Human Recombinant Interleukin 1 Stimulates Collagenase and Prostaglandin E_2 Production by Human Synovial Cells

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Abstract

The pathogenesis and progression of rheumatoid arthritis involves the production of biologically active lymphokines and monokines. Of these, interleukin 1 (IL-1) has been somewhat of a controversial molecule because it seems to evoke various biological responses in several different tissues. In these studies we demonstrate that three biological properties of human monocyte-derived IL-1 (T-lymphocyte activation and human synovial cell prostaglandin E_2 and collagenase production) co-purify. The complementary DNA for the prominent pI 7 form of human IL-1 was expressed, purified, and tested. Any controversy now appears resolved since homogeneous recombinant human IL-1 stimulates prostaglandin E_2 and collagenase from human synovial cells as well as activates T cells in vitro.

Introduction

Mononuclear cell factor (MCF)[1] is a human blood monocyte-derived cytokine that stimulates adherent synovial cells, dermal fibroblasts, chondrocytes, and bone-derived cells to produce increasing amounts of collagenase and prostaglandin E_2 (PGE_2) (1–3). Because of these activities, MCF plays a major role in the pathogenic progression of various arthritides, particularly rheumatoid arthritis (4). In addition to its inflammatory and tissue-destructive activities, MCF shares biochemical and biological properties with lymphocyte-activating factor (LAF) (5), a polypeptide that augments lymphocyte responses to mitogens and antigens. MCF also possesses properties similar to endogenous pyrogen (EP), which produces fever by elevating hypothalamic PGE_2 (6). The current hypothesis is that MCF, LAF, and EP represent the biological activities of either a single peptide or related but distinct proteins grouped together under the name IL-1 (7). IL-1 production is often associated with disease processes. Thus messenger RNA (mRNA) from endotoxin-stimulated human blood monocytes directs oocyte translation of MCF and LAF activity, whereas mRNA from unstimulated blood monocytes lack this property (8–10). We report here that MCF activity coelutes with LAF activity during gel-filtration and elutes from an affinity column of immobilized anti-human EP. Furthermore, homogeneous recombinant human IL-1 stimulates human synovial cells to produce collagenase and PGE_2. Thus, these studies settle any previous dispute since these multiple biological properties do reside in a single polypeptide.

Methods

Human MCF bioassay. Target cells used for the measurement of MCF activity were obtained from freshly dispersed synovium of rheumatoid arthritis patients and from human infant foreskin tissue. Details concerning the culture conditions of these cells have been described previously (8, 9). Various test specimens were diluted in Dulbecco's modified Eagle's medium (DME; Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS) (Gibco) and 5 μg/ml of polymyxin B (Sigma Chemical Co., St. Louis, MO) and then added to synovial cells or dermal fibroblasts (8). After 72 h of incubation, media were removed and assayed for collagenase and PGE_2. Supernatants were treated with trypsin (TRTPCK, Worthington Biochemical Corp., Freehold, NJ), followed by soybean trypsin inhibitor (Worthington Biochemical Corp.). Collagenase activity was measured by incubation with ^{14}C-acetylated salt-soluble rat tail tendon collagen (8). PGE_2 level was measured by a double-antibody radioimmunoassay using an antibody provided by L. Levine (Brandeis University, Waltham, MA) (8). Standard PGE_2 was obtained from Upjohn Corp., Kalamazoo, MI.

Murine LAF assay. Samples were assayed for LAF using the incorporation of [^{3}H]thymidine into thymocytes of 6–8-wk-old C3H/HeJ mice (11) or the murine clonal T cell line, D10.G4.1 (12) stimulated with phytohemagglutinin (PHA, Burroughs Wellcome Co., Triangle Park, NC), 1 μg/ml and in the presence of 5 μg/ml polymyxin B.

MCF preparation. Human peripheral blood mononuclear cells from freshly isolated buffy coats were separated on Ficoll-Hypaque gradients. After 4 h of incubation at 4×10^6 cells/ml in RPMI 1640 supplemented with 2 mM glutamine, 25 mM Hepes, 5% heat-inactivated FCS, 100 μg/ml penicillin, and 100 μg/ml streptomycin, floating cells were removed and adherent cells were washed three times and incubated for 48 h with 1 μg/ml PHA and 10 μg/ml concanavalin A (Pharmacia Fine Chemicals, Piscataway, NJ). Supernatants were dialysed for 48 h against water at 4°C and lyophilized. 50 mg of the lyophilized material was resuspended in 10 mM Tris HCl, pH 7.5, 165 mM NaCl, 5 mM $CaCl_2$, applied to a 2.6 × 90 cm of Ultrogel AcA54 (LKB Produkter; Bromma, Sweden) and eluted with the same buffer at 4°C. Fractions containing MCF activity were pooled and lyophilized.

Preparation of human monocyte interleukin-1 (IL-1). Adherent

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1. *Abbreviations used in this paper:* DME, Dulbecco's modified Eagle's medium; EP, endogenous pyrogen; FCS, fetal calf serum; hrIL-1, human recombinant interleukin 1; IL-1, interleukin 1; LAF, lymphocyte-activating factor; MCF, mononuclear cell factor; PAGE, polyacrylamide gel electrophoresis; PG, prostaglandin; PGE_2, prostaglandin E_2; PHA, phytohemagglutinin.

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monocytes were stimulated with heat-killed *Staphylococcus albus* by previously published methods (13). The crude supernate was purified by elution from the anti-human EP affinity gel followed by gel filtration over Sephadex G-50 (170 × 5 cm). Fractions were assayed for EP and LAF activity. Active fractions in the 15–18-kD range were pooled and subjected to chromatofocusing. Using [^{35}S]methionine labeling of monocytes, this three-step procedure yielded a homogenous 17.5-kD protein as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography that possessed LAF and EP activities (13).

Anti-EP immunoabsorbent. Anti-EP IgG was purified over immunoadsorbents to remove unwanted antibodies (14). The antibody used in the present study was prepared with high specific activity human EP (10) and absorbed before immobilization on Sepharose. 1 ml of phosphate-buffered saline/bovine serum albumin (PBS/BSA) (1 mg/ml BSA) containing 100 mg/ml of lyophilized MCF preparation obtained after AcA54 purification was added to 3 ml of the anti-EP affinity gel and rocked for 2 h at room temperature. The gel was poured into a 1-cm diameter column, washed with PBS, and the MCF was eluted with 3 M NaSCN. Fractions were dialysed against PBS, lyophilized, and resuspended in DME 10% FCS for detecting MCF and LAF activities. Preimmune rabbit IgG bound to Sepharose served as a control column.

Human recombinant IL-1 (hrIL-1). The IL-1 cDNA was expressed by isolating the NcoI-XmnI fragment (bp 295-1,407) from the plasmid pCD 12-1-8 (10) and inserting it into an *Escherichia coli* expression vector. The hrIL-1 expressed is 223 amino acids long and contains 199 of the 269 amino acid long IL-1 precursor form. The alanine amino acid 117 is the NH_2-terminus of the processed IL-1 which is found in supernates of stimulated human blood monocytes (15). The calculated molecular weight of the hrIL-1 is 24,556. hrIL-1 was extracted from the insoluble cellular fraction and purified by sequential ion-exchange and either gel-filtration chromatography or high-performance liquid chromatography. The hrIL-1 was stored at −70°C in physiological saline. The identity of the purified hrIL-1 was confirmed by amino acid composition, the sequence of the amino acid terminal, and by SDS-PAGE (Fig. 1). The endotoxin concentration of the homogeneous hrIL-1 was <20 pg/μg of IL-1 protein using the Limulus amebocyte lysate test (Associates of Cape Cod, Woods Hole, MA). *E. coli* endotoxin as high as 5 μg/ml has no effect in the MCF assay.

Results

Coelution of MCF and LAF activity. Crude MCF elutes from the sizing chromatography column in two peaks; the major peak contains the three biological activities eluted between 14 and 25 kD (Fig. 2 *A*). Another peak of activity eluted between the ovalbumin and the BSA but possessed only collagenase-stimulating and LAF activity but no significant PGE_2-stimulating activity. Active fractions (molecular weight range of 14,000–25,000) were pooled and applied to the anti-EP affinity column. As shown in Fig. 2 *B*, most of the activity was retained on the column and eluted with the addition of 3 M NaSCN. In contrast, when the same material was applied to a control affinity column, only small amounts of PGE_2- and collagenase-inducing activities were eluted from the column after addition of NaSCN, and these are likely due to nonspecific binding to the Sepharose.

Effects of hrIL-1 on production of collagenase and PGE_2 by synovial cells. As shown in Table I, both purified human monocyte-derived IL-1 and hrIL-1 markedly stimulated collagenase and PGE_2 production by human adherent synovial cells. Similar results were obtained using PGE_2 production from dermal fibroblasts (data not shown). The crude *E. coli* extract possessed significantly greater MCF activity when compared with identical dilutions of extract from the same recombinant *E. coli* strain without the IL-1 insert. Two lots of homogeneous hrIL-1 were active in all assays at subnanogram per milliliter concentrations.

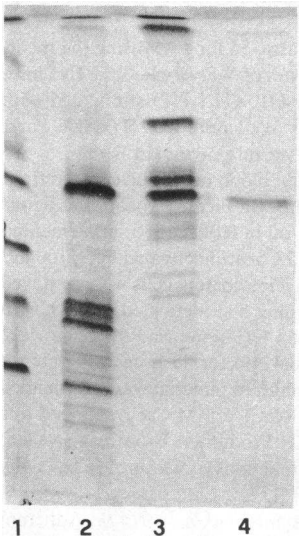

Figure 1. SDS-PAGE (12%, reduced) of hrIL-1 stained with Coomassie blue. Lane *1*, standards; lane *2*, crude extract of IL-1–containing bacterial lysate; lane *3*, lysate after DEAE cellulose chromatography; lane *4*, hrIL-1 (lot 042285) after purification.

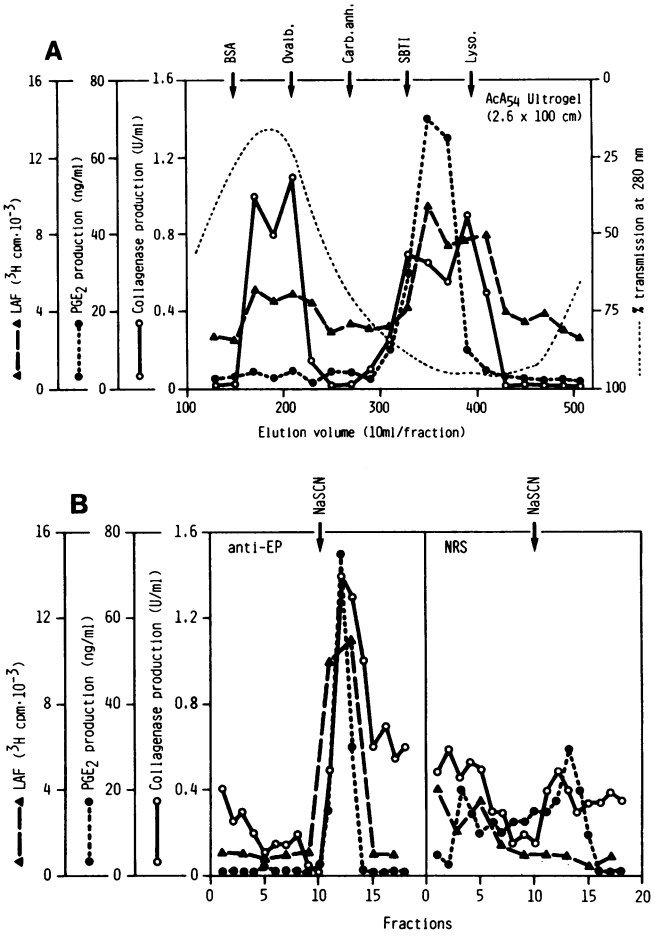

Figure 2. (*A*) Ultrogel AcA54 chromatography of PHA/Con A-stimulated monocyte-macrophage supernatants with MCF and LAF activities as indicated. The thymocyte assay was used for LAF activity. Calibration markers are BSA (bovine serum albumin), ovalb. (ovalbumin), carb. anh. (carbonic anhydrase), SBTI (soybean trypsin inhibitor), lyso. (lysozyme). (*B*) LAF and MCF activity elution profiles from anti-EP affinity column (see Methods). Control column used immobilized preimmune serum.

Table I. Effect of Human Recombinant IL-1 on Collagenase and PGE_2 Production by Human Synovial Cells

Preparations	Concentrations*	Human adherent synovial cells		T cells‡ (index of stimulation)
		PGE_2	Collagenase	
		ng/ml	*U/ml*	
Monocyte-derived IL-1	1:10	667±30	0.22	10.0
	1:100	603±11	0.18	13.5
	1:1,000	187±3	0.08	8.9
	1:10,000	43±10	<0.01	4.7
E. coli extract (with IL-1 insert)	1:1,000	324±17	1.11	30.6
	1:10,000	101±2	0.99	15.3
	1:100,000	28±5	0.40	3.8
E. coli extract (without IL-1 insert)	1:1,000	118±13	0.86	8.3
	1:10,000	9±2	0.19	1.7
	1:100,000	2±1	0.05	1.2
Homogeneous hrIL-1 (lot 69)	500 *ng/ml*	664±63	0.60	9.2
	50 *ng/ml*	268±55	0.24	6.0
	5 *ng/ml*	48±11	0.20	4.0
	0.5 *ng/ml*	41±8	0.09	3.0
Homogeneous hrIL-1 (lot 042285)	1 *μg/ml*	628±42	0.24	16.2
	100 *ng/ml*	352±37	0.19	8.0
	10 *ng/ml*	330±33	0.16	4.7
	1 *ng/ml*	223±14	0.15	2.4
Medium alone		2±1	<0.01	1

* The purified monocyte-derived IL-1 is indicated as dilution of rabbit pyrogenic doses where one dose equals peak fever of 0.6–0.9°C (13). The *E. coli* extracts were diluted after the extraction procedure and the undiluted hrIL-1 protein was estimated on SDS-PAGE to be ~50 μg/ml; the concentration in the assays of the two homogeneous hrIL-1 lots are indicated. ‡ Stimulation index was calculated by setting the PHA response at 1 (11). D10.G4.1 cells were used.

Discussion

To support the hypothesis that MCF and LAF activities are likely due to the same molecule, two methods have been frequently employed: first, the coelution of biological activities during protein purification, and second, the use of apparently homogeneous preparations that possess these biological properties. We initiated our experiments by showing that MCF and LAF activities are found in the same fractions after either gel filtration or antibody affinity chromatography. EP and LAF activities have been followed during protein purifications; apparently homogeneous preparations show a single band on SDS-PAGE (13, 16), and this material also possesses MCF activities. Although both methods have drawbacks, our data support previous observations that MCF and LAF activities are not easily separable. It always seemed likely that MCF and EP were related since MCF production of PGE_2 from synovial cells was biologically similar to EP stimulation of hypothalamic PGE_2 leading to fever (6).

During the course of these studies, human IL-1, as measured by the LAF assay, was cloned (10) and the cDNA expressed in recombinant *E. coli*. As soon as the crude extracts became available, we began testing for MCF activity to help resolve the issue that MCF and LAF reside in the same molecule. Our results now establish that homogeneous hrIL-1 possesses MCF and LAF activities. The ability of a single substance to manifest inflammatory, catabolic, and immunoregulatory activities clarifies an important aspect of the multiple biological functions attributed to IL-1. The hrIL-1 used in the present studies stimulates significant PGE_2 and collagenase activity at <1 ng/ml. To reduce species differences which may influence specific activity, we employed human target cells for MCF activity. The hrIL-1 used in these studies corresponds to the pI 7 form which is the predominant IL-1 isolated from human pathological fluids. It is distinct from the recombinant murine IL-1 which possesses a pI of 5 (17). Although these two forms exhibit <26% amino acid homology, they are related, particularly at the carboxyl terminus. However, the specific activity of the human pI 5 form remains to be established in the human MCF assay.

The clinical and pharmacologic implications of these studies may help explain the chronicity of some arthritides, especially rheumatoid arthritis, since IL-1 results in destruction of connective tissue but is also a source of immunologic stimulation for continued production of antibodies and lymphokines (1–4, 7). Thus therapies designed to alleviate or halt the progression of the disease would best be designed at the level of reduced IL-1 production.

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