

INTERLEUKIN 1 STIMULATION OF COLLAGENASE
PRODUCTION BY CULTURED FIBROBLASTS*

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Interleukin 1 (IL-1) or lymphocyte-activating factor, a monokine released in vitro by cultured monocytes or macrophages, acts on a variety of somatic and immune-related target cells in a genetically unrestricted manner (1-5).

It has long been recognized that fibroblasts appear at sites of inflammatory reactions after macrophages and play a pivotal role in the repair of damaged connective tissue by synthesizing and remodeling components of the matrix (e.g., collagens, fibronectin, and proteoglycans) (6). In this report, we have observed that IL-1 is a potent stimulator of fibroblast collagenase production in vitro. This finding suggests that IL-1 may be a major effector molecule by which macrophages modulate not only fibroblast growth but collagenase production as well (4, 5).

Materials and Methods

IL-1 Purification. In preliminary studies, we found that IL-1 isolated from normal human peripheral blood monocytes was contaminated by lymphokines (personal observation by A. E. Postlethwaite and L. Lachman). However, IL-1 from monoblasts from patients with acute monocytic or myelomonocytic leukemia was free of contaminating lymphokines and its physicochemical properties were identical to those of IL-1 obtained from normal monocytes (7). IL-1 was purified from supernatants from cultures of monoblasts by diafiltration, ultrafiltration, and isoelectric focusing (IEF) as previously described (7, 8). The protein content of IL-1 prepared in this manner was below the limit detectable by the Lowry assay (<50 ng/ml).

High Performance Liquid Chromatography. Analytical gel filtration high performance liquid chromatography (HPLC) was performed on two I-125 protein analysis columns (Waters Associates, Inc., Milford, MA) connected in tandem. The columns were equilibrated and run with 0.0075 M glycylglycine:0.14 M NaCl (GGBS) at pH 7.2.

Analytical anion exchange-HPLC was also performed on a 250-mm × 4.1-mm SynChropak AX300 column (SynChrom, Inc., Linden, IN). The starting buffer was 0.02 M Tris acetate, pH 8, and the limiting buffer was 0.02 M Tris acetate:0.5 M sodium acetate, pH 8.

Thymocyte Proliferation Assay. IL-1 activity was quantitated by measuring the uptake of tritiated thymidine (^3H TdR) by thymocytes from 6- to 10-wk-old Swiss-Webster mice (Charles River Breeding Laboratories, Inc., Boston, MA) (7). Standard errors were <10% of the mean of triplicate determinations.

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Fibroblast Collagenase Production. Cultures of human infant foreskin fibroblasts or HFL-1 cells, an established line of human embryonic lung fibroblasts (Cell Repository Designation, CCL153; American Type Culture Collection, Rockville, MD) were seeded in wells of Falcon 3008 multiwell plates (Falcon Labware, Oxnard, CA) and grown to confluency in maintenance medium (Eagle's minimum essential medium supplemented with nonessential amino acids, 50 $\mu\text{g}/\text{ml}$ ascorbic acid, 100 $\mu\text{g}/\text{ml}$ streptomycin, 1 $\mu\text{g}/\text{ml}$ amphotericin B, and 10% heat-inactivated fetal calf serum [Grand Island Biological Co., Grand Island, NY]) for 72 h at 37°C in a humidified atmosphere that contained 5% CO_2 . Fibroblasts were serum-starved for three additional days. Medium was then removed from each well and replaced with 200 μl serum-free maintenance medium plus 100 μl of sample, and the fibroblasts were cultured for 24 h.

Harvested fibroblast culture supernatants were preincubated with trypsin to activate latent collagenase as follows: Aliquots (200 μl) of culture supernatant were incubated at 25°C for 30 min with 20 μl trypsin (TRTPCK; Worthington Biochemical Corp., Freehold, NJ), final concentration 10 $\mu\text{g}/\text{ml}$. After incubation, trypsin was inactivated by adding a fourfold excess of soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO) in a volume of 20 μl .

Collagenase Assay. Collagenolytic activity in fibroblast culture supernatants was quantitated by using a modification of a microassay previously described by Johnson-Wint (9). The substrate, type I calf skin collagen, was radiolabeled with ^{14}C acetic anhydride according to the method of Cawston and Barrett (10). In the original description of the microassay the collagen substrate was dispensed into flat-bottomed wells of microtiter plates and allowed to dry for several days to form a film (9). We modified the assay by keeping the substrate in gel form. Gels in wells were allowed to form in a humidified atmosphere at 37°C for 24 h before the addition of test samples. After the addition of samples (200 μl), plates were incubated for 24 h at 37°C in a humidified atmosphere that contained 5% CO_2 . An aliquot (150 μl) was then removed from each well and subjected to scintillation counting. For each assay, the total counts released by 100% lysis of the collagen gels by bacterial collagenase (Worthington Biochemical Corp.) and the background counts released by gels incubated with maintenance medium were determined.

The collagenase activity of each sample applied to each 25 μl ^{14}C collagen gel was calculated as follows: percent gel lysis = [(cpm sample - mean cpm medium)/(mean cpm bacterial collagenase - mean cpm medium)] \times 100. The standard error for gel lysis values $>5\%$ was $<10\%$ of the mean of triplicate determinations.

Tritiated Amino Acid Incorporation. In some experiments, fibroblast cultures in Falcon 3008 multiwell plates were pulsed for 24 h with 5 μCi of a mixture of ^3H amino acids (Amersham Corp., Arlington Heights, IL). Harvested culture supernatants and trypsinized cell layers were exposed to cold 10% trichloroacetic acid (TCA). The precipitates were collected under suction on Whatman 3 MM scintillation pads (Arthur H. Thomas Co., Philadelphia, PA), washed three times with 10 ml of cold TCA, dried, and subjected to scintillation counting.

DNA Quantitation. The DNA content of fibroblast monolayers was measured by the method of Kissane and Robins (11).

Results

IL-1 Stimulation of Collagenase Production. Different doses of IEF-purified IL-1 were tested for their ability to stimulate thymocyte proliferation and fibroblast collagenase production (Fig. 1). IL-1 stimulated collagenase production at concentrations that triggered thymocyte proliferation, which suggests that both cell types exhibit similar sensitivities to IL-1 (Fig. 1).

Because IL-1 can stimulate the replication of subconfluent fibroblasts *in vitro*, it was essential to determine whether the increase in collagenase production induced by IL-1 was accompanied by an increase in fibroblast growth (4, 5). Increased collagenase production by confluent fibroblasts exposed to IL-1 was not accompanied by an increase in cell layer DNA (Table I). Therefore, IL-1 does not appear to increase production of collagenase simply by stimulating fibroblast growth.

Inhibition of fibroblast protein synthesis by cycloheximide resulted in a loss of the ability of IL-1 treated fibroblasts to produce increased levels of collagenase (Table II).

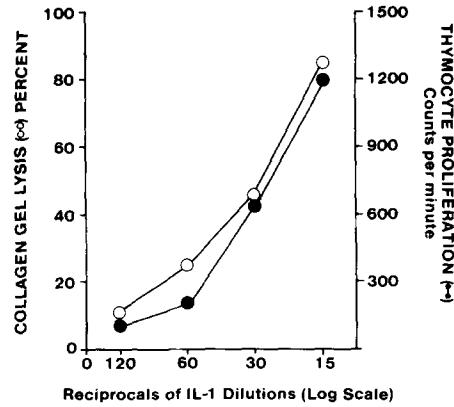


FIG. 1. Human IL-1 was partially purified (diafiltration, ultrafiltration, and isoelectric focusing) from supernatants of cultures of monoblasts-monocytes from a patient with acute monocytic leukemia. IL-1 was added to cultures of mouse thymocytes and foreskin fibroblasts at the dilutions indicated. Collagenase production by fibroblasts and thymocyte proliferation were measured.

TABLE I
Effect of Interleukin 1 on DNA Content of Confluent Fibroblasts

Condition*	DNA per well μg	Collagenase activity
		Percent gel lysis
Fibroblasts + saline	1.06 ± 0.04	0.5 ± 0.3
Fibroblasts + IL-1	0.94 ± 0.03	82.3 ± 1.2

* Foreskin fibroblasts at confluency in monolayer cultures were incubated for 24 h with and without IL-1 (1:20 dilution). Collagenolytic activity was measured in the culture supernatants, and the DNA content of the fibroblast monolayers was determined as described in Materials and Methods.

TABLE II
Interleukin 1 and Fibroblast Protein Synthesis

Condition*	³ H amino acid incorporation		Collagenase activity
	Supernatant cpm	Cell layer cpm	Percent gel lysis
Fibroblasts + saline	310 ± 12	435 ± 25	2 ± 0.1
Fibroblasts + saline + cycloheximide	30 ± 2	47 ± 4	0.6 ± 0.2
Fibroblasts + IL-1	257 ± 19	354 ± 17	94.8 ± 1.5
Fibroblasts + IL-1 + cycloheximide	30 ± 3	74 ± 5	0.1 ± 0.2

* Cycloheximide (7.5 μg/ml) was added for 24 h to cultures of HFL-1 fibroblasts with and without IL-1 (1:20 dilution). Trypan blue exclusion in all cultures was >95%. All samples were added to sextuplet wells of Falcon 3008 multiwell plates containing HFL-1 cells. Triplicate wells for each sample were pulsed with ³H amino acids (see Materials and Methods). Supernatants from fibroblast cultures in the remaining triplicate wells were assayed for collagenase activity.

This suggests that protein synthesis is required for IL-1 to induce the production or release of collagenase from fibroblasts.

HPLC Studies. It was possible that IEF-purified IL-1 contained a contaminating monokine that was responsible for the observed stimulation of collagenase production. In an effort to resolve this issue, IEF-purified IL-1 was subjected to further analysis by high resolution gel filtration- and anion exchange-HPLC. Eluted column fractions

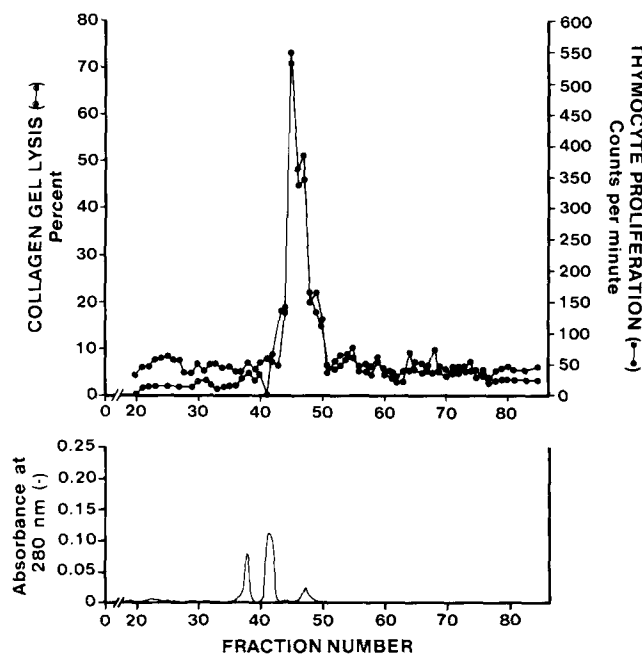


FIG. 2. A sample (0.3 ml) of IEF-purified IL-1 was applied to two I 125 protein analysis columns connected in tandem. Columns were run at a flow rate of 1 ml/min as described in Materials and Methods. Fractions (0.4 ml) were collected and tested at a 1:6 dilution for their effect on foreskin fibroblast collagenase production and thymocyte proliferation.

were tested for their ability to stimulate fibroblast collagenase production and thymocyte proliferation. When IEF-purified IL-1 was fractionated on the gel filtration-HPLC columns, fractions containing maximal IL-1 activity also stimulated fibroblast collagenase production (Fig. 2). The two activities were superimposable on the chromatogram as a single major peak (Fig. 2).

IEF-purified IL-1 also eluted from the ion exchange-HPLC column as a single major peak between 21 and 27% of the salt gradient (Fig. 3). Fractions that stimulated fibroblast collagenase production eluted in the same region of the gradient (Fig. 3). Taken together, these HPLC data indicate that IL-1 possesses the ability to stimulate fibroblast collagenase production.

Discussion

Human IL-1 stimulates collagenase production by fibroblasts in a dose-dependent manner. Analysis of IEF-purified IL-1 by high resolution gel filtration- and anion exchange-HPLC revealed that thymocyte proliferation and fibroblast collagenase-stimulating activities could not be dissociated. These data indicate that human IL-1 possesses, in addition to its previously recognized properties, the ability to enhance collagenase production by connective tissue fibroblasts.

It should be noted that IL-1 from the murine macrophage cell line P388D₁ has been shown to be capable of stimulating collagenase production by adherent rheumatoid synovial cells, and that a human mononuclear cell factor previously recognized to have the same effect on synovial cells has some properties in common with IL-1 obtained from the P388D₁ cell line (3, 12-14). Adherent rheumatoid synovial cells are

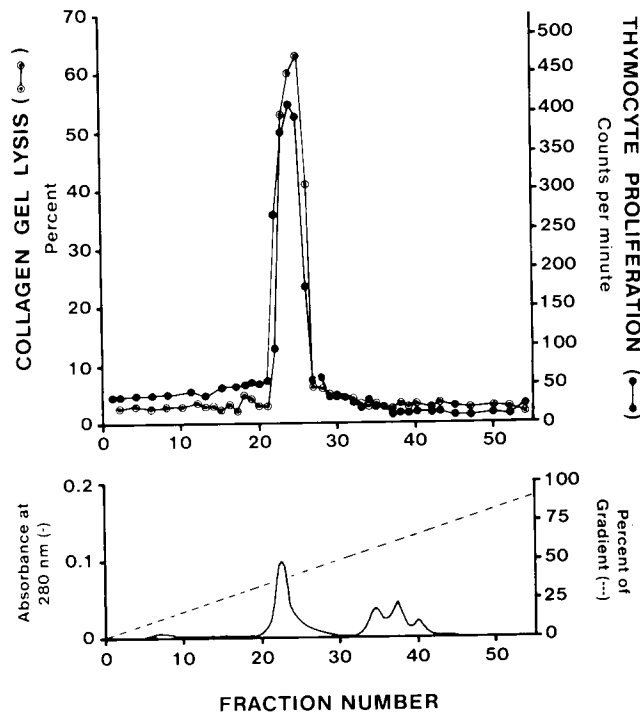


FIG. 3. A sample (0.3 ml) of IEF-purified IL-1 was applied to a SynChropak AX300 anion-exchange column and eluted with a linear salt gradient at a flow rate of 1 ml/min as described in Materials and Methods. Fractions (0.5 ml) were collected and tested at a 1:6 dilution for their ability to stimulate HFL-1 fibroblast collagenase production and thymocyte proliferation.

morphologically and functionally different from connective tissue fibroblasts (e.g., more heterogeneous, larger, and they produce more collagenase and less collagen than fibroblasts), and their true nature and origin remain to be defined (3). In addition, IL-1 from P388D₁ cells is chemically and physically different from human IL-1, as evidenced by the fact that their isoelectric points and protease sensitivities are not the same (7, 8, 15, 16).

Because a variety of different substances (e.g., antigen-antibody complexes, phagocytized particles, antigen- or mitogen-stimulated lymphocytes, lymphokines, and microbial-derived components) have been reported to stimulate the release of IL-1 from macrophages *in vitro*, it is conceivable that IL-1 could be released by monocytes-macrophages at sites of inflammatory reactions of diverse etiologies (1). IL-1 may serve an important role in regulating collagenase production by fibroblasts and thereby influence the remodeling of collagen at sites of inflammation *in vivo*.

Summary

Interleukin 1 is a monokine that exerts biological effects on a variety of target cells *in vitro*. In this report, interleukin 1 has been found to be capable of stimulating collagenase production by cultured dermal fibroblasts. The concentrations of interleukin 1 that stimulate fibroblast collagenase production are similar to those that stimulate mouse thymocyte proliferation. Analyses by high performance liquid chromatography indicate that interleukin 1, rather than a contaminating monokine, is

responsible for this effect on fibroblasts. Interleukin 1, released in vivo by macrophages infiltrating sites of tissue damage or inflammation, may function to stimulate the release of collagenase by connective tissue fibroblasts.

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