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Interleukin-1 α –induced proteolytic activation of metalloproteinase-9 by human skin

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Abstract

Background—Increased activity of matrix metalloproteinase-9 (MMP-9) has been well documented in many diseases associated with inflammation, such as chronic wounds, bullous pemphigoid, liver failure, and tumor metastases. The mechanism for the proteolytic activation of pro-MMP-9 in human tissue still remains unknown.

Methods—We investigated this mechanism through reconstitution of an inflammatory condition in normal human skin, and epidermal and dermal cells derived from skin. Normal human skin was cultured with exogenous cytokines associated with inflammation and tissue repair. MMP-9 induction and activation were measured, and potential mechanisms were probed by inhibitors.

Results—Pathophysiologic concentrations of interleukin (IL)-1 α rapidly induced pro-MMP-9 synthesis by human skin. In contrast, IL-1--induced activation of pro-MMP-9 was a slow process, which required 3 days. Tumor growth factor- β induced pro-MMP-9 but failed to promote activation of the precursor. When the skin was stimulated with the combination of tumor growth factor- β and IL-1 α , substantial induction and activation of pro-MMP-9 occurred. This IL-1 induced activation of pro-MMP-9 was observed in intact skin but not in isolated dermal fibroblasts or keratinocytes. IL-1--induced activation of pro-MMP-9 was inhibited by chymostatin, a chymotrypsinlike proteinase inhibitor. Furthermore, IL-1 α decreased tissue inhibitor of metalloproteinase 1 without changing MMP-9 activator activity.

Conclusions—The proteolytic activation of pro-MMP-9 in skin inflammatory diseases likely occurs via a pathway including IL-1 α . The activation is mediated by downregulation of tissue inhibitor of MMP-1 and involves an as yet unidentified chymotrypsinlike proteinase.

Inflammation is an essential and immediate reaction in response to injury and infection. Proinflammatory cytokines are mediators that modulate cell proliferation, migration, and neovascularization.¹ Uncontrolled or excessive inflammation, however, results in diverse diseases including chronic wounds, tumor metastasis, psoriasis, and arthritis.² Persistent inflammation has long been associated with nonhealing wounds.³ Elevated levels of interleukin (IL)-1 and tumor necrosis factor- α (TNF- α) also have been linked to deficient wound healing.^{4,5} A higher concentration of IL-1 α has been observed in cancers with liver metastasis.⁶ The mechanisms whereby these mediators induce these pathophysiologic conditions are not known.

The matrix metalloproteinases (MMPs) are a family of extracellular or transmembrane proteinases that share several common structural features including a prodomain and a zinc-containing, core catalytic domain.⁷ MMP-9, also named gelatinase B or type IV collagenase, is believed to participate in the remodeling of basement membrane.⁸ Like most MMPs,

nascent, 92-kDa MMP-9 is secreted as a zymogen whose latency is maintained by the interaction of a conserved cysteine in the N-terminal prodomain with the zinc within the catalytic domain. Proteolytic activation of the 92-kDa pro-MMP, an irreversible process, is mediated through cleavage of the prodomain, which leads to the dissociation of the cysteine in the N-terminal domain from the zinc atom, a process called the “cysteine-switch.”⁹ Active MMP-9, with an apparent molecular weight of 82-kDa (the human version), has been found in many types of metastatic cancer.^{10,11} The presence of MMP-9 in tumor metastases was further demonstrated by MMP-9 gene knockout mice showing a decreased rate of tumor metastases.¹²

MMP-9 is expressed rarely in adult human tissues, while MMP-2, another gelatinase, is expressed constitutively. However, pro-MMP-9 is expressed quickly after cutaneous trauma, and concentrations decline with the progression of healing.^{13–16} In chronic wounds, pro-MMP-9 is overexpressed persistently, along with the converted zymogen, active MMP-9 (82-kDa).^{17–20} MMP-9 is almost always associated with inflammatory conditions. For instance MMP-9 is expressed in rheumatoid arthritis and psoriatic arthritis.^{21,22} Because of the activity of MMP-9 on the degradation of the basement membrane components such as type IV and type VII collagen, the pathophysiology of chronic wounds may include the excessive destruction of the extracellular matrix that is essential for reepithelialization.

Although the proteolytic activation of pro-MMP-9 has been documented extensively in many pathologic conditions, the cellular factors that control this process have not been identified. In the present study, we identified IL-1 α as a potent factor inducing the proteolytic activation of pro-MMP-9 in human skin. In addition, the conversion of pro-MMP-9 is mediated by an as yet unidentified skin-associated chymotrypsinlike proteinase.

METHODS

Material

Cytokines were purchased from R&D Systems (Minneapolis, Minn). The antibodies against human tissue inhibitor of metalloproteinase 1 (TIMP-1) and MMP-9 were purchased from Chemicon International (Temecula, Calif), antibody for mast cell α -chymase from NeoMarkers (Fremont, Calif), chymostatin from Roche Molecular Biochemicals (Indianapolis, Ind), immobilon-P from Millipore (Bedford, Mass, enhance chemiluminescence from Amersham Pharmacia (England), gelatin from Sigma Chemical Co (St. Louis, Mo), and gelatin Sepharose-4B from Pharmacia (Uppsala, Sweden).

Organ culture and cytokine stimulation of human skin

Our procedure has been reported previously in detail.²³ In brief, normal human skin was obtained from the discarded tissue of patients undergoing reconstructive or aesthetic surgery; this procedure was approved by the University of Southern California Institutional Review Board #012022. Full-thickness skin was decontaminated by incubation in 200 U/mL penicillin G sodium, 200 U/mL streptomycin sulfate, and 0.5 mg/mL amphotericin B in Dulbecco modified Eagle medium (DMEM) for 14 hours at 4°C. The skin was cut into squares of 0.25 cm² and incubated in DMEM at 37°C with 5% CO₂ for 6 hours. To decrease the effects of endogenous soluble factors in the skin induced by the harvesting process, we changed the medium 3 times during the 6-hour incubation. Finally, the skin pieces were floated in 2 mL DMEM with specific cytokines and were maintained at 37°C with 5% CO₂. The experiment was repeated with the use of skin from different donors (n = 3).

Preparation and culture of human dermal fibroblasts and keratinocytes

Dermal fibroblasts and keratinocytes were isolated from normal, full-thickness, or partial-thickness human skin as described in the previous paragraph.^{4,24} The isolated fibroblasts at the early passages ($n < 5$) were cultured in DMEM containing 10% fetal bovine serum, penicillin (100 U/mL), streptomycin sulfate (100 μ g/mL). To simulate the tissue environment, we embedded the dermal fibroblasts in type I collagen as an attached lattice.²⁴ The cell density for the collagen lattices was 100,000 cells/cm³ with 0.25 mL gel loaded into each well in a 24-well plate. The primary keratinocytes at early passages ($n < 3$) were cultivated in a 24-well plate with complete keratinocyte growth medium (KGM). The cells were washed, and the medium was replaced with serum-free DMEM for the fibroblasts and keratinocyte basal medium for the keratinocytes.

Preparation of pro-MMP-9

The transformed human keratinocytes (IKC) (kindly provided by Dr David T. Woodley at the University of Southern California) were grown in KGM to confluence. The cells were treated with 2 ng/mL TNF- α in keratinocyte basal medium for 4 days under standard culture conditions. In this condition, most of the gelatinase secreted in the conditioned medium is the 92-kDa pro-MMP-9.²³ The conditioned medium was collected, centrifuged at 4000g for 5 minutes, passed through a 5-mL gelatin Sepharose-4B column. The resin was washed with 400 mmol/L NaCl, 0.5% Triton X100 in 50 mmol/L TRIS, pH 7.5. The bound gelatinase was eluted by 2 mol/L urea followed by dialysis against buffer containing 100 mmol/L NaCl and 50 mmol/L TRIS, pH 7.5. The identity of MMP-9 was confirmed by zymography and Western blot analysis using an antibody against MMP-9.

Measuring pro-MMP-9 activation

Normal human skin ($n = 21$) was stimulated with or without IL-1 α at 10 ng/mL in DMEM for 64 hours. The resulting conditioned medium and primed tissue were separated. The MMP-9 in the conditioned medium was depleted by passing through the gelatin Sepharose-4B column. The skin tissue was further extracted by 2.5% Triton X100. As shown previously, the pro-MMP-9-converting enzyme activity is mostly retained in the Triton-insoluble fraction of skin tissue.²³ To assay the converting activity, we incubated 80 μ L of the precleaned conditioned medium or the suspension of tissue fractions with 15 μ L of purified pro-MMP-9 together with 5 μ L 100 mmol/L CaCl₂. After 16 hours of incubation at 37°C, the incubated samples were resolved by gelatinolytic zymography to measure relative amounts of active and pro-MMP-9.

Partial purification of pro-MMP-9--converting enzyme(s)

After the skin tissue was cultured in DMEM for 2 to 3 days, we found the pro-MMP-9 activator was increased. The cultivated skin was homogenized on ice and extracted by 2.5% Triton X100 for 24 hours at 4°C. The insoluble tissue fraction was extracted subsequently by 6 mol/L urea for another 24 hours. The resulting insoluble tissue fraction was then washed with Sodium-Tris (NT) buffer containing 100 mmol/L NaCl and 50 mmol/L TRIS, pH 7.5. Finally, the pro-MMP-9--converting activity was extracted by 2 mol/L NaCl. This final soluble fraction was subjected to dialysis against NT buffer. The protein concentration in the final extract was measured by bicinchoninic acid (Sigma), and the converting activity was measured as described in the previous section. For the inhibition experiments, 2.5 μ L chymostatin or the indicated concentrations of other inhibitors were added to the system containing 80 μ L extract and 15 μ L of purified pro-MMP-9 and 2.5 μ L of 200 mmol/L CaCl₂. After incubation was completed, the resulting mixture was resolved by zymography to measure relative amounts of active and pro-MMP-9.

Gelatinolytic zymography

The conditioned medium or other experimental reaction products were mixed with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer in the absence of a reducing agent. The protein was resolved by electrophoresis in 10% polyacrylamide gel containing 0.1% (w/v) gelatin at 4°C with 120 V for 16 hours. After electrophoresis, the SDS in the gel was removed by incubation with 2.5% Triton X-100 for 30 minutes and then washed with water. The gelatinolytic activity was developed in a buffer containing 5 mmol/L CaCl₂, 150 mmol/L NaCl, and 50 mmol/L TRIS, pH 7.5, for 16 hours at 37°C. The gelatinolytic activity was then visualized by staining with Coomassie blue R-250 and recorded by digital camera. The experiment was repeated 3 times.

Western blot analyses

The conditioned medium and skin extracts were resolved by a reducing SDS-PAGE (15%). The protein was transferred to immobolin-P (Millipore). The protein blot was blocked by 5% nonfat milk, followed by exposure to the primary antibodies. The bound first antibodies were probed by peroxidase-conjugated secondary antibodies, which were subsequently detected by enhanced chemiluminescence.

RESULTS

IL-1 α induces activation of pro-MMP-9 in organ-cultured human skin

To simulate the inflammatory state, we stimulated normal, full-thickness human skin with IL-1 α and other cytokines such as tumor growth factor- β (TGF- β), IL-6, IL-8, and platelet-derived growth factor (PDGF) individually or in combination (1 ng/mL concentration of TGF- β and 10 ng/mL concentration of the other cytokines; $n = 4$). After 4 days of culture, the conditioned medium was resolved by zymography (Fig 1). Seventy-two kilodaltons of pro-MMP-2 was expressed constitutively to a similar extent by all the tissues regardless of exogenous cytokines and, thus, provided an internal control. In contrast, very little 92-kDa pro-MMP-9 was expressed without stimulation but was induced by TGF- β . The other cytokines tested (such as IL-6 and IL-8) were without effect. IL-1 α alone promoted the conversion of 92-kDa pro-MMP-9 into the active 82-kDa form. As was found during stimulation by TNF- α , the addition of TGF- β to IL-1 α further augmented the formation of 82-kDa MMP-9.

We then measured the timing of induction and activation of pro-MMP-9. Normal human skin was cultured in DMEM with or without IL-1 α at 2 concentrations (0.1 and 1 ng/mL; $n = 3$). This range of concentration of IL-1 α was chosen as it falls within those observed in patients having inflammation associated with chronic wounds or osteoarthritis, in which MMP-9 expression is a marker.^{25,26} Conditioned medium was sampled at 24, 48, and 72 hours for zymographic analysis. Without exogenous IL-1 α , 92-kDa pro-MMP-9 was present without active 82-kDa MMP-9 (Fig 2). We believe that this result may reflect the effect of endogenous cytokines produced by the skin tissue in response to the culturing process. With the addition of IL-1 α at 0.1 ng/mL, pro-MMP-9 was markedly induced and detected readily at 48 hours. An additional 24 hours (3 days total) was required for the occurrence of the “activated” 82-kDa MMP-9. At the higher dose of IL-1 α (1 ng/mL), more active MMP-9 was generated. This time-course experiment also suggests the character of the potential pro-MMP-9 activator. The 24-hour lag between induction of pro-MMP-9 and its conversion to the active form suggests IL-1 either slowly induces an activator or promotes down-regulation of an inhibitor. In contrast, induction of the zymogen was found immediately in response to IL-1 α stimulation.

IL-1 α induces pro-MMP-9 but does not stimulate its conversion to the active form by dermal fibroblasts and keratinocytes

Initially, we attempted to determine whether IL-1 α --stimulated expression and proteolytic activation of pro-MMP-9 observed in human skin could be carried out by skin-derived dermal fibroblasts or epidermal keratinocytes alone. Primary human epidermal keratinocytes ($n = 4$) were stimulated by IL-1 α (10 ng/mL), TNF- α (10 ng/mL), and TGF- β (1 ng/mL) individually or in combination. The range of concentrations chosen in the experiment was determined by our previous work; concentrations at which the saturation effect was observed were chosen. As shown in Figure 3, a very small amount of 92-kDa active MMP-9 was observed in the basal state; IL-1 α enhanced pro-MMP-9 expression (Fig 3, A). IL-1 α , in contrast to TNF α or TGF- β , was a less-potent inducer of the zymogen by keratinocytes. This finding may reflect the relative lesser abundance of cytokine receptors on the keratinocytes. The combination of IL-1 α with TGF- β had an additive effect on the induction of pro-MMP-9 although no active 82-kDa MMP-9 was produced.

We then tested dermal fibroblasts ($n = 4$). To simulate the dermal environment, we embedded primary dermal fibroblasts in type I collagen lattices. The lattices were stimulated with IL-1 α , TGF- β , TNF- α , PDGF, IL-6, and IL-8 alone or in combination. In contrast to keratinocytes, fibroblasts produced a completely different array of gelatinase activities (Fig 3, B). The 72- and 62-kDa MMP-2, which were absent in the keratinocytes, were expressed abundantly by the dermal fibroblasts. IL-1 α alone or in combination with TGF- β had little effect on pro-MMP-9 induction, while TNF- α in combination with TGF- β promoted the induction of pro-MMP-9. These results indicate that the factor(s) responsible for the activation of pro-MMP-9 by human skin are absent in these pure cell cultures and may originate from other resident skin cells. Alternatively, these cells may generate specific inhibitors. To further investigate the possibility of cell interactions as a cause for pro-MMP-9 activation, we cocultured keratinocytes and dermal fibroblasts, and stimulated them with these same cytokines. Similarly, we observed only the induction of pro-MMP-9 but not its proteolytic activation (data not shown).

Skin tissue associated pro-MMP-9--converting enzyme

We reasoned that the activation of pro-MMP-9 by IL-1 α in human skin could be achieved by the induction of a specific converting enzyme, which then cleaves the prodomain. To address this question, we stimulated skin with IL-1 α at various concentrations for 48 hours ($n = 3$), followed by analysis of the converting activities in both the conditioned medium and the skin tissue (Fig 4, A). After culture, the skin tissues were washed by NT buffer containing 100 mmol/L NaCl in 50 mmol/L TRIS, pH 7.5, to remove the secreted factors. The tissue was further extracted by 2% Triton X100. On the basis of our previous experience, the pro-MMP-9--converting enzyme activity was retained in the Triton-insoluble fractions. To assay for the converting activities, we incubated the skin tissue and conditioned medium with purified pro-MMP-9 as the substrate. As shown in Figure 4, B, pro-MMP-9 was converted to its active form by the skin tissue but not by the conditioned medium. Importantly, human skin with or without previous cytokine treatment was equally competent to activate the pro-MMP-9, indicating that the activator is expressed constitutively. In addition, these results imply that the unidentified activator is not a secreted factor but rather is tightly associated with the skin tissue itself. The absence of a direct cytokine regulation also suggests that the regulation of IL-1 α --mediated activation may occur via an inhibitor: that is, the cytokine may downregulate an inhibitor, while the activator is expressed persistently in the skin. We have suggested previously that TIMP-1, an inhibitor controlling pro-MMP-9 activation, is downregulated by TNF- α .²⁷

The tissue-associated pro-MMP-9--converting enzyme is a chymotrypsinlike protease

To characterize the unidentified pro-MMP-9 activator, we tested a variety of proteinase inhibitors on the skin-mediated activation of pro-MMP-9. Among the inhibitors for aspartic proteinase, MMP, and serine proteinase, we found that chymostatin, an inhibitor for chymotrypsinlike proteinase, effectively blocked the proteolytic activation. Normal human skin ($n = 3$) was simultaneously stimulated by IL-1 α (10 ng/mL) and TGF- β (1 ng/mL), with or without chymostatin at 0.02 and 0.2 mmol/L concentrations. Conditioned medium was sampled after culture for 48 and 72 hours (Fig 5, A). Without an inhibitor, the pro-MMP-9 was partially converted into 82-kDa active form on day 2 in the organ culture, while the conversion was totally completed after culture for 72 hours. Chymostatin at 0.02 mmol/L showed initial inhibition as evidenced by accumulation of 92-kDa pro-MMP-9. At 0.2 mmol/L, chymostatin completely attenuated the conversion of pro-MMP-9. Inhibition of the activation of pro-MMP-9 by chymostatin is not likely to be due to a direct cellular toxic effect because the induction of pro-MMP-9 was found even in the presence of the inhibitor. Further, the conversion of 72-kDa pro-MMP-2 to the active 62-kDa form was normal and not affected by the chymostatin.

To prove the direct inhibition by chymostatin rather than a metabolic conversion of the inhibitor in the organ culture, we performed the same test in cell-free skin tissues ($n = 3$). As shown in Figure 5, B, chymostatin at 10 μ mol/L totally inhibited the skin extract-mediated activation of pro-MMP-9. This concentration is within the typical inhibitory range of the chymotrypsinlike proteinases (Roche). This experiment indicates the as yet unidentified pro-MMP-9 activator is likely a tissue-associated, chymotrypsinlike proteinase.

IL-1 α downregulates TIMP-1

The above experiments showed that IL-1 α could induce activation of pro-MMP-9 in organ cultures of human skin. However, as shown in Figure 4, the skin-associated activator is present persistently and is not directly regulated by IL-1 α . Taken together, these results suggest that this cytokine may directly regulate an inhibitor, which in turn determines the conversion of pro-MMP-9, rather than induce the activator per se. Others have found that TIMP-1 can associate physically with pro-MMP-9.²⁸ We hypothesized that IL-1 α may downregulate TIMP-1, which releases pro-MMP-9 from the inhibitor for access to the tissue-associated activator. Therefore, we assayed to determine if IL-1 α could downregulate TIMP-1 in a skin organ culture. Normal human skin ($n = 3$) was stimulated with IL-1 α at an increased concentration for 64 hours, as shown in Figure 2. The conditioned medium and skin extracts were subjected to Western blot analysis with monoclonal antibody for TIMP-1 (Fig 6). TIMP-1 was expressed constitutively in the basal state (in the absence of IL-1) and was secreted primarily into the medium rather than bound to the skin tissue. As hypothesized, IL-1 α indeed downregulated expression of TIMP-1 in a dose-dependent manner. Importantly, the concentration at which IL-1 α decreased TIMP-1 levels correlated well with the cytokine-induced activation of pro-MMP-9 (Fig 2). Thus, the conversion of pro-MMP-9 induced by IL-1 α was tightly associated with downregulation of TIMP-1. In fact, in a previous report, we demonstrated the inhibition of pro-MMP-9 activation by TIMP-1.²⁷ On the other hand, TIMP-2 is not expressed in the organ cultured skin but is expressed constitutively by dermal fibroblasts and keratinocytes (data not shown).

DISCUSSION

In this study, we found that IL-1 α induced the conversion of the 92-kDa pro-MMP-9 into its active 82-kDa form in cultured human skin. Further, this proteolytic activation was sensitive to chymostatin, indicating the activator is a tissue-associated, chymotrypsinlike proteinase. However, the cytokine (IL-1 α) apparently does not induce directly the activator in the human

skin. Rather, the IL-1 α -induced conversion of pro-MMP-9 occurred through the downregulation of an inhibitor, likely TIMP-1. These findings imply a direct link of a specific cytokine (IL-1 α) to MMP-9 activation in the pathogenesis of inflammatory conditions such as cancer metastases and chronic wounds.

Although the proteolytic activation of pro-MMP-9 has been observed extensively in such diverse diseases as chronic wounds, liver failure, cancer metastasis, and arthritis, the cellular factors controlling the conversion have not been elucidated previously. Because there is a close association between inflammation and MMP-9 activation in these conditions, we postulated that cytokines may have a prominent role in regulation of this process. After screening a panel of cytokines, including proinflammatory cytokines and growth factors, we previously identified TNF- α to be a potent stimulator for MMP-9 activation in the human skin.²³ On the basis of the similar biologic functions and intracellular signaling pathways controlled by TNF- α and IL-1 α , we postulated the latter would also promote MMP-9 activation, which, in fact, appears to be true. Moreover, IL-1 α , like TNF- α , does not induce directly the putative pro-MMP-9 activator. We believe that the activator is expressed constitutively or may be secreted simply in response to physical trauma. At the present time, we have no specific candidate for this unidentified activator. Determining its identity will be the subject of our future work through extraction and chromatography, including inhibitor-conjugated affinity purification. Conclusive identification of the *in vivo* activator must come from its direct isolation from human tissue.

We propose that the activator is a tissue-associated, chymotrypsinlike proteinase on the basis of the following evidence: (1) The conversion of the 92-kDa form into the active 82-kDa form mediated by skin tissue is inhibited by chymostatin, a specific inhibitor for chymotrypsin. In support of this conclusion, we found that 1-chloro-3-tosylamido-4-phenyl-2-butanone (TPCK), another inhibitor for chymotrypsin, can also inhibit this conversion.²⁷ (2) The activator failed to be extracted by nonionized Triton X100 and urea but could be extracted by NaCl. This suggested the activator is likely bound to tissue through ionic bonds. This extraction property also gives us a method to isolate and identify the activator from human skin.

We speculated initially that the conversion of pro-MMP-9 by IL-1 α /TNF α was through activator induction. Our results, however, suggest that the activator is present constantly in the skin and not regulated by TNF- α /IL-1 α . Instead, soluble factors secreted from skin prevent activation. As shown in Figure 1, TGF- β alone induced pro-MMP-9 but did not generate the 82-kDa activated form. Only after these soluble factors were removed, could the skin convert pro-MMP-9. Taken together, these findings indicate that activation of pro-MMP-9 by IL-1 α is likely through downregulation of an inhibitor. In this report, we propose TIMP-1 is the likely inhibitor. This conclusion is based on 3 facts: (1) TIMP-1 binds pro-MMP-9 at its C-terminus.²⁹ (2) The activation of pro-MMP-9 by IL-1 α correlates with downregulation of TIMP-1. (3) We found previously that recombinant TIMP-1 can inhibit directly the skin-mediated conversion of pro-MMP-9.²⁷

There is other supporting evidence for the role of TIMP-1 as the regulator of MMP-9 in human diseases. TIMP-1 is upregulated in acute wounds, a condition in which most MMP-9 is retained in the 92-kDa form.³⁰ In contrast, TIMP-1 was absent in chronic wounds, where the 82-kDa active MMP-9 is present.³¹ This absence of TIMP-1 when activated MMP-9 is increased provides further support for our hypothesis that the downregulation of TIMP-1 is essential for MMP-9 activation. This observation also suggests that treatment of chronic and nonhealing wounds with TIMP-1 or its analogue might facilitate wound healing. Obviously, much work will be necessary to support this concept.

CONCLUSION

IL-1 facilitates the activation of MMP-9 in human tissue. The mechanism appears to be through the decrease of the endogenous inhibitor TIMP-1. Blockage of IL-1 or direct inhibition of the pro-MMP-9 activator by chymostatin might be useful interventions in conditions in which excess MMP-9 is thought to have pathologic consequences.

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Fig 1.

IL-1 α -induced proteolytic activation of pro-MMP-9 by human skin. The full-thickness skin was cultured in DMEM with or without TGF- β (1 ng/mL) or IL-1 α (10 ng/mL), IL-6 (10 ng/mL), IL-8 (10 ng/mL), and PDGF (10 ng/mL) as well as their combination (*left panel*). After 4 days of culture, the gelatinase activities in the conditioned medium were resolved by zymography, as described in Methods. The identities of MMP-9 and MMP-2 were confirmed previously by Western blot analyses. The active and pro-MMP-2/MMP-9 are indicated by arrows (*right panel*). *IL*, Interleukin; *PDGF*, platelet-derived growth factor; *TGF*, tumor growth factor; *MMP*, matrix metalloproteinase.

Fig 2.

Concentration dependence and time course of IL-1 α -induced activation of pro-MMP-9. Full-thickness skin was cultured in DMEM with IL-1 α at 0.1 ng/mL (*center panel*) and 1 ng/mL (*right panel*). (Control, *left panel*.) At the indicated intervals, the conditioned medium was sampled for zymographic analysis. Considerable activation is not seen until 72 hours. The active and pro-MMP-2/MMP-9 are indicated by *arrows* (*right panel*). *IL*, Interleukin.

Fig 3.

Cytokine-mediated induction of pro-MMP-9 without activation by epidermal keratinocytes (A) and dermal fibroblasts (B). Human dermal fibroblasts were embedded in type I collagen as attached lattices. Primary epidermal keratinocytes were isolated from normal human skin, and the early passages were used in the experiment. The lattices or cells were stimulated by the cytokines individually or in combination in DMEM. After culturing for 68 hours, the conditioned medium was subjected to zymographic analysis. No activation of MMP-9 was noted. *TNF*, Tumor necrosis factor; *IL*, interleukin; *TGF*, tumor growth factor; *PDGF*, platelet-derived growth factor; *MMP*, matrix metalloproteinase.

Fig 4.

Proteolytic activation of pro-MMP-9 is mediated by a skin-associated factor. **A**, Normal human skin was primed with IL-1 α at the indicated concentrations for 48 hours. Then skin tissue and conditioned medium were separated. The skin tissue was extracted by 2.5% Triton X100. **B**, To assay the activation, we incubated purified pro-MMP-9 with either conditioned medium or skin tissue for 16 hours. The resulting reaction was resolved by zymography.

Fig 5.

Chymostatin inhibits IL-1 α -mediated activation of pro-MMP-9. Normal human skin was cultured in DMEM with IL-1 α and TGF- β to maximally promote pro-MMP-9 expression and activation. **A**, Chymostatin was added at the indicated concentration. Conditioned medium from 48 and 72 hours were resolved for gelatinolytic zymography. Note decreased MMP-9 activation. **B**, Chymostatin also inhibited directly pro-MMP-9 activation by cell-free tissue fractions (B). Note a lack of 82-kDa MMP-9 after chymostatin treatment.

Fig 6. IL-1 α downregulates TIMP-1. Normal human skin was cultured in DMEM with IL-1 α at the indicated concentrations for 64 hours. The protein concentration in the conditioned medium (*left panel*) and skin extracts (*right panel*) was normalized and then resolved by SDS-PAGE. TIMP-1 was measured by Western blot analysis.