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Generation of Biologically Active IL-1β by Matrix Metalloproteinases: A Novel Caspase-1-Independent Pathway of IL-1β Processing[1]

Uwe Schönbeck, François Mach, and Peter Libby[2]

Biologic activity of IL-1β requires processing of the inactive precursor, a function generally ascribed to IL-1β-converting enzyme (caspase-1). However, alternative mechanisms of IL-1β activation have been postulated in local inflammatory reactions. Expression of IL-1β and matrix metalloproteinases (MMPs) frequently occurs simultaneously at sites of inflammation. We describe here that stromelysin-1 (MMP-3), as well as the gelatinases A (MMP-2) and B (MMP-9), processes recombinant human IL-1β precursor (pIL-1β) into biologically active forms. Detection of both pIL-1β processing and biologic IL-1β activity demonstrated different processing capacities of the respective MMPs. Conversion of pIL-1β by stromelysin-1 required coincubation for at least 1 h, and biologic activity faded after 8 h to 24 h. Gelatinase A was less effective in processing pIL-1β, requiring at least 24 h of coincubation. In contrast, gelatinase B processed pIL-1β within minutes, resulting in immunoreactive products as well as biologic activity stable for 72 h. In addition, prolonged incubation of mature IL-1β with stromelysin-1, and to a lesser extent also with gelatinases, but not with interstitial collagenase, resulted in the degradation of mature IL-1β. None of the MMPs processed the second isoform of IL-1, IL-1α. The present study indicates a biphasic regulation of IL-1β activity by MMPs: a caspase-1-independent pathway of IL-1β activation and inhibition of IL-1β activity by degrading the mature cytokine. The balance of the respective MMPs and pIL-1β might regulate the long term appearance of IL-1β activity at sites of acute or chronic inflammation. ***The Journal of Immunology,*** **1998, 161: 3340–3346.**

Interleukin 1β, a key component of the cytokine network, possesses diverse biologic effects contributing to acute and chronic inflammation (1). As originally described for activated monocytes (2, 3), IL-1β is synthesized as a cell-associated 33-kDa precursor protein. In the precursor form, IL-1β essentially lacks biologic effects (3–6). In contrast to IL-1α, biologic activity of IL-1β requires processing into the mature 18-kDa protein (3, 6, 7). This processing involves proteolytic removal of the N-terminal portion of the precursor protein (8–10) and is associated with IL-1β-converting enzyme (ICE; or caspase-1), an intracellular cysteine protease that cleaves the precursor at position Asp^{116}-Ala^{117} (11–14). The function of caspase-1 in the release of mature IL-1β (mIL-1β)[3] is well established, and several in vitro as well as in vivo studies have shown that cells that do not express caspase-1, such as fibroblasts and keratinocytes (15–17), as well as caspase-1-deficient mice (18, 19), lack the capacity to release mIL-1β. However, recent in vivo studies of local inflammation in caspase-1-deficient mice as well as in human keratinocytes suggest caspase-1-independent mechanisms of IL-1β activation (20, 21). Aside from caspase-1, other proteases capable of cleaving the IL-1β precursor (pIL-1β) include bacterial enzymes (22), trypsin, or chymotrypsin (5, 6, 23), as well as the physiologically more relevant enzymes, leukocyte elastase (6) and granzyme A (24). Except for granzyme A, all of these proteases cleave the pIL-1β N terminal of the caspase-1 cleavage site, resulting in fragments of >18 kDa.

IL-1β is an autoregulated protein, inducing its own expression (25, 26). Despite a decade of study on the mechanism(s) of activation and catabolism of the IL-1β protein, surprisingly little is known about the fate of active IL-1β. However, regulation of the local level of this potent and pleiotropic mediator (1, 27, 28) would seem crucial to avoid a self-perpetrating positive feedback loop of this autoinducible mediator.

Overexpression of IL-1β occurs in various inflammatory diseases, including rheumatoid arthritis and atherosclerosis (1), conditions that also involve induction of matrix metalloproteinases (MMPs) (29–33). Different members of the MMP family contribute to the matrix degradation, including collagenases (such as interstitial collagenase, MMP-1), gelatinases (such as gelatinase A (MMP-2) and B (MMP-9)), or stromelysins (such as stromelysin-1, MMP-3) (34, 35). Cells associated with the diseases mentioned above, such as macrophages and vascular endothelial and smooth muscle cells, overexpress these MMPs at the sites of inflammation (30, 36, 37). Indeed, cytokines such as IL-1β regulate the expression and activation of MMPs, a scenario that sets the stage for cross-regulation among these two classes of effectors of inflammation.

Since active MMPs as well as active IL-1β colocalize at sites of inflammation and since the activation of IL-1β does not necessarily depend on the presence of caspase-1, the current study tested the hypothesis that MMPs can modulate IL-1β activity. We report

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[3] Abbreviations used in this paper: mIL-1β, recombinant mature human IL-1β (18 kDa); MMP, matrix metalloproteinase; pIL-1β, recombinant human IL-1β precursor (33 kDa); APMA, *p*-aminophenylmercuric acetate; TIMP-1, tissue inhibitor of matrix metalloproteinases 1.

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here that the matrix metalloproteinases stromelysin-1, gelatinase A, and gelatinase B, but not interstitial collagenase, convert the inactive pIL-1β into biologically active forms. Upon prolonged exposure, MMP-3 further degrades IL-1β, yielding loss of biologic activity. These results suggest a novel role for these enzymes in the regulation of IL-1β activity, e.g., at sites of acute or chronic inflammation.

Materials and Methods

Reagents

Recombinant human MMP-2 and MMP-9 as well as the tissue inhibitor of MMP-1 (TIMP-1) were purchased from Calbiochem (San Diego, CA). The gelatinases were obtained as *p*-aminophenylmercuric acetate (APMA)-activated, truncated enzymes (Calbiochem cat. No. PF023 and PF024). MMP-1 and MMP-3 were obtained as zymogens from Merck Research Laboratories (Rahway, NJ) and were activated by incubation with APMA (10 μM) for 3 h at 37°C. The APMA treatment presumably produces active MMP-1 by cleavage of the Val^{82}-Leu^{83} (38) and active MMP-3 by cleavage of the His^{82}-Phe^{83} bond (39, 40). APMA was removed by G-25 column chromatography. Activation of the enzymes was confirmed by Western blotting as well as in hydrolysis assays employing the fluorogenic substrate MocAc-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH_2 for MMP-1; NFF-2 (MocAc-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH_2) for MMP-2/-9; and NFF-3 (MocAc-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2) (all from Peptide Institute, Osaka, Japan) for MMP-3. The substrate (1 μM) and the respective rMMP (200 ng) were incubated for 3 h at 37°C and fluorescence intensity determined in a fluorescence spectrometer (Perkin-Elmer, Norwalk, CT). All MMPs employed in the present study were activated via APMA treatment and share similar levels of enzymatic activity as determined by cleavage of the respective fluorogenic substrate. Polyclonal rabbit anti-IL-1β and anti-IL-1α Ab were obtained from Upstate Biotechnology (Lake Placid, NY). Recombinant human caspase-1 was prepared as described previously (41). The secondary goat anti-rabbit Ab was purchased from Jackson Immunoresearch (West Grove, PA). The nucleotide [^{3}H]thymidine was obtained from Dupont-NEN (Boston, MA).

Processing assay

To study processing, recombinant human pIL-1β or mIL-1 (both at 50 ng) was incubated with the indicated concentrations of the respective rMMP for the indicated times. Specificity of pIL-1β processing by MMPs was analyzed in processing assays performed in the absence or presence of TIMP-1 (1 μg/ml). All processing assays were performed in a final volume of 50 μl. For Western blot analysis, processing was stopped by heating the samples (10 min, 95°C) in 10 μl SDS-PAGE (5×) sample buffer (0.2 M Tris, 5% glycerol, 0.1% SDS, 3% β-mercaptoethanol, 0.1 mg/ml bromophenol blue, final concentrations). For detection of IL-1β activity, the processing assay was stopped by adding excess rTIMP-1 (1 μg/ml) and freezing the samples. The presence of TIMP-1 did not affect the assay (see also Fig. 1).

Western blot analysis

The preparations of the processing assays were separated by standard SDS-PAGE under reducing conditions and transferred to polyvinylidene fluoride (PVDF) membranes using a semidry blotting apparatus (3.0 mA/cm^2, 30 min; Bio-Rad, Hercules, CA). Blots were blocked for 2 h, and first and second Abs were diluted in 5% defatted dry milk/PBS/0.1% Tween 20. After 1 h of incubation with the respective primary Ab (1:1000), blots were washed four times for 15 min in PBS/0.1% Tween 20, and the secondary peroxidase-conjugated goat anti-rabbit Ab (1:10,000) was added for another hour. Finally, after four washings (20 min, PBS/0.1% Tween 20), detection of the Ag was conducted using the enhanced chemiluminescent detection method according to the manufacturer's recommendations (Dupont-NEN), and subsequent exposure of the membranes to x-ray film.

IL-1β activity assay

The IL-1-dependent murine thymocyte cell line D10.G4.1 (kindly provided by Dr. Andrew Lichtman, Brigham and Women's Hospital) was cultured in RPMI 1640 (BioWhittaker, Walkersville, MD) supplemented with 1% L-glutamine, 1% penicillin/streptomycin, 20 μM 2-ME, 25 mM HEPES, and 10% FBS. For detection of IL-1β activity, D10.G4.1 cells were washed twice (400 × *g*, 10 min, 4°C), and 50-μl aliquots of the cell suspension (100,000 cells/ml) were plated in 96-well flat-bottom microtiter plates (Falcon, Lincoln Park, NJ). For the assay, 50 μl of the samples were added in the absence or presence of the neutralizing IL-1β Ab (1 μg/ml; Endogen, Woburn, MA) to triplicate cultures for 72 h. Finally, cells were exposed to tritiated thymidine ([^{3}H]thymidine, 5 μCi/well) during the final 24 h, then harvested on glass fiber filter strips (Cambridge Technology, Watertown, MA), and [^{3}H]thymidine incorporation (dpm per culture ± SD) was analyzed in a liquid scintillation counter (Beckman, Fullerton, CA). The mean of triplicate cultures was determined. For quantification of IL-1β activity, [^{3}H]thymidine incorporation was calibrated to a standard of recombinant human mIL-1β (10 fg/ml–10 ng/ml) added to D10.G4.1 indicator cells.

Results

Processing of the recombinant pIL-1β by human stromelysin-1, gelatinase A, and gelatinase B, but not interstitial collagenase

To explore the capability of matrix metalloproteinases to process the inactive 33-kDa pIL-1β, representatives of three classes of MMPs, interstitial collagenase (MMP-1), stromelysin-1 (MMP-3), and the gelatinases A (MMP-2) and B (MMP-9), were incubated with pIL-1β, and processing was followed by Western blot analysis. Active stromelysin as well as the gelatinases A and B, but not interstitial collagenase, processed pIL-1β (Fig. 1). However, preincubation of stromelysin-1, gelatinase A, and gelatinase B with TIMP-1, a specific inhibitor of matrix metalloproteinases, before coincubation with the cytokine, blocked pIL-1β processing. The specificity of the TIMP-1-mediated inhibition was demonstrated in processing assays employing chymotrypsin or caspase-1. TIMP-1 inhibited neither chymotrypsin- nor caspase-1-mediated cleavage of pIL-1β (Fig. 1).

Processing of the inactive pIL-1β into biologically active IL-1β by stromelysin-1 depended on concentration and time

Processing of the inactive pIL-1β does not necessarily produce the biologically active cytokine, a property of only certain C-terminal fragments. The Ab used here for Western blot analysis was raised against the mature form of IL-1β, indicating generation of C-terminal fragments by stromelysin-1, gelatinase A, and gelatinase B. To determine whether these fragments possess biologic activity, pIL-1β was incubated with various concentrations of the respective MMP for up to 72 h, and the preparations were analyzed by immunoblotting as well as by an IL-1 activity assay. Incubation of pIL-1β with interstitial collagenase did not produce detectable fragments or IL-1β activity even after 72 h (Fig. 2, *A* and *C*) and use of up to 10 μg/ml of the enzyme (Fig. 3, *A* and *C*). The pIL-1β itself showed no autodegradation for the times tested. Processing of pIL-1β by stromelysin-1 required at least 1 h of incubation, as demonstrated by Western blot analysis (Fig. 2*B*). The processed bands detected had a m.w. of ~28 and 14 kDa. The 28-kDa band was detected before the lower m.w. cleavage product. The precursor band became fainter after 3 h and disappeared after 24 h of coincubation, concurrently with the processed bands. IL-1β activity was detected after 1 h of incubation with stromelysin-1 and declined after ~24 h, in parallel with the 14-kDa band detected by immunoblotting. The inhibitory IL-1β Ab abrogated this biologic activity. In addition to its dependence on the amount of time elapsed, processing of pIL-1β also depended on the concentration of stromelysin-1. Detection of both processed bands in Western blot analysis and IL-1β activity required the incubation of the precursor with at least 1 μg/ml of stromelysin-1 (Fig. 3, *B* and *C*). In contrast, interstitial collagenase did not process pIL-1β in any of the concentrations tested.

Analysis of the processing capacities of gelatinases revealed different potentials for gelatinase A and B to cleave pIL-1β into biologically active forms. Coincubation of pIL-1β with gelatinase A yielded a barely detectable 16-kDa and a faint 10-kDa processed product. In addition, the detection of precursor conversion required a high gelatinase A concentration (10 μg/ml) (Fig. 4*A*) for at least

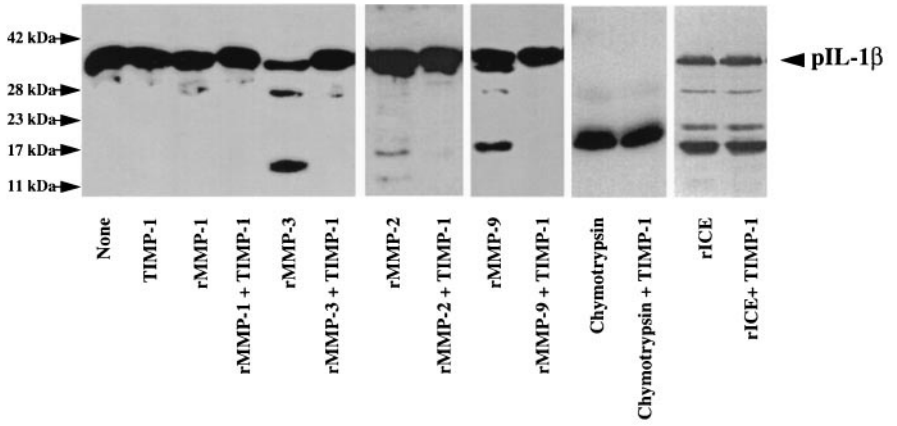

FIGURE 1. Human recombinant stromelysin-1, gelatinase A, and gelatinase B, but not interstitial collagenase, process pIL-1β. Human recombinant pIL-1β (1 μg/ml, final concentration) was incubated with recombinant interstitial collagenase (rMMP-1; 3 h), stromelysin (rMMP-3; 3 h), gelatinase A (rMMP-2; 24 h), or gelatinase B (rMMP-9; 3 h) (all at 1 μg/ml final concentration) in the absence or presence of TIMP-1 (1 μg/ml, final concentration). As a control, chymotrypsin (2 h) or recombinant human caspase-1 (30 min) (both at 1 μg/ml final concentration) was coincubated with pIL-1β in the presence and absence of TIMP-1 (1 μg/ml, final concentration). Aliquots of these preparations were applied to SDS-PAGE and subsequently immunoblotted with anti-IL-1β Ab. Incubation of pIL-1β with TIMP-1 alone did not affect the appearance of the cytokine. The position of the m.w. markers is indicated (kDa). Similar data were obtained in four independent experiments.

24 h to 48 h of coincubation (Fig. 5*A*). In contrast, pIL-1β processing by gelatinase B required an ~1000-fold lower concentration of the enzyme (0.1 μg/ml MMP-9; Fig. 4*B*) and occurred within minutes (Fig. 5*B*). Gelatinase B converted the precursor into a prominent 17-kDa form and a minor 26-kDa band. Studies in which equal amounts of rMMP-9 (1 μg/ml) were added every

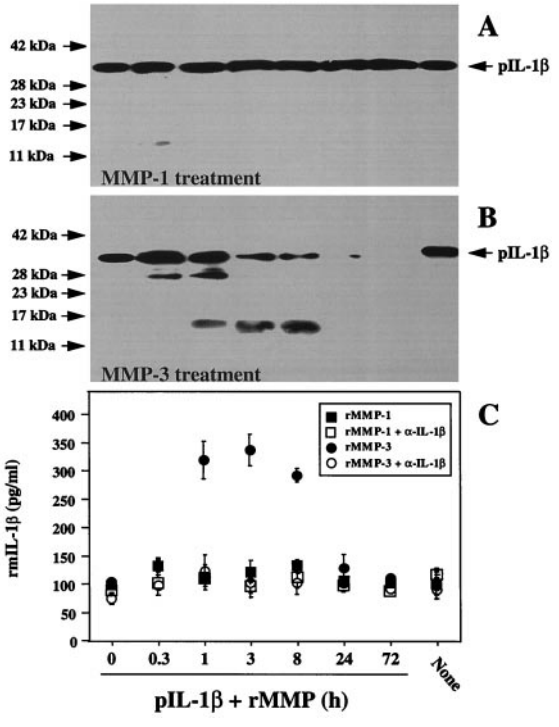

FIGURE 2. Stromelysin-1 time dependently converts pIL-1β into its biologically active form. Human recombinant pIL-1β (1 μg/ml, final concentration) was incubated for the indicated times with recombinant interstitial collagenase (rMMP-1) or stromelysin-1 (rMMP-3) (both at 1 μg/ml, final concentration). Aliquots of these preparations were applied to SDS-PAGE and subsequent immunoblotting with the anti-IL-1β Ab (*A* and *B*) or were added in the presence or absence of the blocking IL-1β Ab (α-IL-1β, 1 μg/ml, final concentration) to cultures of D10.G4.1 indicator cells (*C*). [^{3}H]Thymidine incorporation was measured and biologic IL-1β activity determined as described in *Materials and Methods*. pIL-1β alone (None) was added for control purposes. The position of the m.w. markers is indicated (kDa). Similar data were obtained in three independent experiments.

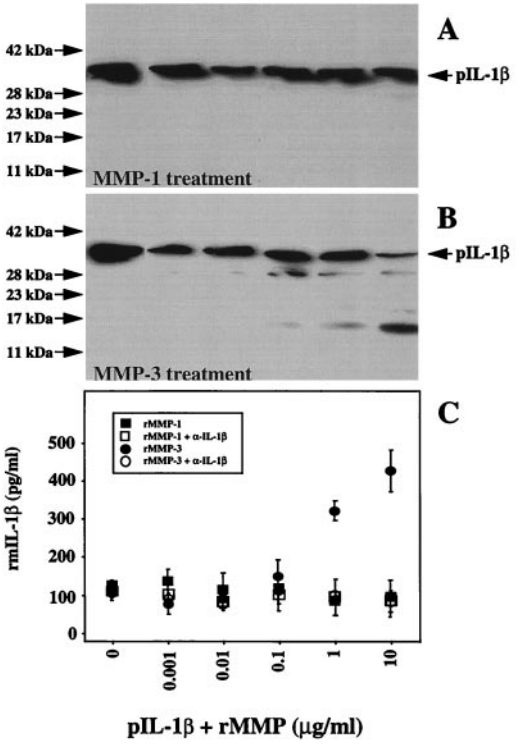

FIGURE 3. Stromelysin-1 concentration-dependently activates pIL-1β. Human recombinant pIL-1β (pIL-1β, 1 μg/ml, final concentration) was incubated for 3 h with the indicated concentrations of recombinant interstitial collagenase (rMMP-1) or stromelysin-1 (rMMP-3). Aliquots of these preparations were applied to SDS-PAGE and subsequently immunoblotted with anti-IL-1β Ab (*A* and *B*) or added in the presence or absence of the IL-1β Ab (α-IL-1β, 1 μg/ml, final concentration) to cultures of D10.G4.1 indicator cells (*C*). [^{3}H]Thymidine incorporation was measured and biologic IL-1β activity determined as described in *Materials and Methods*. The position of the m.w. markers is indicated (kDa). Similar data were obtained in three independent experiments.

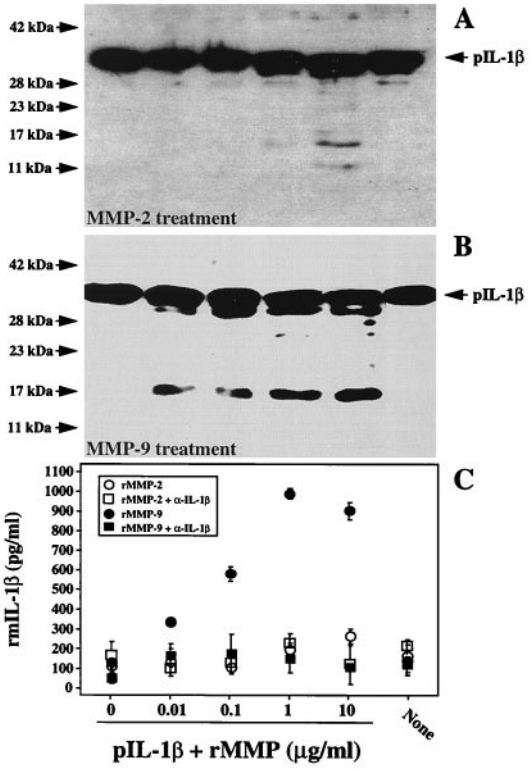

FIGURE 4. Gelatinases A and B concentration-dependently convert pIL-1β. Human pIL-1β (1 μg/ml, final concentration) was incubated 3 h with the indicated concentrations of active recombinant gelatinase A (rMMP-2) or gelatinase B (rMMP-9). Aliquots of these preparations were applied to SDS-PAGE and subsequently immunoblotted with anti-IL-1β Ab (*A* and *B*) or added to cultures of D10.G4.1 indicator cells (*C*). [^{3}H]thymidine incorporation was measured and biologic IL-1β activity determined as described in *Materials and Methods*. The position of the m.w. markers are indicated (kDa). Similar data were obtained in three independent experiments.

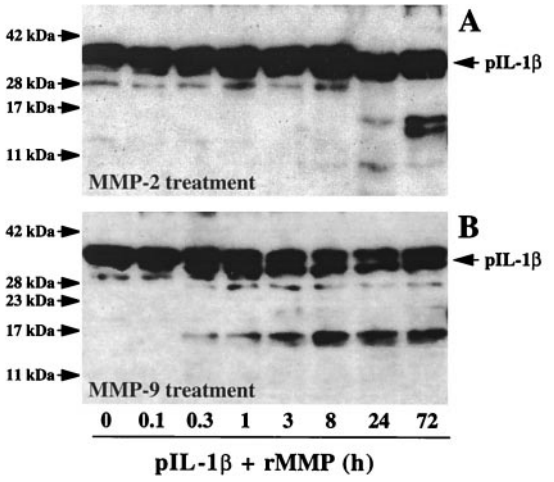

FIGURE 5. Gelatinases A and B time dependently convert pIL-1β. Human recombinant pIL-1β (1 μg/ml, final concentration) was incubated for the indicated times with active recombinant gelatinase A (*A*) or gelatinase B (*B*) (both at 1 μg/ml final concentration), applied to SDS-PAGE, and subsequently immunoblotted with anti-IL-1β Ab. The position of the m.w. markers are indicated (kDa). Similar data were obtained in three independent experiments.

8 h to the processing assay (for a total time of 24 h, data not shown) revealed extended cleavage of pIL-1β into the 17-kDa immunoreactive form, indicating a limited capacity of the rMMP-9 enzyme employed to extend processing activity for more than 8 to 12 h. The concentration dependence of the generation of biologic IL-1β activity by gelatinase A and gelatinase B paralleled that of the protein processing (Fig. 4*C*).

Stromelysin-1 effectively degrades mIL-1β

In view of the disappearance of the precursor, as well as processed IL-1β protein and activity, after several hours of incubation of the precursor with stromelysin-1, we further explored whether MMPs can affect the presence and activity of human mature IL-1β. Incubation of recombinant mIL-1β with stromelysin-1, but not with interstitial collagenase, resulted in degradation of the mature 18-kDa protein in Western blot analysis (Fig. 6, *A* and *B*). Almost no intermediate bands were detected during this degradation. Corresponding to the Western blot analysis, IL-1β activity declined after 1 h of coincubation and disappeared after ~3 h to 8 h of incubation with stromelysin-1. Detection of IL-1β activity after coincubation with interstitial collagenase (Fig. 6, *A* and *C*) resembled the kinetics obtained with mIL-1β alone. Gelatinase A as well as gelatinase B also demonstrated some mIL-1β processing capacity, resulting in ~14, 10, and 5 kDa fragments, as well as a 13-kDa fragment, respectively (Fig. 6, *D* and *E*). However, complete degradation of the cytokine did not occur even when the enzymes were applied for 72 h or were repeatedly applied (every 8 h for 24 h) to the processing assay (data not shown). Neither interstitial collagenase and stromelysin-1 (Fig. 6*C*, *right*) nor gelatinase A and gelatinase B (Fig. 6*F*, *right*) affected the appearance of the second isoform of IL-1, IL-1α.

Discussion

The cytokine IL-1β plays a pivotal role in a variety of acute and chronic inflammatory states, conditions also associated with overexpression of MMPs (30–33). The present study reports the consequences of interaction between the biologically inactive pIL-1β and mIL-1β with different classes of MMPs. In particular, MMP-9, along with IL-1β, a prominent product of stimulated macrophages, activated pIL-1β. Only one of the enzymes tested, interstitial collagenase, did not process the recombinant human pIL-1β. The IL-1β activity obtained with stromelysin-1, gelatinase A, or gelatinase B is presumably mediated by the 14-, 16-, or 17-kDa C-terminal product, respectively, since higher m.w. products, such as the ~28-kDa fragments obtained with stromelysin-1 and gelatinase B, have been shown by others to lack substantial activity (3, 6–10). Aside from granzyme A-mediated processing (24), the products shown in this report are the only reported fragments smaller than the products obtained by caspase-1-mediated conversion. Other proteases such as trypsin, chymotrypsin, cathepsin G, or elastase process the pIL-1β N-terminal of the Asp^{116}-Ala^{117} site (5, 6, 22, 23). Similar to these proteases, the MMP-generated IL-1β products possessed less activity than mature, caspase-derived IL-1β. However, production of active IL-1β by MMPs might occur at sites of local inflammatory reactions. Recent studies by Fantuzzi et al. (20) demonstrated that under pathologic conditions displaying inflammatory aspects, caspase-1-deficient mice can mediate IL-1β-dependent reactions. Furthermore, production of biologically active IL-1β in these mice occurred at the same level as found in wild-type mice. Although the mediator of this caspase-1-independent pIL-1β processing in the inflammatory model was unknown, the authors postulated a conversion in the vicinity of the Asp^{116}-Ala^{117} site and invoked the involvement of leukocyte-derived serin proteases, such as chymase, elastase, or granzyme A.

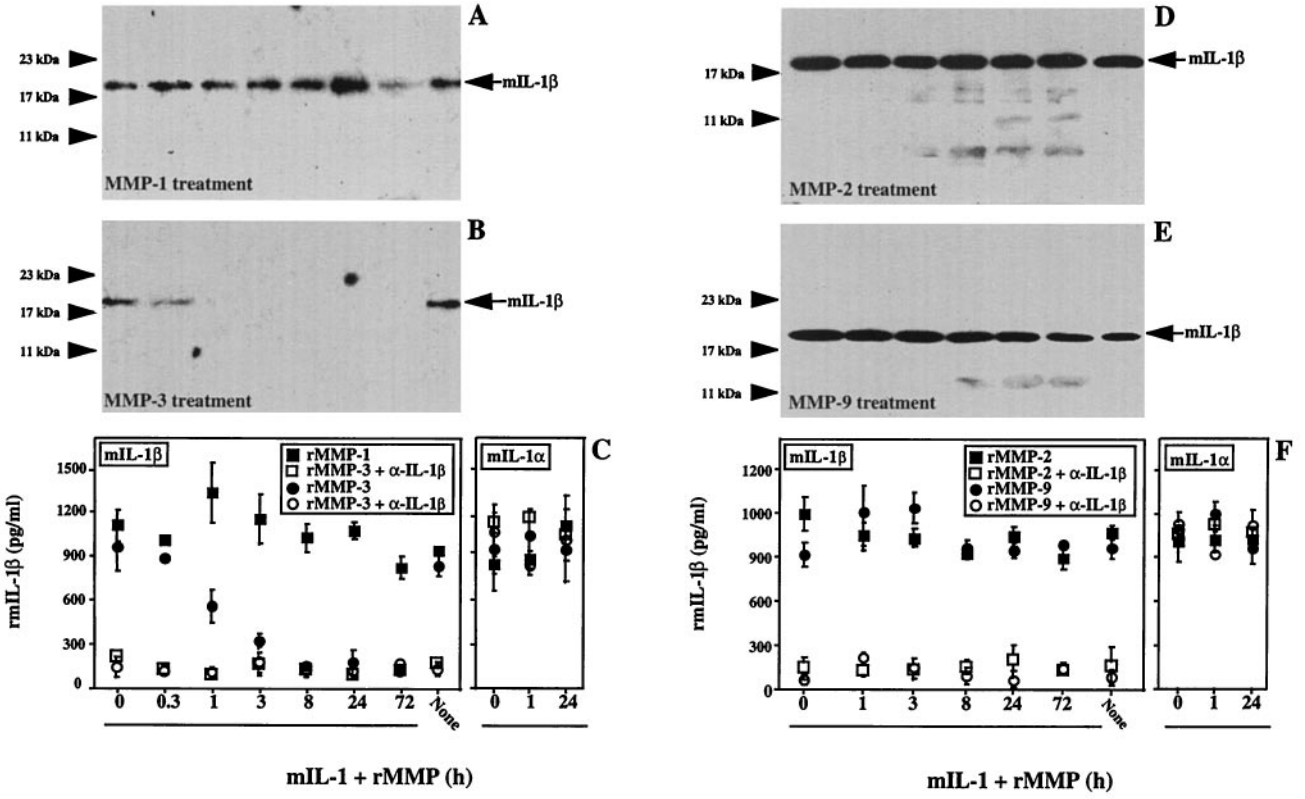

FIGURE 6. Stromelysin time dependently degrades mature, active IL-1β. Human recombinant mIL-1β or mIL-1α (both at 1 μg/ml final concentration) were incubated for the indicated times with recombinant interstitial collagenase (rMMP-1), stromelysin-1 (rMMP-3), gelatinase A (MMP-2), or gelatinase B (MMP-9) (all at 1 μg/ml final concentration). *A*, *B*, *D*, and *E*, Aliquots of these preparations were applied to SDS-PAGE and subsequently immunoblotted with anti-IL-1β Ab. *C* and *F*, Furthermore, aliquots diluted 1:1000 were added in the presence or absence of the blocking IL-1β Ab (α-IL-1β, 1 μg/ml, final concentration) to cultures of D10.G4.1 indicator cells. [^{3}H]Thymidine incorporation was measured and biologic IL-1β activity determined as described in *Materials and Methods*. Recombinant human mIL-1β was added as control (None). The position of the m.w. marker is indicated (kDa). Similar data were obtained in three independent experiments.

The induction of IL-1β activity in a caspase-1-deficient mice was unexpected, since these mice have major defects in the production of mIL-1β, even under endotoxin activated conditions (18, 19). Conceivably, distinct pathologic conditions, probably displaying inflammatory aspects and/or tissue alterations, are required for induction of caspase-1-independent mechanisms of IL-1β processing.

In addition to participating in normal homeostasis and developmental remodeling of connective tissues, MMPs appear to contribute, by their proteolytic activity, to the tissue damage seen in inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, and atherosclerosis, as well as tumor metastasis (29, 30, 42, 43). Overexpression of interstitial collagenase, stromelysin-1, gelatinase A, and gelatinase B, as well as of cytokines such as IL-1β, characterizes sites of inflammation (30, 34, 36, 44–49). Within these diseases, the concentrations of MMPs as well as cytokine correlates with those used in the present study. Between 0.5 and 350 μg/ml MMP-1, MMP-2, MMP-3, and MMP-9 have been detected in the serum or at sites of chronic inflammation such as rheumatoid arthritis or atherosclerosis, describing MMP-3 $>$ MMP-2/-9 $\gg$ MMP-1 as the most prominent MMP (50–55). Furthermore, patients with chronic inflammatory diseases bear 0.1 to 10 ng/ml of IL-1β in serum, but up to the μg/ml range at the inflammatory site (56–58). Cells associated with these diseases include fibroblasts and smooth muscle cells. Both cell types express but do not process pIL-1β, due to the lack of caspase-1 (15, 16) or the expression of a caspase-1 inhibitory activity (41), respectively. Thus, caspase-1-independent activation of pIL-1β via MMPs may be of major importance in these cell types. Keratinocytes of the human plantar stratum corneum process IL-1β by a caspase-1-independent pathway. These caspase-1-deficient cells produce IL-1β activity in the normal epidermis in vivo (21). Keratinocytes, like fibroblasts, can express MMPs (59–61), particularly at sites of inflammation. The presence of pro-IL-1β in the cytoplasm and the predominantly extracellular presence of active forms of MMPs does not render irrelevant the findings reported herein. Sites of chronic inflammation are generally characterized by substantial cell turn-over and apoptotic and necrotic cell death. Such processes likely permit interaction between active MMPs and the pIL-1β. In addition, both activation of MMPs and processing of the pIL-1β can occur within the cytoplasm, indicating their possible intracellular interaction.

In addition to the generation of IL-1β activity, the present study also provides evidence for participation of MMPs in IL-1β degradation. IL-1β has diverse and potent actions, including the ability to induce its own gene expression (25, 26). Thus, the presence of active IL-1β might trigger a positive feedback loop, requiring an inhibitory limb to avoid excessive propagation. IL-1β is one of the classical inducers of MMPs, including interstitial collagenase, stromelysin-1, and the gelatinases A and B, in various cell types (46, 62, 63). The presence of active IL-1β might thus promote the expression of MMPs, which could govern IL-1β action by catabolizing the mature cytokine.

In our study, different MMPs expressed different potentials for processing the IL-1β protein. Gelatinase B very effectively processed pIL-1β, yielding stable products and biologic activity.

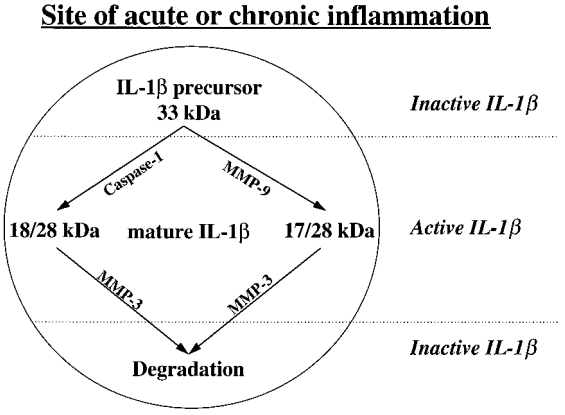

FIGURE 7. Regulation of IL-1β activity at sites of inflammation.

Stromelysin-1 required higher concentrations and several hours of coincubation to convert the precursor, but was the only MMP tested that was capable of completely degrading biologically active forms of IL-1β. The present data suggest operation of a novel pathway of IL-1β regulation, independent of caspase-1. We propose (Fig. 7) that at sites of acute and chronic inflammation, the presence of MMP-9 yields biologic IL-1β activity, whereas MMP-3 can degrade the active form of the mediator, leading to its inactivation. These alternative routes of IL-1β activation and catabolism illustrate 1) the redundancy of regulation of the biologic effects of this multipotent cytokine, 2) the importance of inhibitory as well as stimulatory control, and 3) that therapeutic strategies targeting caspase-1 or MMPs must take these complexities into consideration.

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