Prostaglandin E_2 and Collagenase Production by Fibroblasts and Synovial Cells Is Regulated by Urine-derived Human Interleukin 1 and Inhibitor(s)

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Abstract

Interleukin 1 (IL-1) possesses multiple biological activities that may be blocked selectively by different inhibitors. Some known inhibitors block the lymphocyte activating factor (LAF/IL-1) but not the mononuclear cell factor (MCF/IL-1) measured by its capacity to stimulate prostaglandin E_2 (PGE_2) and collagenase production. The presence of IL-1 in vivo may be difficult to detect due to the presence of inhibitor(s) and the level of the inhibitor(s) may vary depending upon pathological conditions. We have found that urine from three patients with monocytic leukemia (M5) contained high levels of inhibitor(s) of MCF/IL-1, whereas urine of normal subjects did not contain significant amounts. Urine from two patients with other blood neoplasic diseases also contained little inhibitory activity. The MCF/IL-1 inhibitor(s), which also acts on human recombinant IL-1 β, is ~25–35 kD, is not retained on concanavalin A-Sepharose column and can be partially destroyed with urea and boiling. At this stage of the purification the fraction containing the MCF/IL-1 inhibitor(s) also inhibits the LAF/IL-1 assay. However, this inhibitor(s) is probably distinct from other inhibitors already described.

Introduction

Interleukin 1 (IL-1)[1] possesses multiple biological activities; the IL-1 complementary (c)DNA has been cloned and expressed in *Escherichia coli* (1–3). In the immune response IL-1, as defined by lymphocyte-activating factor (LAF), triggers interleukin 2 production. In chronic inflammation, IL-1 defined as mononuclear cell factor (MCF) stimulates various types of cells (e.g., rheumatoid synovial cells and fibroblasts) to produce prostaglandin E_2 (PGE_2) and collagenase, both of which reflect mechanisms of tissue destruction (4–8). IL-1 has been found in synovial fluid (9, 10), serum (11, 12), and urine (13, 14). However, the control of IL-1 activities and its regulation in vivo is poorly understood. Inhibitors of LAF/IL-1 have been revealed in urine (13, 14), serum (15), and cell culture supernatant (16–19), but no in vivo inhibitory activity against MCF/IL-1 has been described as yet. We now report MCF/IL-1 activities (~67, ~35, and ~17 kD) and MCF/IL-1 inhibitor(s) (~25 kD) in urine from patients with monocytic leukemia. These findings may have important implications since such inhibition could counteract the stimulation of collagenase and PGE_2 and suggest a possible balance in regulation of tissue destruction.

Methods

Patients and urine collection. The diagnosis of acute monocytic leukemia (M5 of the French American British classification) (20) was established in three patients. B.M. was a 44-yr-old woman hospitalized for fever without infection. H.P. was a 74-yr-old man hospitalized for high fever and pneumonia. F.C. was a 41-yr-old woman presenting with loss of weight, high fever, and asthenia without infection. Two other patients with neoplasic diseases and high fever (T lymphoma and chronic myelomonocytic leukemia) and five normal donors without fever were also investigated. Urine (400 ml) was collected before any treatment. After addition of 20 μg/ml of gentamycin, urine was dialyzed at 4°C against bidistilled water (3.5 kD, Spectrapore, Spectrum Medical Industries, Inc., Los Angeles, CA). Urine was then concentrated ~20-fold with Aquacide II (Calbiochem-Behring Corp., La Jolla, CA), centrifuged at 9,000 *g* for 30 min at 4°C and stored at −20°C.

MCF/IL-1 preparation. MCF/IL-1 was generated in the supernatant of concanavalin A (Con A, 0.1 μg/ml; Pharmacia Fine Chemicals, Piscataway, NJ) and phytohemagglutinin (0.1 μg/ml Wellcome Research Laboratories, Kent, England) stimulated peripheral blood mononuclear cells (2.10^6 cells/ml, 72 h in RPMI 1640, 10% fetal calf serum, FCS). The supernatant was purified on ultrogel AcA54 gel filtration monitored by the collagenase- and PGE_2-stimulating activity (21). Using human recombinant IL-1 β as a standard, the activity of the MCF/IL-1 preparation in this study was equivalent to 100 pg/ml or 2 U in the C_3H/HeJ mouse thymocyte proliferation assay. One unit represents the 50% stimulation in the assay (6).

PGE_2- and collagenase-stimulating activity and inhibitory test. Dialyzed concentrated urines were tested at various dilutions in Dulbecco's modified Eagle's medium (DME) containing 10% FCS, penicillin 100 U/ml, and streptomycin 100 μg/ml for PGE_2 and collagenase production by dermal fibroblasts or synovial cells (20,000 cells/200 μl per well) in presence or in absence of partially purified MCF/IL-1. After 72 h of incubation in humidifed atmosphere (37°C, 95% air/5% CO_2) cell free supernatants were assayed for PGE_2 by radioimmunoassay (4) and for collagenase activity using ^{14}C-acetylated reconstituted collagen fibrils (8).

Gel chromatography of urine. Dialyzed ~20-fold concentrated urine (15 ml) was chromatographed on ultrogel AcA54 gel with a buffer con-

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1. *Abbreviations used in this paper:* Con A, concanavalin A; DME, Dulbecco's modified Eagles medium; hrIL-1 β, human recombinant IL-1 β; IL-1, interleukin 1; LAF, lymphocyte-activating factor; MCF, mononuclear cell factor; PGE_2, prostaglandin E_2.

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taining 10 mM Tris-HCl (pH 7.5), 165 mM NaCl and 5 mM $CaCl_2$ and eluted fractions (10 ml/fraction) were assayed (dilution 1:5 in DMEM, 10% FCS) in the presence of partially purified MCF/IL-1. A 40–80% $(NH_4)_2SO_4$ precipitated crude urine was chromatographed on a Con-A Sepharose (Pharmacia Fine Chemicals) column equilibrated in 0.05 M Tris-HCl (pH 7.4), 0.2 M NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$. An elution gradient was performed with 0.3 M α-methyl-D-mannoside and fractions were tested in the MCF/IL-1 and LAF/IL-1 assays.

Heat and urea treatment. Active inhibitory AcA54 fractions were boiled for 10 min at 100°C, centrifuged, and tested. Similar fractions were treated with 8 M urea for 1 h, dialyzed against H_2O, and tested.

LAF/IL-1 was measured in the C_3H/HeJ mouse thymocyte proliferation assay (6). Human recombinant IL 1 β (hrIL-1 β) was expressed in *E. coli* at Biogen SA, Geneva, Switzerland. The pyrogen content was <40 pg/mg protein (Wingfield, P., M. Payton, J. Tavernier, M. Barnes, K. Rose, M. G. Simona, S. Demczuk, K. Williamson, and J.-M. Dayer, manuscript in press. *Eur. J. Biochem.*).

Results

Biological activities of concentrated-dialyzed urine. Dialyzed and concentrated urine from B.M. was tested for MCF/IL-1 activity and inhibition against a partially purified MCF/IL-1 preparation. No MCF/IL-1 activity could be detected at any dilution (Fig. 1). In contrast a marked inhibition of PGE_2- and collagenase-stimulating activity induced by a partially purified preparation of MCF/IL-1 was observed up to a dilution of 1:160. This inhibition was partially overcome by increasing amounts of MCF/IL-1. Fibroblast number and morphology were unchanged and viability in cultures exposed to urine and/or to MCF/IL-1 was >95% by trypan blue dye exclusion test.

Biochemical characterization. To determine whether MCF/IL-1 activity can be masked by an inhibitor, urine from two patients (B.M. and H.P.) was fractionated on ultrogel AcA54 gel. Eluted fractions were tested in presence of a constant concentration of MCF/IL-1. MCF/IL-1 activity was eluted in two major peaks ~67 kD, ~35 kD (Fig. 2 *A*) and one additional peak ~17 kD for H.P. (Fig. 2 *B*). The same fractions that exhibited activity in presence of exogenous MCF/IL-1 were found to effectively contain MCF/IL-1 when tested in the absence of exogenous MCF/IL-1 (Fig. 2 *A*). In contrast, a significant inhibition of MCF/IL-1 activity was found close to the soybean trypsin inhibitor (SBTI; 21.5 kD) marker. Since the simultaneous presence of inhibitory and stimulatory MCF/IL-1 activity could be detected in different fractions of urine, the two fractions (B.M.) containing the MCF/IL-1 activity (fractions 16 and 22 or 23) were combined with fractions containing the inhibitory activity (fractions 30 + 31). The fractions containing the inhibitory activity completely inhibited the fractions containing the stimulatory activity as well as the activity of exogenous partially purified MCF/IL-1 alone, as demonstrated by PGE_2 and collagenase production by fibroblasts (Fig. 3). The inhibitory fractions added to human recombinant IL-1 β (hrIL-1 β) alone also inhibited the PGE_2 and collagenase stimulation. When the Con-A Sepharose inhibitory fraction (1:40 dilution) was mixed with 250 pg/ml of hrIL-1 β the value of PGE_2 was 6±1 ng/ml. In the same experiment the basal and the hrIL-1 β stimulated values were, respectively, 8±2 and 340±25 ng/ml. To examine whether the inhibitor(s) was a glycoprotein, urine from H.P. was precipitated with 40–80% ammonium sulfate and the precipitate was chromatographed on a Con A-Sepharose column. As shown in Fig. 4 a major peak of inhibitory activity against LAF/IL-1 and MCF/IL-1 was observed before the elution gradient with α-methyl-D-mannoside. The inhibitory peak inhibitory for LAF/IL-1 observed at the end of the elution is due to the free α-methyl-D-mannoside inhibiting the action of the lectin in the LAF assay, since the end buffer alone was also inhibitory. However, we cannot exclude the possibility that other inhibitory material is masked by the α-methyl-D-mannoside. To determine whether the inhibitor(s) was a protein fractions (AcA54) con-

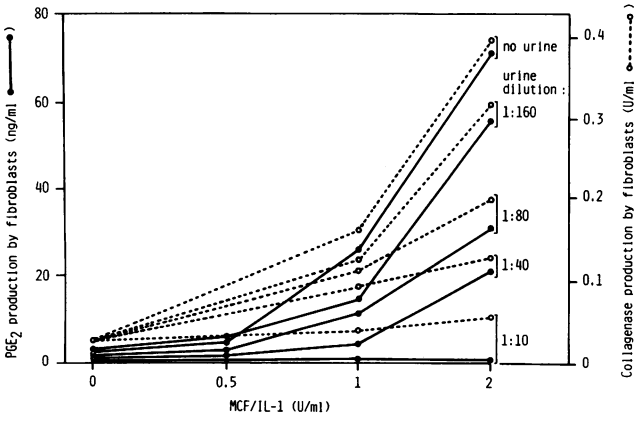

Figure 1. Effects of dialyzed concentrated urine (B.M.) on PGE_2 and collagenase production by fibroblasts in presence or absence of MCF/IL-1. Concentrated (~20-fold) and dialyzed urine (cut off 3,500 mol wt) was mixed with purified MCF/IL-1 and added to fibroblasts (20,000 cells/200 μl per well). After 72 h of incubation cell-free supernatants were assayed for PGE_2 (●) and collagenase (○).

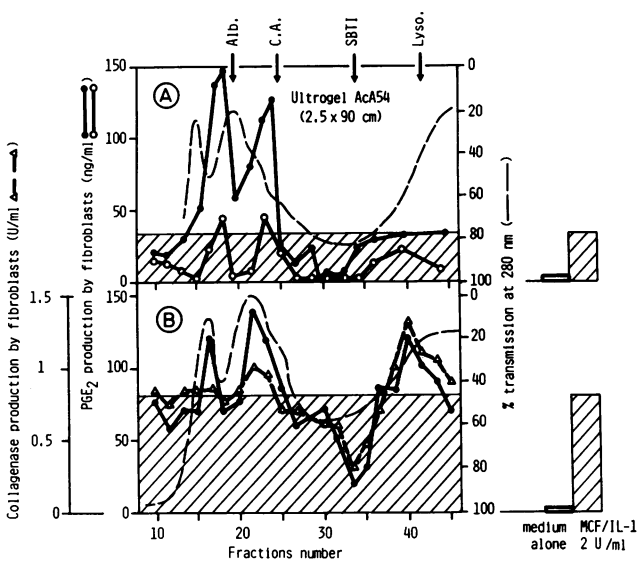

Figure 2. MCF/IL-1 activity and MCF/IL-1 inhibitory activity in chromatographed urine. Dialyzed ~20 fold-concentrated urine (15 ml) from B.M. (*A*) and H.P. (*B*) was chromatographed on ultrogel AcA54 gel buffer and eluted fractions (10 ml/fraction) were assayed (dilution 1:5 in DME, 10% FCS) for inhibition of partially purified MCF/IL-1 as described in Fig. 1. Shaded area represents the level of PGE_2 and collagenase production induced by MCF/IL-1 alone without adding urine samples. (*A*) Eluted fractions were also tested for PGE_2-stimulating activity without addition of exogenous MCF/IL-1 (○). □ and ▩ represent, respectively, the PGE_2 and collagenase production by unstimulated cells (medium alone) or stimulated cells (MCF/IL-1, 2 units). Marker proteins are albumin (Alb. 67 kD), carbonic anhydrase (C.A. 30 kD), soybean trypsin inhibitor (SBTI 215 kD), and lysozyme (Lyso. 14.4 kD).

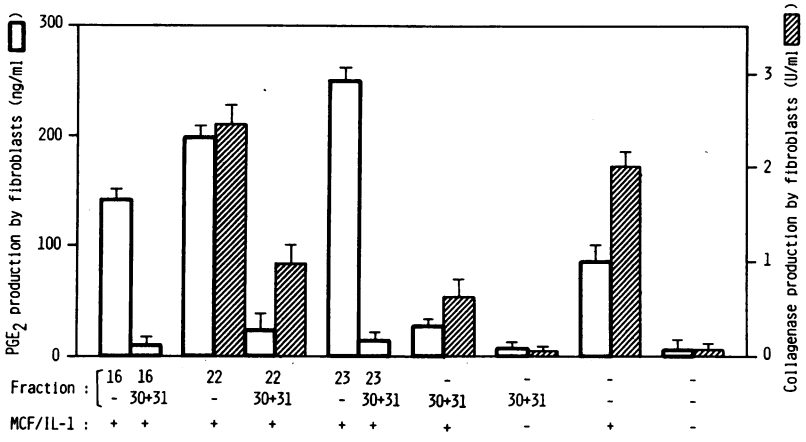

Figure 3. PGE_2 and collagenase-inhibitory activity of chromatographed urine (B.M.). Fractions 16, 11, and 23 containing MCF/IL-1 activity and fractions 30 and 31 containing MCF/IL-1 inhibitory activity were mixed at equal volumes (50 μl/50 μl) and diluted 1:5 with DME, 10% FCS containing purified MCF/IL-1. After 72 h incubation on fibroblasts media were removed and assayed for PGE_2 and collagenase. Values represent mean±SEM (n = 3).

taining inhibitory activity were treated with 8 M urea or boiled for 10 min. These two treatments reduced the inhibitory activity by 54.5 and 78.6%, respectively (Table I).

Patient study. The urine from three patients with monocytic leukemia, two patients with blood neoplasic diseases and five normal subjects were compared for MCF/IL-1 and MCF/IL-1 inhibitory activity. The dialyzed urines and the chromatographed fractions containing the peak of the MCF/IL-1 activity or MCF/IL-1 inhibitory activity were measured in percentages of stimulation of PGE_2 production by fibroblasts and in percentages of inhibition of the activity of MCF/IL-1 stimulated fibroblasts. MCF/IL-1 activity was tested in the absence of additional MCF/IL-1 (Table II). All urine from patients with monocytic leukemia showed a marked inhibitory activity in crude dialyzed and chromatographed material as well as a stimulatory activity when chromatographed. Urines from other patients and normal subjects exhibited significantly lower activities in both inhibitory and stimulatory effects.

Discussion

Previous studies on urine of normal subjects and patients with high fever have reported LAF/IL-1 inhibitors in unfractionated and purified material (13, 14–22). However, these inhibitors found in vivo did not seem to affect MCF/IL-1 as defined by PGE_2 production-stimulating activity (23). In contrast, in a preliminary report, we found that urine from a monocytic leukemia patient (M5) contained inhibitor(s) for MCF/IL-1 (24). A similar biological action has recently also been described in an in vitro study in which an inhibitor of MCF/IL-1 and LAF/IL-1 was found to be produced by monocytes exposed to adherent immune complexes (25).

We now report that unfractionated urines from three patients with monocytic leukemia (M5) contain high levels of inhibitors for MCF/IL-1. In comparison, two other groups of individuals were also studied: two febrile patients with other neoplasic diseases (T lymphoma and chronic myelomonocytic leukemia) and five normal subjects. It is interesting that the same type of observation was made in these two groups although the inhibition/stimulation measured was much lower as compared to that of the M5 patients. Among the five normal subjects only one showed a slight inhibitory activity (10%). At the present stage of purification only semiquantitative measurements have been performed. In the unfractionated urine of our patients we did not detect the presence of spontaneous MCF/IL-1 activity but

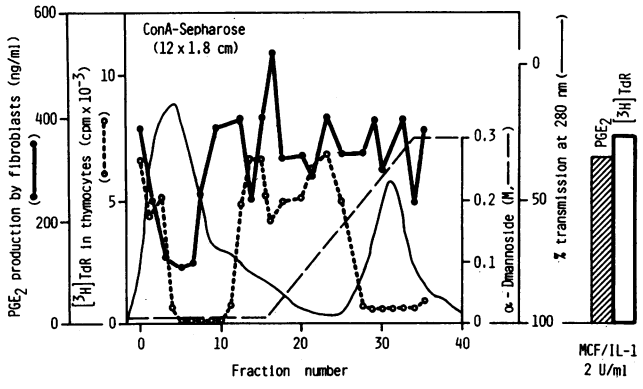

Figure 4. Fresh urine (30 ml from H.P.) was precipitated between 40 and 80% $(NH_4)_2SO_4$. The precipitate 2.85 mg was chromatographed on Con A-Sepharose in 0.05 M Tris-HCl (pH 7.4), 0.2 M NaCl, 1 mM $MgCl_2$, 1 mM CaC_2. An elution gradient was performed with 0.3 M α-D-methyl mannoside. Eluted fraction (5 ml/fraction) were diluted 1:40 and assayed for PGE_2-stimulating activity on dermal fibroblasts (●) and for urine thymocyte proliferation. Bars represent the MCF/IL-1 stimulation alone for PGE_2 (■) and thymocyte proliferation (□).

Table I. Urea and Heat Treatment of the MCF/IL-1 Inhibitor(s)

Test sample	Synovial cells PGE_2	MCF/IL-1 inhibition
	ng/ml	*Percent*
Medium alone	14±2	
IL-1/MCF	145±26	0
Fraction 32 AcA54	15±5	89.7
[Fraction 32 + buffer] + [MCF/IL-1]	5±3	96.6
[Fraction 32 + 8 M urea] + [MCF/IL-1]	66±7	54.5
[Buffer + 8 M urea] + [MCF/IL-1]	153±13	−5.5
[Fraction 32 boiled] + [MCF/IL-1]	31±18	78.6

Fraction 32 (B.M.) containing the inhibitory activity was treated for 1 h with 8 M urea and dialyzed against H_2O. The same fraction was also boiled for 10 min. Values represent mean±SD of three determinations.

Table II. MCF/IL-1 and MCF/IL-1 Inhibitor(s) in Urine of Patients

Patients	Urine*		
	Dialyzed	Chromatographed	
	MCF/IL-1 inhibition‡	MCF/IL-1 stimulation§	MCF/IL-1 inhibition‡
	Percent	*Percent*	*Percent*
Monocytic leukemia ($n = 3$)	88±15 (3/3)	50±23 (2/2)	92±14 (2/2)
Other blood neoplastic diseases ($n = 2$)	25±16 (2/2)	30±20 (2/2)	23±13 (2/2)
Normal subjects ($n = 5$)	2±1 (1/5)	7±5 (3/3)	6±3 (1/3)

* Urine was dialyzed and chromatographed (AcA54 ultrogel) and the fractions containing the peak of the stimulatory or inhibitory activity were pooled separately and added (1:20 dilution) to fibroblast cultures for measurement of PGE_2 production. ‡ MCF/IL-1 in urine was assessed by the PGE_2 production-stimulating activity in absence of additional MCF/IL-1 and expressed as percentage of stimulation over medium alone. 100% represents the maximum PGE_2 stimulation obtained with a standard concentration of MCF/IL-1 alone. PGE_2 values are 25±8 ng/ml in unstimulated cells and 252±45 ng/ml in MCF/IL-1–stimulated cells. § MCF/IL-1 inhibitory activity was measured by the inhibition of the stimulation of PGE_2 production obtained by a standard concentration of MCF/IL-1. In the first column the total number of patients studied is in parentheses. Given in the three columns are the number of patients showing inhibitory or stimulatory activities over the number of patients studied. Values represent mean±SD.

such an activity was very strong following gel filtration. In addition to the absence of inhibitory activity against MCF/IL-1 by the urine-derived LAF/IL-1 inhibitor described by others (23) there was already evidence that the LAF/IL-1 inhibitor(s) was not active in the MCF/IL-1 bioassay. It has been shown that the production of LAF/IL-1 was hampered by inhibitors in supernatants of U937 monocytic cell line, but in contrast the MCF/IL-1 activity was not overcome by these LAF/IL-1 inhibitors (16–18). This indicates that the urinary inhibitor(s) found in our study is different from the one produced by U937 cells or described in urine (23) or liver extracts (26).

It is interesting to note that the urinary and the liver homogenate inhibitor of LAF/IL-1 inhibitors previously described (23, 26) both have a PGE_2-stimulating activity. In addition 1-antitrypsin has been shown to modulate the biological activity of LAF/IL-1, although inhibition requires high concentrations of α_1-antitrypsin (2–4 mg/ml) (27). We have not found inhibition of MCF/IL-1 by adding 0.2 and 2 mg/ml of α_1-antitrypsin to our preparations.

To determine whether the urinary MCF/IL-1 inhibitor(s) is a molecule similar to other glycoproteins with inhibitory activity in the lymphocyte proliferation assay (22) a 40–80% ammonium sulfate–precipitated urine was chromatographed on a Con A-Sepharose gel. The inhibitory activity was not retained on the gel in contrast to uromodulin. The molecular weight (85 kD) also differs from our inhibitor. The MCF/IL-1 activity was not revealed by this chromatography step indicating that MCF/IL-1 coelutes with the inhibitor(s). When the inhibitory fractions of the Con A-Sepharose column were fractionated on an ultrogel AcA54 the MCF/IL-1 activity was then revealed (data not shown). To determine whether the inhibitor(s) was a protein, the AcA54 inhibitory fraction was treated with 8 M urea or boiled. The inhibitory activity was partially destroyed indicating that the inhibitor(s) was very resistant and heat stable. Despite the inconclusive data of Table II, the molecular weight suggests the existence of a protein moiety.

We have also observed inhibitory activity in a low molecular weight range (<5,000, data not shown). This may be due to other substances or breakdown products. At this stage of the purification the fraction containing the MCF/IL-1 inhibitor(s) also contains LAF/IL-1 inhibitor(s). The inhibitor(s) is also acting in presence of human recombinant IL-1 β.

Our observations taken together with the information from others suggest the existence of different inhibitors of different IL-1 biological activities. Only after purification and precise characterization of the inhibitor(s) will it be possible to analyze and quantify the kinetics and balance of MCF/IL-1 and MCF/IL-1 inhibitors in urine that might mirror local events in tissues. This observation represents to our knowledge the first description of an in vivo human natural inhibitor(s) of MCF/IL-1 activity assessed by the stimulation of collagenase and PGE_2 production by fibroblasts and synovial cells. It remains to be established at which cellular level the inhibition takes place. Recent experiments performed with this inhibitor suggest that the MCF/IL-1 inhibition occurs at the receptor level (P. Seckinger, J. Lowenthal, K. Williamson, J.-M. Dayer, and R. MacDonald. Manuscript in preparation). This inhibitor(s) therefore could be considered as an important natural molecule for modulating connective tissue destruction.

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