fig. 5). Because collagenase-1 expression was assessed soon
after plating, inhibition of enzyme production was not due to
accumulation of keratinocyte-derived basement membrane
proteins. These data indicate that contact with type I collagen
is not sufficient to induce collagenase-1 expression in keratino-
cyes; the cells must also have the ability to migrate. Con-
versely, keratinocyte migration in and of itself does not induce
collagenase-1 expression. For example, collagenase-1 is not
expressed by keratinocytes involved in re-epithelialization of
lesions with an intact basement membrane, such as suction
blisters or early bullous pemphigoid blisters (3, 4). In addition,
collagenase-1 is not expressed by cultured keratinocytes on
gelatin, even though these cells can migrate on this substrate
(5). Thus, induction of collagenase-1 requires both contact of
the keratinocytes with the appropriate matrix stimulus (native
collagen) and the ability of the cells to migrate.

In summary, our findings suggest that, as keratinocytes
move from the basement membrane onto the dermal matrix,
contact with native type I collagen mediates induction of collagenase-1. The invariable expression of collagenase-1 by migrating basal keratinocytes in all forms of wounds indicates that the activity of this MMP serves a critical and required function during re-epithelialization. In contrast, collagenase-1 is not expressed in squamous or basal cell carcinomas (41, 42), and cells derived from squamous cell carcinomas did not express collagenase-1 in response to collagen or other stimuli (Table II). These findings suggest that the proteolytic requirements of normal epithelial repair differ from those needed for invasion of carcinoma cells.

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