Role of Metalloproteases in the Release of the IL-1 type II Decoy Receptor*

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The IL-1 type II receptor (decoy RII) is a nonsignaling molecule the only established function of which is to capture IL-1 and prevent it from interacting with signaling receptor. The decoy RII is released in a regulated way from the cell surface. Here, we reported that hydroxamic acid inhibitors of matrix metalloproteases inhibit different pathways of decoy RII release, including the following: (*a*) the slow (18 h) gene expression-dependent release from monocytes and polymorphonuclear cells exposed to dexamethasone; (*b*) rapid release (minutes) from myelomonocytic cells exposed to tumor necrosis factor, chemoattractants, or phorbol myristate acetate; (*c*) phorbol myristate acetate-induced release from decoy RII-transfected fibroblasts and B cells. Inhibition of release was associated with increased surface expression of decoy RII. Inhibitors of other protease classes did not substantially affect release. However, serine protease inhibitors increased the molecular mass of the decoy RII released from polymorphonuclear cells from 45 to 60 kDa. Thus, irrespective of the pathway responsible for release and of the cellular context, matrix metalloproteases, rather than differential splicing, play a key role in production of soluble decoy RII.

IL-1[1] is the name of two polypeptide mediators (IL-1α and IL-1β) that are among the most potent and multifunctional cell activators described in immunology and cell biology literature. The spectrum of action of IL-1 encompasses cells of hematopoietic origin, from immature precursors to differentiated leukocytes, vessel wall elements, and cells of mesenchymal, nervous, and epithelial origin (1, 2). The production and action of IL-1 are regulated by multiple control pathways, some of which unique to this cytokine. This complexity and uniqueness is best represented by the term "IL-1 system" (3). The IL-1 system consists of two agonists, IL-1α and IL-1β, a specific activation system (IL-1-converting enzyme), a receptor antagonist produced in different isoforms (4, 5), and two high affinity surface binding molecules (3).

Two receptors for IL-1, termed type I and type II (RI and RII, respectively), usually coexpressed in different cell types, have been identified and cloned (6–8). An accessory protein, which increases the binding affinity of RI for IL-1β, has recently been identified (9). IL-1 signaling activity appears to be mediated exclusively via the RI and the accessory protein (9, 10–12), whereas the IL-1RII has no signaling property and acts in myelomonocytic cells as a decoy for IL-1 (decoy RII), inhibiting its activity by preventing IL-1 from binding to the signaling RI (3, 13).

The decoy RII is released *in vitro* and is found in biological fluids in a variety of pathophysiological condition (14–17). Two main pathways of regulation of decoy RII release have been identified. Anti-inflammatory signals (*e.g.* glucocorticoid hormones) augment decoy RII gene expression and eventually release in myelomonocytic cells (18, 19). Over a period of 18 h, dexamethasone increases the number of monocyte surface RII from 171 to 3742 receptors/cell, and about 150×10^6 molecules are released in the culture medium over the same period of time by 20×10^6 monocytes (19). A second pathway of regulation of the decoy RII involves the rapid (5 min) shedding from the cell surface. This rapid pathway of release is activated by chemoattractants, reactive oxygen intermediates, phorbol myristate acetate (PMA), and TNF (20–22). The present study was designed to assess whether proteolytic enzymes are involved in the gene expression-dependent and -independent pathways of decoy RII. The identification of an mRNA transcript encoding a released version of decoy RII (23) raised the issue of the relative contribution of proteolytic shedding *versus* differential splicing in production of soluble decoy RII. The results presented here show that metalloprotease inhibitors block all pathways of soluble decoy RII production and, by implication, that proteolytic shedding is a dominant mechanism for generation of soluble decoy RII.

EXPERIMENTAL PROCEDURES

Cells—Human PMNs were separated from the peripheral blood of human healthy donors by Percoll gradient centrifugation (24). Briefly, whole blood was fractionated by Ficoll gradient centrifugation (Seromed-Biochem KG, Berlin, Germany), and PMNs, collected from the pellet, were layered on top of 62% Percoll (Pharmacia & Upjohn, Uppsala, Sweden) after a centrifugation at 1500 rpm for 20 min at room temperature. PMN pellets (≥98% pure as assessed by morphology) were resuspended at 10^7 cells/ml in RPMI 1640 medium (Seromed-Biochem KG) with 2 mM glutamine (Seromed-Biochem KG). Human monocytes were separated from the peripheral blood of human healthy donors by Percoll gradient centrifugation (24). Briefly, whole blood was fractionated by Ficoll-Hypaque gradient centrifugation (Seromed-Bio-

[1] The abbreviations used are: IL, interleukin; RI, IL-1 type I receptor; RII, IL-1 type II receptor; PMN, polymorphonuclear cell; TNF, tumor necrosis factor; PMA, phorbol myristate acetate; fMLP, formyl-Met-Leu-Phe; α₁-AT, α₁-antitrypsin inhibitor; MCP-1, monocyte chemotactic protein-1; DSS, disuccinimidyl suberate; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody; FCS, fetal calf serum.

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chem KG), and mononuclear cells, collected from the ring, were layered on top of 46% Percoll (Pharmacia & Upjohn) after a centrifugation at 2000 rpm for 30 min at room temperature. Monocytes (≥98% pure as assessed by morphology) were resuspended and incubated (5×10^6 cells/ml) for 40 h in RPMI 1640 medium with 2 mM glutamine and 30% autologous serum on hydrophobic plates (Petriperm Hydrofob, Heraeus, Osterode, Germany).

Human fibrosarcoma cells (line 8387) were transfected with pCEP4β episomal expression vector containing the IL-1RII full-length cDNA (25). 83C7 refers to cells containing the IL-1Rll. 83C7 cells have 2×10^3 (clone A) or 4×10^4 (clone B) IL-1β binding sites/cell. The receptor released by 83C7 cells is similar in size to that of monocytes (see below) and was identified as the decoy RII by cross-linking, antibody blocking, and Western analysis (19).

The 1H7 cell line is a subline of Epstein-Barr virus-positive Burkitt lymphoma line Raji obtained by limiting dilution and selection for high expression of decoy RII (26).

Reagents—The metalloprotease inhibitor BB-94 was from British Biotechnology (Oxford, United Kingdom); L,680833 (a specific elastase inhibitor) and CT1418 and Ro31,9790 (two metalloprotease inhibitors) were from Pharmacia and Upjohn (Nerviano, Italy); PMA and fMLP were from Sigma; α1-AT and E-64 were from Calbiochem; TNFα was from BASF Knoll (Ludwigshaven, Germany). Media and reagents contained less than 0.125 units/ml of endotoxin as checked by Limulus Amebocyte Lysate assay (BioWhittaker, Walkerville, MD).

IL-1 Binding—The assay was conducted as described (20). Briefly, 12×10^6 cells were incubated with or without the indicated stimuli in serum-free medium at 37 °C in 5% CO_2 for 20 min in polypropilene 50-ml conical tubes (Falcon-Beckton Dickinson Labware, Lincoln Park, NJ). Cells were then washed with binding buffer (RPMI 1640 medium, 0.2% bovine serum albumin, pH 7.4), and 4×10^6 cells were incubated with 800 pM ^{125}I-labeled IL-1β (specific activity, 180 μCi/μg; NEN Life Science Products, Bad Homburg, Germany) in the presence or absence of a 100-fold molar excess of cold cytokine in 50 μl of binding buffer at 4 °C for 4 h in polystyrene 96-well round-bottomed microplates (Falcon) on a shaking platform. Preliminary experiments showed that binding reached a plateau at 4 h under these conditions. To separate bound from free ^{125}I-labeled IL-1β, cells were resuspended, transferred to Eppendorf tubes, washed in binding buffer, resuspended in 70 μl of binding buffer, layered on the top of a 200-μl cushion of 20% sucrose (Merck), 1% bovine serum albumin in 400-μl polypropylene tubes (Beckman Instruments), and centrifuged at 10,000 rpm for 30 s at room temperature. The cellular pellets were counted in a γ-counter.

Affinity Cross-linking—30×10^6 cells were incubated with protease inhibitors and stimuli in 1 ml of RPMI 1640 medium at 37 °C for 20 min. Medium was recovered and concentrated 10 times by membrane filtration (Amicon, Beverly, MA; cut-off, 10,000). 100 μl were added to 1 nM ^{125}I-labeled IL-1β, and incubated at 4 °C for 4 h. After addition of 1 mM disuccinimidyl suberate (DSS) (Pierce) at 4 °C for 30 min, samples were analyzed by 8% SDS-PAGE under reducing conditions, and dried gels were exposed to autoradiography for 1–3 days. Nonspecific binding was determined by adding a 200-fold molar excess of unlabeled IL-1β, 10 μg/ml M1 (blocking mAb anti-IL-1 RI), or M22 (blocking mAb anti-IL-1 RII) (kindly provided by Dr. J. E. Sims (Immunex)) (data not shown).

Western Blot Analysis—The supernatants from 5×10^6 83C7 cells were collected, concentrated, subjected to 10% SDS-PAGE, and then blotted onto a nitrocellulose filter (Stratagene, La Jolla, CA). Incubation with primary and secondary antibodies was carried out according to standard protocols. A mouse mAb against decoy RII (clone 8/5)[2] was used as primary antibody. Immunoreactive protein bands were revealed by a chemiluminescence-based procedure (ECL Detection, Amersham International, Little Chalfont, UK) according to the manufacturer's instructions.

Chemotaxis Assay—The chemotactic response of human circulating PMNs to fMLP was tested as described (27, 28). Briefly, 25 ± 1 μl of fMLP, diluted in 1% FCS-RPMI 1640 medium, were seeded in the lower compartment of the chemotaxis chamber, and 50 μl of cell suspension (1.5×10^6/ml) were seeded in the upper compartment. The two compartments were separated by a 5-μm pore size polyvinylpirrolidone-free polycarbonate filter (Nucleopore Corp., Pleasant, CA). Chambers were incubated at 37 °C in air with 5% CO_2 for 90 min. At the end of the incubation, filters were removed, fixed, and stained with Diff-Quik (Harleco, Gibbstown, NJ), and five high power oil immersion fields were counted.
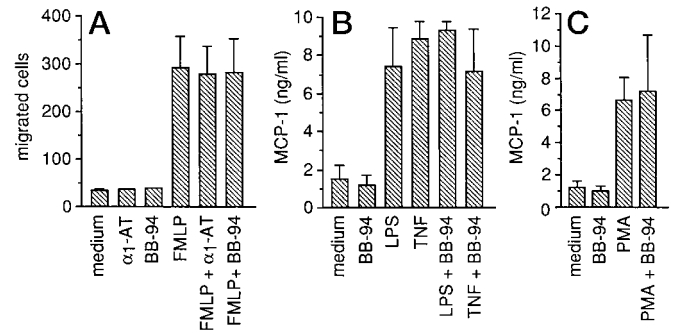
[2] G. Peri, unpublished data.

FIG. 1. **Effect of BB-94 on PMN and monocyte functions.** *A*, effect of BB-94 and α1-AT on fMLP-induced PMN chemotaxis: PMNs were incubated with α1-AT (100 μg/ml) or BB-94 (1 μg/ml), and then fMLP chemotactic response was tested (see "Experimental Procedures"). Data are expressed as migrated cells (means with range of two different donors). *B*, effect of BB-94 on MCP-1 production induced by TNF and lipopolysaccharide in monocytes: monocytes were incubated with medium alone or with lipopolysaccharide (10 ng/ml) or TNF (50 ng/ml) with or without BB-94 (1 μg/ml) for 24 h at 37 °C in RPMI 1640 medium with 1% FCS, and then MCP-1 production was tested (see "Experimental Procedures"). Data are expressed as ng/ml of MCP-1 released by 2×10^5 monocytes (mean with range of two different donors). *C*, effect of BB-94 on MCP-1 production induced by PMA in IL-1 decoy RII-transfected cell line 8387: cells were incubated with medium alone or PMA (50 ng/ml) with or without BB-94 (1 μg/ml) for 24 h at 37 °C in RPMI 1640 medium with 1% FCS, and then MCP-1 production was tested (see "Experimental Procedures"). Data are expressed as ng/ml of MCP-1 released by 4×10^4 cells (mean with range of two different experiments).

MCP-1 Production—Cells were exposed for 24 h to various stimuli or to medium alone. The supernatants were collected and tested for MCP-1 production by a recently developed sandwich enzyme-linked immunosorbent assay with a sensitivity limit of 30 pg/ml (29).

Statistical Analysis—Statistical significance was assessed by the Tukey test.

RESULTS

Effect of Protease Inhibitors on Cellular Function—In a preliminary series of assays, we examined whether the protease inhibitors used in this study affected cellular functions unrelated to proteolytic activity. BB-94, the matrix metalloprotease inhibitor used most extensively in the present study, and α1-AT did not affect PMN chemotaxis, lipopolysaccharide-induced MCP-1 production in monocytes, or PMA-activated MCP-1 production in 83C7 cells (Fig. 1).

Effect of Protease Inhibitors on Rapid Release of IL-1 Decoy RII in PMNs—IL-1 decoy RII is expressed on PMNs as integral transmembrane protein, and it is released very rapidly in the supernatant after treatment of the cells with TNF, chemoattractants, and PMA. To ask what type(s) of enzyme is involved in the cleavage of this molecule, the effects of various protease inhibitors on the release of decoy RII was tested. As shown in Fig. 2A, TNF induced a rapid and drastic decrease of IL-1 binding to PMNs ($17 \pm 7\%$ of binding compared with 100% of control binding). The metalloprotease inhibitor BB-94, an hydroxamic acid-based inhibitor of metalloproteases, strongly inhibited the TNF action ($84 \pm 4\%$). Two other specific metalloprotease inhibitors (CT1418 and Ro31,9790) blocked the action of TNF, although less strongly than BB-94 ($67 \pm 19\%$ for CT1418 and $59 \pm 2\%$ for Ro31,9790). Inhibitors of other classes of proteases, such as serine proteases (α1-AT and L,680833) and cystein proteases (E-64), did not affect TNF-induced release of IL-1 decoy RII. The increase in the amount of IL-1 binding at the level of cell-associated receptor was reflected by a concomitant decrease of the soluble receptor in the supernatant as shown in Fig. 2B. More precisely, TNF increased the level of a soluble IL-1 binding molecule of 45 kDa, as expected. BB-94 strongly inhibited TNF action; surprisingly, treatment
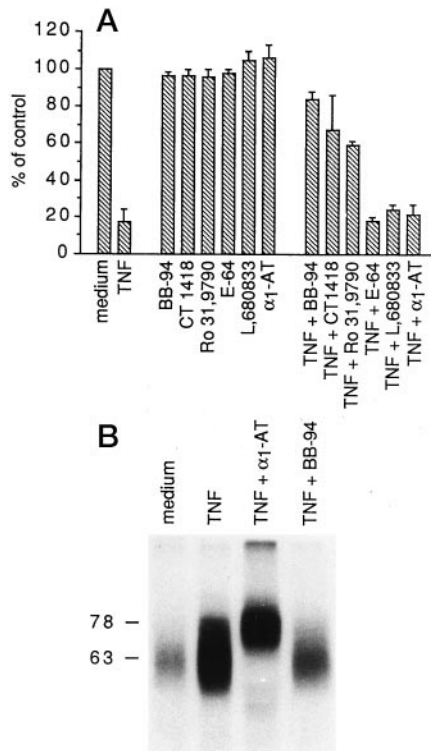
Metalloprotease-mediated Release of IL-1 Decoy Receptor

FIG. 2. **Effect of protease inhibitors on TNF-induced rapid re-lease of the IL-1 decoy RII in PMNs.** *A*, effect on surface binding: 10×10^6 PMNs were incubated with BB-94 (1 μg/ml), CT1418 (10 μM), Ro 31,9790 (10 μM), E-64 (10 μM), L,680833 (2 μM), or α_1-AT (100 μg/ml) with or without TNF (50 ng/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium and then examined for IL-1 binding. Data are shown as percentage of control of specific binding of IL-1 (mean $\pm$ S.D. of three different donors). *B*, effect on release in the supernatant: 30×10^6 PMNs were cultured with or without TNF (50 ng/ml) in the presence of absence of BB-94 (1 μg/ml) and α_1-AT (100 μg/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium. Supernatants were then recovered, concentrated, mixed with ^{125}I-labeled IL-1β, cross-linked with DSS, and then analyzed by SDS-PAGE. Nonspecific binding was determined by adding a 200-fold molar excess of unlabeled IL-1β, 10 μg/ml M1 (block-ing mAb anti-IL-1 RI), or 10 μg/ml M22 (blocking mAb anti-IL-1 RII) (kindly provided by Dr. J. E. Sims (Immunex)) (data not shown).

of PMNs with α_1-AT, a serine protease inhibitor, did not cause inhibition of TNF action but shifted the size of the released decoy RII from 45 to 60 kDa, the same molecular mass observed in monocytes and IL-1 decoy RII-transfected cells (19, 25). L,680833, a specific elastase inhibitor, also caused the shift in the released decoy RII from 45 to 60 kDa (data not shown). Chemoattractant molecules, such as fMLP and PMA, induced rapid release of decoy RII in PMNs (20, 22). We tested the effect of BB-94 on release of IL-1 decoy RII induced by these two stimuli. As shown in Fig. 3, fMLP and PMA induced rapid release of decoy RII as reflected by decrease in binding to cells ($32 \pm 7\%$ and $48 \pm 4\%$ for fMLP and PMA, respectively). As expected, BB-94 inhibited the release induced by fMLP and PMA ($81 \pm 11\%$ and $83 \pm 2\%$ of control values, respectively).

Effect of Protease Inhibitors on Rapid Release of IL-1 Decoy RII in Monocytes—Monocytes also express high quantities of surface decoy RII (19). TNF induces rapid (10 min) release of IL-1 decoy RII (22). When we tested the effects of different protease inhibitors (Fig. 4*A*), we found that only metallopro-tease inhibitors, such as BB-94, CT1418, and Ro31,9790, inhib-ited the action of TNF. In particular, BB-94 restored the IL-1 binding capacity of TNF-treated cells to $93 \pm 12\%$ of control, compared with $26 \pm 10\%$ of cells treated with TNF alone; CT1418 and Ro31,9790 also caused a significant inhibition of TNF action ($92 \pm 8\%$ for CT 1418 and $81 \pm 9\%$ for Ro 31,9790).
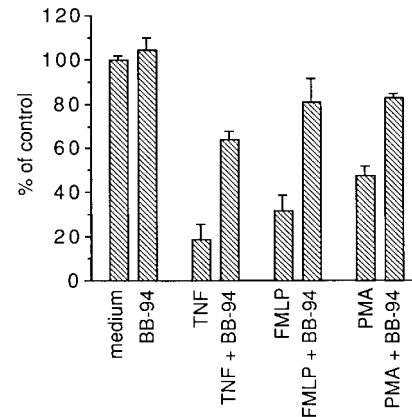

FIG. 3. **Effect of protease inhibitors on fMLP- and PMA-in-duced release of the IL-1 decoy RII in PMNs.** 10×10^6 PMNs were incubated with TNF (50 ng/ml), fMLP (10^{-7} M), or PMA (50 ng/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium with or without BB-94 (1 μg/ml) and then examined for IL-1 binding. Data are shown as percentage of control of specific binding of IL-1 (mean $\pm$ S.D. of three different donors).

FIG. 4. **Effect of protease inhibitors on TNF-induced release of the IL-1 decoy RII in monocytes.** *A*, effect on surface binding: 10×10^6 monocytes were incubated with BB-94 (1 μg/ml), CT1418 (10 μM), Ro31,9790 (10 μM), E-64 (10 μM), or L,680833 (2 μM), with or without TNF (50 ng/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium and then examined for IL-1 binding. Data are shown as percentage of control of specific binding of 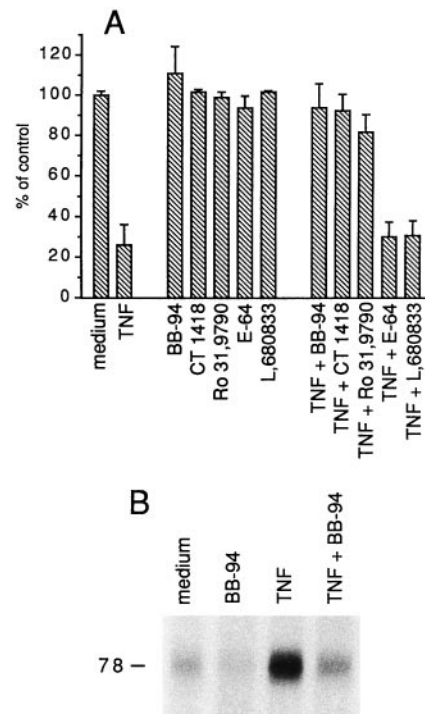IL-1 (mean $\pm$ S.D. of three different donors). *B*, effect on release in the supernatant: 30×10^6 monocytes were cultured with or without TNF (50 ng/ml) in the presence or absence of BB-94 (1 μg/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium. Supernatants were then recovered, concentrated, mixed with ^{125}I-labeled IL-1β, cross-linked with DSS, and then analyzed by SDS-PAGE.

Other protease inhibitors, such as L,680833 and E-64, had no effect on IL-1 decoy RII release. As shown in Fig. 4*B*, TNF-stimulated monocytes released a 60-kDa soluble IL-1 decoy RII, and BB-94 inhibited the shedding of soluble receptor.

Effect of Protease Inhibitors on Dexamethasone-induced IL-1 Decoy RII Release in PMNs and Monocytes—In agreement with previous reports (18, 19), dexamethasone augmented the sur-
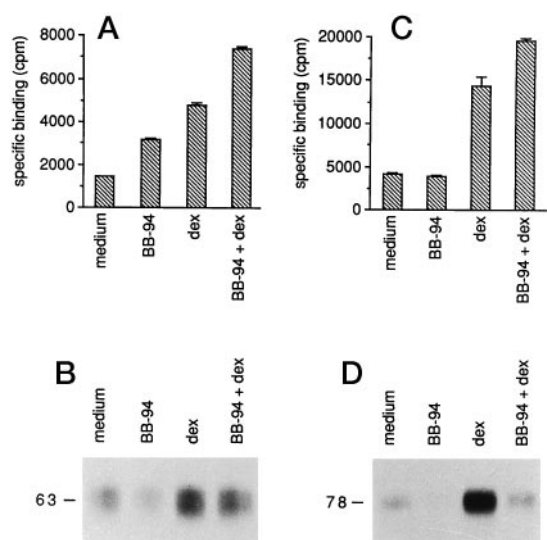
FIG. 5. **Effect of protease inhibitors on dexamethasone-induced release of the IL-1 decoy RII in PMNs and monocytes.** *A,* effect on PMN surface binding: 10×10^6 PMNs were incubated with or without dexamethasone (10^{-6} M) in the presence or absence of BB-94 (1 μg/ml) for 18 h at 37 °C in RPMI 1640 medium with 10% FCS and then examined for IL-1 binding. Data are shown as cpm of specific binding of ^{125}I-labeled IL-1β of a representative experiment out of three experiments performed. *B,* effect on release in the supernatant by PMNs: 30×10^6 PMNs were cultured with or without dexamethasone (10^{-6} M) in the presence or absence of BB-94 (1 μg/ml) for 18 h at 37 °C in serum-free RPMI 1640 medium. Supernatants were then recovered, concentrated, mixed with ^{125}I-labeled IL-1β, cross-linked with DSS, and then analyzed by SDS-PAGE. *C,* effect on monocyte surface binding: 10×10^6 monocytes were incubated with or without dexamethasone (10^{-6} M) in the presence or absence of BB-94 (1 μg/ml) for 18 h at 37 °C in RPMI 1640 medium with 10% FCS and then examined for IL-1 binding. Data are shown as cpm of specific binding of ^{125}I-labeled IL-1β of a representative experiment out of three experiments performed. *D,* effect on release in the supernatant by monocytes: 30×10^6 monocytes were cultured with or without dexamethasone (10^{-6} M) in the presence or absence of BB-94 (1 μg/ml) for 18 h at 37 °C in serum-free RPMI 1640 medium. Supernatants were then recovered, concentrated, mixed with ^{125}I-labeled IL-1β, cross-linked with DSS, and then analyzed by SDS-PAGE.

face expression of the decoy RII (reflected by increased specific binding) in both monocytes and PMNs (Fig. 5, *A* and *C*). Concomitantly, dexamethasone augmented the release of soluble versions of decoy RII of 45 and 60 kDa for PMNs and monocytes, respectively (Fig. 5, *B* and *D*). BB-94 blocked release of the decoy RII from dexamethasone-treated myelomonocytic cells and concomitantly augmented binding on the cell surface.

Effects of Protease Inhibitors on Release from a B Lymphoma and IL-1 Decoy RII Transfected Fibroblasts—The results discussed so far were obtained with myelomonocytic cells. It was important to assess whether metalloproteases play a central role in decoy RII release in a different cellular context as well. We therefore examined the effect of metalloprotease inhibitors on decoy RII release from B cells (cell line 1H7, a subline of an Epstein-Barr virus-positive Burkitt lymphoma line Raji, selected for high expression of IL-1 decoy RII (26)) and transfected fibroblasts (transfected cell line 8387). Both of these cell lines released large amounts of soluble decoy RII spontaneously and after stimulation with PMA. As shown in Figs. 6*B,* 6*C,* and 7*B,* BB-94 inhibited the spontaneous and PMA-induced release of the decoy RII in transfected fibroblast cells and 1H7 cells. Concomitantly, BB-94 blocked the PMA-induced reduction of IL-1 binding in these nonmyeloid cellular contexts (Figs. 6*A* and 7*A*). The capacity of BB-94 to block decoy RII release from transfected fibroblasts was observed by both cross-linking and Western analysis.
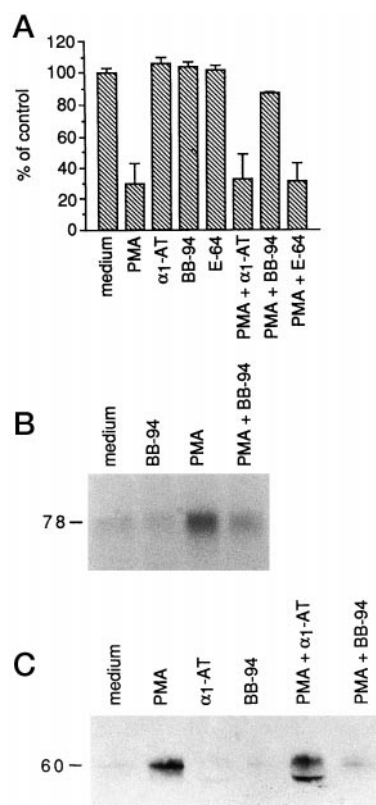

FIG. 6. **Effect of protease inhibitors on PMA-induced release of the IL-1 decoy RII on IL-1 decoy RII-transfected 8387 cell line.** *A,* effect on surface binding: 3×10^6 83C7 cells were incubated with α_1-AT (100 μg/ml), BB-94 (1 μg/ml), or E-64 (10 μM), with or without PMA (50 ng/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium and then examined for IL-1 binding. Data are shown as percentage of control of specific binding of IL-1 (mean with range of two different experiments). *B,* effect on release in the supernatant (cross-linking): 10×10^6 83C7 (clone A; 2×10^3 receptors/cell) cells were cultured with or without PMA (50 ng/ml) in the presence or absence of BB-94 (1 μg/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium. Supernatants were then recovered, concentrated, mixed with ^{125}I-labeled IL-1β, cross-linked with DSS, and then analyzed by SDS-PAGE. *C,* effect on release in the supernatant (Western blotting): 10×10^6 83C7 (clone B; 4×10^4 receptors/cell) cells were cultured with or without PMA (50 ng/ml) in the presence or absence of BB-94 (1 μg/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium. Supernatants were then recovered, concentrated, run on 10% SDS-PAGE, and then analyzed by Western blot with anti-RII mAb 8/5.

DISCUSSION

The results presented here show that one or more enzyme of the metalloprotease class play a key role in the production of soluble versions of the decoy RII. Different inhibitors of metalloproteases inhibited release of decoy RII, whereas agents which act on serine proteases or cysteine proteases had little or no effect. Metalloprotease inhibitors did not affect various functions of the cells used in the present study, including PMN chemotaxis, lipopolysaccharide-induced cytokine production in monocytes, and PMA-activated cytokine production in transfected fibroblasts. It is therefore unlikely that the capacity of different metalloprotease inhibitors to block decoy RII release is related to effects other than target enzyme inhibition.

The decoy RII is present in biological fluids, with augmented levels in inflammatory conditions (16, 17) and in culture supernatants (18, 19). Previous studies had identified two general pathways through which environmental signals activate release of the decoy RII in myelomonocytic cells. Several molecules with anti-inflammatory activity (glucocorticoids, IL-4, and IL-13) augmented gene expression and subsequently surface levels of decoy RII: an increased surface expression is
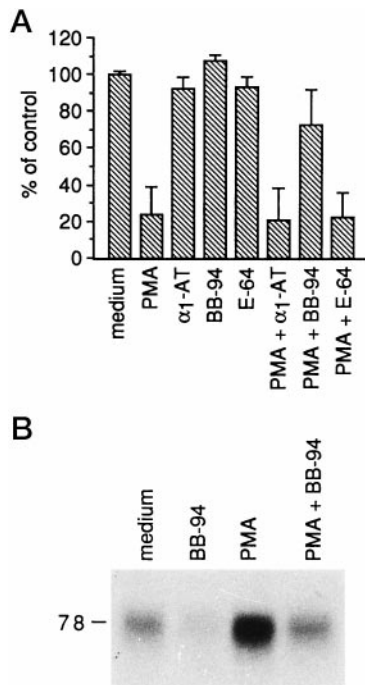
FIG. 7. **Effect of protease inhibitors on PMA-induced release of the IL-1 decoy RII on 1H7 cell line.** *A*, effect on surface binding: 3×10^6 1H7 cells were incubated with α_1-AT (100 μg/ml), BB-94 (1 μg/ml), or E-64 (10 μM), with or without PMA (50 ng/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium and then examined for IL-1 binding. Data are shown as percentage of control of specific binding of IL-1 (mean with range of two different experiments). *B*, effect on release in the supernatant: 10×10^6 cell were cultured with or without PMA (50 ng/ml) in the presence or absence of BB-94 (1 μg/ml) for 20 min at 37 °C in serum-free RPMI 1640 medium. Supernatants were then recovered, concentrated, mixed with ^{125}I-labeled IL-1β, cross-linked with DSS, and then analyzed by SDS-PAGE.

associated with increased release (18, 19). A second pathway is gene expression- and protein synthesis-independent: decoy RII release is activated in about 5 min by chemoattractants, reactive oxygen intermediates, and TNF (20–22). In the present study, metalloprotease inhibitors blocked decoy RII release irrespective of the pathway of induction and the cellular context.

The soluble decoy RII present in biological fluids was classically described as a 45-kDa molecule (16–18). *In vitro*, we have described two forms of released decoy RII, a 45-kDa molecule released from PMNs (18, 20, 22) and a 60-kDa form released from monocytes and transfected fibroblasts (19, 25). Intriguingly, given the central role of monocytes in inflammation, the 60-kDa version of decoy RII had not previously been described in biological fluids. The results reported here may provide a reasonable explanation for this apparent discrepancy. Serine protease inhibitors had little or no effect on decoy RII release. However, serine protease inhibitors modified the size of decoy RII released from PMNs. In the presence of the inhibitors, PMNs released a 60-kDa, rather than 45-kDa, form of the decoy RII. Thus one could speculate that all cell types release primarily a 60 kDa version of the decoy RII via metalloproteases, and then extracellular serine proteases produced by PMNs and possibly other cellular elements process it to the 45-kDa form found *in vivo*. The extracellular domains of different membrane proteins, including pro-TNFα, p75 and p55 TNF receptor, IL-6-receptor, Fas-ligand, L-selectin, pro-transforming growth factor α, and thyrotropin receptor are shed in the supernatant in different cell type, by one or more metalloproteases (30–38). A mutant Chinese hamster ovary cell line, defective in the shedding of several unrelated membrane pro-

teins, was recently described, suggesting a common system for membrane protein shedding (39). On the other hand, no sequence similarity can be found in the cleavage site of released proteins (39). Studies based on mutational analysis of the cleavage site of L-selectin, p55 TNF receptor, and pro-TNFα (40–42) showed that the proteolytic processing of molecules might depend on the secondary structural characteristics of the cleavage domain and might not require strict sequence specificity. The observation that the juxtamembrane region of the decoy RII shows no primary sequence similarity to other released receptors does not necessarily imply the involvement of different enzyme systems.

Soluble receptors of different cytokines fulfill different functions (43, 44). For instance, soluble IL-6 receptor allows interaction of IL-6 with the signal-transducing gp130 chain (45, 46), whereas soluble TNF receptor usually blocks TNF (47) and is currently undergoing clinical evaluation (48). The released IL-1 decoy RII does not facilitate signal transduction (10) and presumably acts as a systemic buffering system for IL-1 (3, 13).

A splice variant of the decoy RII mRNA, encoding a molecule without a transmembrane segment, has been identified (23). The results reported here show that metalloprotease inhibitors block release and augment surface expression of the decoy RII, irrespective of the inducing agent and the cellular context. Therefore, these observations suggest that proteolytic shedding, rather than differential RNA splicing, is the dominant pathway for generation of soluble decoy RII.

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