Volume 269, number 1, 93-95 FEBS 08734 August 1990

Tumor necrosis factor bifunctionally regulates matrix metalloproteinases and tissue inhibitor of metalloproteinases (TIMP) production by human fibroblasts

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Received 11 June 1989

The production of tissue inhibitor of metalloproteinases (TIMP) in human uterine cervical fibroblasts was increased by human recombinant tumor necrosis factor α (hrTNF) at a low concentration (0.005 ng/ml) but the elevated synthesis was suppressed in a dose-dependent manner at higher concentrations (up to 50 ng/ml). In contrast, the production of collagenase (EC 3.4.24.7) and stromelysin was stimulated at all the corresponding concentrations. In contrast, human recombinant interleukin-1α (hrIL-1, 10 ng/ml) coordinately induced these enzymes and TIMP production. The reduction of the elevated TIMP production by TNF was not due to the inhibition of TIMP secretion. These results suggest that TNF modulates the extracellular matrix degradation in human fibroblasts bifunctionally by the suppression of TIMP production in addition to the acceleration of matrix metalloproteinases production. Furthermore, the fact that TNF and IL-1 differently controlled the production of TIMP suggests that the signal pathway of TNF for TIMP production is different from that of IL-1.

Tumor necrosis factor; Matrix metalloproteinase; Collagenase; Stromelysin; Tissue inhibitor of metalloproteinase; Interleukin-1; Human fibroblast

1. INTRODUCTION

Tumor necrosis factor (TNF), a cytokine secreted from monocytes in response to immune and inflammatory stimuli or endotoxin, induces the necrosis of tumors in vivo and tumor cell killing in vitro [1]. It also exerts other biological effects on the various cell types [2,3]. TNF plays a major role in the inflammatory processes by enhancing the destruction of extracellular matrix components [4,5], in which matrix metalloproteinases (MMPs), collagenase (MMP-1) and stromelysin (MMP-3) mainly participate [6]. These actions of TNF [4,5] are similar to those of another inflammatory cytokine interleukin-1 (IL-1) [7,8]. Thus, the intracellular signal transduction pathway for collagenase production by TNF may be analogous to that of IL-1. Recently, it is proposed that the advancement of the matrix breakdown is caused by the imbalance of metalloproteinases and their inhibitor, TIMP [9]. However, it is not clearly known how TNF participates in the production of TIMP in the connective tissue cells.

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Abbreviations: TNF, tumor necrosis factor; hrTNF, human recombinant TNFα, TIMP, tissue inhibitor of metalloproteinases; MMP, matrix metalloproteinase; IL-1, interleukin-1; hrIL-1, human recombinant IL-1α; MEM, Eagle's minimum essential medium; FBS, fetal bovine serum; LAH, lactalbumin hydrolysate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

In this paper, we have investigated the effects of TNF, in comparison with those of IL-1, on TIMP and MMPs production from human fibroblasts. TNF was found to modulate bidirectionally the collagenolysis in fibroblasts by the acceleration of MMPs production and the suppression of TIMP production, whereas IL-1 accelerated both MMPs and TIMP production coordinately.

2. MATERIALS AND METHODS

Eagle's minimum essential medium (MEM) was purchased from Grand Island Biological Co., Grand Island, NY, USA. Fetal bovine serum (FBS) was obtained from Whittaker Bioproducts Inc., Walkersville, MD, USA. Alkaline phosphatase-conjugated donkey anti-(sheep IgG)IgG and rabbit anti-(mouse IgG)IgG, 5-bromo-4-chloro-3-indolyl phosphate, Nitro blue tetrazolium and monensin were obtained from Sigma Chemical Co., St. Louis, MO, USA. Protein A-Sepharose CL-4B was obtained from Pharmacia LKB, Uppsala, Sweden. L-[^{35}S]methionine (800 Ci/mmol) was from American Radiolabeled Chemicals, St. Louis, MO, USA. Human recombinant TNFα (hrTNF) (2.55×10^6 units/mg) and human recombinant IL-1α (hrIL-1) (2×10^7 units/mg) were kindly supplied by Dainippon Pharmaceutical Co., Suita, Osaka, Japan. Sheep anti-(human synovial procollagenase) and anti-(human synovial stromelysin) antibodies were provided by Dr H. Nagase from the University of Kansas Medical Center, Kansas City, KS, USA. Mouse monoclonal anti-(bovine TIMP)IgG was purchased from Fuji Chemical Industries, Tokyo, Japan. Other reagents used were the same as in the previous paper [8].

2.1. *Cell culture and treatment*

Human uterine cervical fibroblasts and human chorionic fibroblasts were prepared and maintained in MEM/10% (v/v) FBS as described previously [8]. The confluent cells were treated with hrTNF or hrIL-1 in the serum-free MEM containing 0.2% (w/v) lactalbumin

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00145793/90/$3.50

hydrolysate (LAH) in order to examine MMPs and TIMP production.

2.2. *Assay for collagenase activity*

Collagenase was assayed by the fibril assay using [^{14}C]acetylated collagen as described previously [10]. Procollagenase in the culture medium was activated by treating with 80 μg/ml trypsin at 25°C for 10 min followed by 360 μg/ml excess of soybean trypsin inhibitor prior to the assay. One unit of collagenase hydrolyzes 1 μg of substrate/min at 37°C.

2.3. *Western blotting*

TIMP, procollagenase and prostromelysin were analyzed by Western blotting. The samples were first subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 12.5% or 10% acrylamide slab gels [11] and then proteins were electrotransferred to a nitrocellulose filter. Immunoreactive TIMP, procollagenase or prostromelysin was visualized indirectly by the reaction of 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium with the alkaline phosphatase-conjugated second antibody bound to the antigen-antibody complex in the filter as described previously [8].

2.4. *Labeling with L-[^{35}S]methionine, immunoprecipitation and fluorography of TIMP*

After treating confluent human uterine cervical fibroblasts with hrIL-1 or hrTNF, cells were washed once with methionine-free MEM and then incubated with L-[^{35}S]methionine (10 μCi/ml) in the same medium. After a 2 h incubation, medium was collected (medium fraction). Cells were harvested by scraping with a rubber policeman, sonicated in the presence of 1 mM diisopropyl phosphorofluoridate and 5 mM EDTA, and centrifuged. Labeled culture media and cell extracts were incubated with mouse anti-(bovine TIMP)IgG, and then the antigen-antibody complexes were isolated by coupling to protein-A Sepharose. Control incubations contained nonimmune mouse IgG under the same condition. Immunoprecipitable TIMP was subjected to SDS-PAGE and analyzed by fluorography after impregnating the gel with EN^{3}HANCE (Dupon, USA) and exposed to Kodak Omat AR X-ray film (Eastman Kodak Co.) at −80°C.

3. RESULTS AND DISCUSSION

When human fibroblasts were treated with hrTNF in culture, the apparent collagenolytic activity in culture media of human chorionic cells increased in a dose-dependent manner. Similarly, hrIL-1 enhanced the collagenolytic activity (Table I). These cytokines also stimulated human skin fibroblasts to produce much collagenolytic activity (data not shown). The collagenolytic activity, however, was not detected with uterine cervical fibroblasts even after the treatment of cells with hrTNF or hrIL-1. This was due to the similar enhancement of TIMP production by these cytokines (see Fig. 2). Their promotive effects are similar to those reported for MMPs production previously [4,5]. Dayer et al. [4] reported that TNF stimulates collagenase production from human synovial cells and dermal fibroblasts. An increase in gelatinase and collagenase production from rat granulation tissue in culture by TNF was reported by Nakagawa et al. [5]. However, these investigators monitored the production of collagenase or gelatinase by measuring their enzymic activities in culture media but the amounts of MMPs and their inhibitor, TIMP, produced after the TNF treatment were not examined. In order to understand the balance between MMPs and TIMP upon stimulation of

Table I

Effect of hrTNF on the collagenase activity in culture media of human fibroblasts

Treatment	Concentration (ng/ml)	Collagenase activity (units/ml)	
		Chorionic fibroblasts	Cervical fibroblasts
Control	-	0.04 ± 0.03	n.d.
hrTNF	0.01	0.05 ± 0.05	n.d.
	1	0.64 ± 0.50	n.d.
	100	1.04 ± 0.08***	n.d.
hrIL-1	10	0.40 ± 0.11*	n.d.

Confluent human uterine cervical fibroblasts at eight passage were treated with hrTNF or hrIL-1 in 0.2% (w/v) LAH/MEM three times for 6 days and human chorionic fibroblasts at fifth passage were treated as described above for 2 days. The harvested culture media were performed for the assay of collagenase activity. Data are the mean value ± SD of three wells.

* and ***; significantly different from control ($P<0.05$ and $P<0.001$, respectively). n.d. = not detectable.

connective tissue cells we examined, using the immunoblotting technique, the amounts of MMPs and TIMP proteins produced by the cultured fibroblasts following the hrTNF or hrIL-1 treatment. As shown in Fig. 1A, both hrTNF and hrIL-1 increased the production of procollagenase in the culture media in a dose-dependent manner. Similarly, the production of prostromelysin, which has the ability to degrade various connective tissue matrix components [12] and recently has been characterized as an endogenous activator for procollagenase [13,14], was stimulated by these cytokines in a dose dependent manner (Fig. 1B). The ability of hrTNF or hrIL-1 to induce the production of MMPs was also confirmed with human chorionic cells (data not shown). In contrast, the effects of hrIL-1 and hrTNF on the TIMP production were different from each other. As shown in Fig. 2A, hrIL-1 stimulated the

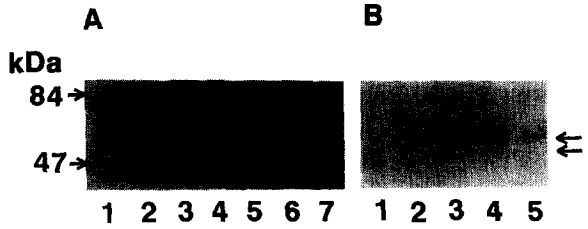

Fig. 1. Effect of hrTNF on the production of procollagenase and prostromelysin from human fibroblasts. Confluent uterine cervical fibroblasts at eight passage (A) and sixth passage (B) were treated with hrTNF or hrIL-1 in 0.2% (w/v) LAH/MEM for 2 days. The harvested culture media (1.5 ml) were mixed with 0.3 ml of 20% (w/v) trichloroacetic acid. The precipitates were collected by centrifugation, dissolved in 20 μl of reducing sample buffer and subjected to SDS-PAGE. The bands of procollagenase (A) or prostromelysin (B) were visualized by immunoblotting as described in section 2. (A) Lane 1, no treatment; lanes 2–4, hrIL-1 (0.001, 0.1 and 10 ng/ml, respectively); lanes 5–7, hrTNF (0.005, 0.5 and 50 ng/ml, respectively). (B) Lane 1, no treatment; lanes 2–4, hrTNF (100, 10 and 1 ng/ml, respectively); lane 5, hrIL-1 (10 ng/ml).

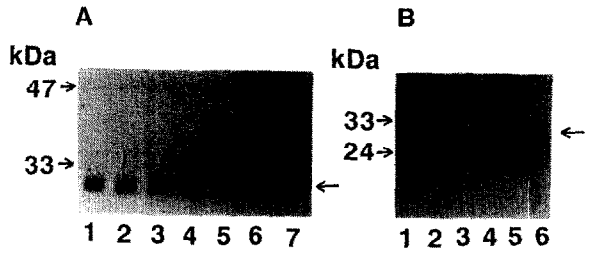

Fig. 2. Effect of hrTNF on TIMP production by human fibroblasts. Confluent uterine cervical fibroblasts at eight passage (A) and chorionic cells at eighth passage (B) were treated with hrTNF or hrIL-1 for 2 days. The harvested culture media were subjected to the immunoblotting for TIMP as described in Fig. 1. (A) (10% acrylamide gel): lane 1, no treatment; lanes 2–4, hrIL-1 (0.001, 0.1 and 10 ng/ml, respectively); lanes 5–7, hrTNF (0.005, 0.5 and 50 ng/ml, respectively). (B) 12.5% acrylamide gel): lane 1, no treatment, lanes 2–4, hrTNF (0.01, 1 and 100 ng/ml, respectively); lanes 5 and 6, hrIL-1 (1 and 10 ng/ml, respectively).

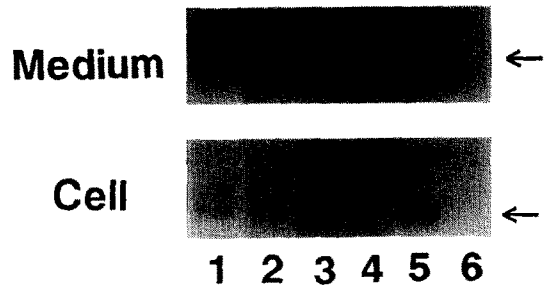

Fig. 3. Effect of hrTNF on the secretion of [^{35}S]methionine-labeled TIMP from human uterine cervical fibroblasts. Fibroblasts at sixth passage were preincubated with hrTNF (0.1 or 100 ng/ml) for 24 h and then labeled with [^{35}S]methionine for 2 h. The harvested culture media and cell extracts were subjected to immunoprecipitation, SDS-PAGE and fluorography as described in section 2. Lane 1, no treatment; lane 2, hrIL-1 (100 ng/ml); lanes 3 and 4, hrTNF (0.1 and 100 ng/ml, respectively); lane 5, hrIL-1 (100 ng/ml) plus monensin (10 μM); lane 6, hrIL-1 (100 ng/ml) precipitated using nonimmune IgG.

production of TIMP from uterine cervical fibroblasts, whereas the effects of hrTNF on these cells were biphasic; at a low concentration (0.005 ng/ml, lane 5) of TNF early enhanced the production of TIMP, but at high concentrations of hrTNF (up to 50 ng/ml) as high levels of TNFα in synovial fluids of RA [15] the increased TIMP production was reduced in a dose-dependent manner. It is unclear why TNF biphasically regulates the TIMP production in uterine cervical cells. In contrast, hrTNF consistently suppressed the TIMP production in human chorionic fibroblasts as shown in Fig. 2B. These results indicate that hrTNF differentially regulates the TIMP and MMPs production in human fibroblasts. Thus, the hrTNF-enhanced collagenolytic activity in culture media is due not only to the increased collagenase production but also to the decreased synthesis of TIMP.

In order to clarify the mechanism by which the decreased TIMP production was caused, we investigated the influence of hrTNF on the TIMP secretion. After the treatment of uterine cervical fibroblasts with hrTNF (0.1 or 100 ng/ml) for 24 h, cells were incubated with L-[^{35}S]methionine in methionine-free MEM for 2 h, and immunoreactive ^{35}S-labeled TIMP in both media and cell extracts were analyzed by SDS-PAGE followed by fluorography. As shown in Fig. 3, a low concentration (0.1 ng/ml) of hrTNF significantly increased TIMP production (lane 3) as well as hrIL-1 (lane 2). However, at a higher concentration of hrTNF (100 ng/ml, lane 4) the synthesis of TIMP was significantly suppressed as well as Western blotting analysis (Fig. 3, Medium). The decrease in ^{35}S-labeled TIMP in the medium was not due to the inhibition of TIMP secretion as the intracellular levels of ^{35}S-labeled TIMP were indistinguishable except for the cells with monensin treatment. These results suggest that TNF regulates the TIMP synthesis either at the transcriptional and/or translation level.

In conclusion, this is the first report that TNF affects the TIMP synthesis from human fibroblasts. Thus TNF fifunctionally regulates the collagenolysis in fibroblasts by the suppression of TIMP production in addition to the acceleration of matrix metalloproteinases production. This is an actual observation since the advanced collagenolysis in connective tissue are considered to result from the imbalance of MMPs and TIMP [9]. Therefore, TNF may play an important role in pathological and physiological destruction of connective tissue components as well as IL-1.

Acknowledgements: We are grateful to Dr H. Nagase from the University of Kansas Medical Center, Kansas City, KS, USA, for useful suggestions and critical reading of the manuscript.

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