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Purification and Biochemical Characteristics of Two Distinct Human Interleukins 1 from the Myelomonocytic THP-1 Cell Line

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Received October 29, 1985; Revised Manuscript Received January 9, 1986

ABSTRACT: An effective induction protocol for the production of interleukin 1 (IL 1) by human myelomonocytic cell line (THP-1) cells was developed, and two biochemically distinct human IL 1 peptides were purified. Lipopolysaccharide, silica, and hydroxyurea by themselves did not induce IL 1 production, but these three stimulants in combination had a synergistic effect on the production of IL 1 by THP-1 cells. A 17-kilodalton (kDa) form of human IL 1 with a p*I* of 7.0 (IL 1-β) was purified to homogeneity by sequential chromatography on DEAE-Sephacel, Sephacryl S-200, CM high-performance liquid chromatography (HPLC), and hydroxyapatite HPLC. The recovery of IL 1-β activity was 45%, and the specific activity was 2.3×10^7 units/mg. Both IL 1-β and a second 17-kDa IL 1 moiety with a p*I* of 5.0 (IL 1-α) were also extracted from stimulated THP-1 cells and purified to homogeneity by sequential chromatography on Sephacryl S-200, ion exchange HPLC, and hydroxyapatite HPLC. The recovery of IL 1-β from cell extracts was 5.6%, and the specific activity was 3×10^7 units/mg. In contrast, only 0.85% of IL 1-α was recovered with a specific activity of 5.3×10^7 units/mg. The amino acid sequence of the amino-terminal end and amino acid composition of purified IL 1-β obtained from both culture supernatants and cell extracts of THP-1 cells showed complete identity with those of IL 1-β from culture supernatants of normal human monocytes, suggesting that normal human monocytes and THP-1 cells produce identical intracellular human IL 1-β and release the same activity into the culture supernatant. The amino composition of IL 1-α in cells extracts of THP-1 cells was noticeably different from that of IL 1-β but closely resembled the predicted amino acid composition of the 17-kDa C-terminal portion deduced from the published cDNA nucleotide sequence of IL 1-α, further supporting the existence of two distinct human IL 1 genes. In conclusion, the purification and biochemical characterization of two distinct human IL 1 moieties (IL 1-α and IL 1-β) from a human myelomonocytic cell line establish that this leukemic cell line produces two distinct IL 1 moieties that are biologically and biochemically identical with the products of normal human monocytes. Consequently, THP-1 cells provide a good source of IL 1 that is identical with that produced by normal human monocytes.

We have previously reported the purification of human IL 1-β[1] (p*I* 7.0, molecular mass 17 kDa) from culture supernatants of normal human peripheral blood adherent cells (monocytes) and that such purified IL 1-β has thymocyte comitogenic activity, fibroblast proliferation activity, acute phase protein inducing activity, and endogenous pyrogen activity (Matsushima et al., 1985a). The amount of purified IL 1-β that could be obtained from normal human monocytes was very limited. For example, 5 L of supernatants from $\sim 5 \times 10^9$ cultured adherent cells that were derived from 25×10^9 peripheral blood mononuclear cells yielded only 4 μg of IL 1 (Matsushima et al., 1985a). But, this did not suffice for studies of the primary structure and possible posttranslational modifications of human IL 1. Although the purification of human IL 1 from human cell lines could potentially provide large amounts of IL 1, only a few papers report low levels of IL 1 production by human cell lines (Palacios et al., 1982; Krakauer & Oppenheim, 1983). Furthermore, March et al. have recently cloned two distinct (acidic and neutral) human IL 1 (IL 1-α and IL 1-β) cDNA coding for two distinct human IL 1 proteins (March et al., 1985), which probably correspond with the p*I* 5.0 and 7.0 peaks of natural human IL 1 activity detected by isoelectrofocusing (Matsushima, 1985b). However, so far, there is no direct evidence of the existence of human IL 1-α in either culture supernatants or cell extracts. We have, therefore, developed an efficient induction protocol

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[1] Abbreviations: IL 1, interleukin 1; FCS, fetal calf serum; LPS, lipopolysaccharide; HU, hydroxyurea; D-PBS, Dulbecco's phosphate-buffered saline; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; NaPB, sodium phosphate buffer; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; 2-ME, 2-mercaptoethanol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride.

for obtaining a larger amount of IL 1 activity utilizing a human myelomonocytic cell line (THP-1) (Krakauer & Oppenheim, 1983) and report here the purification and biochemical characteristics of two distinct human IL 1 moieties present in culture supernatants and cell extracts of THP-1 cells.

Materials and Methods

Culture Conditions for Human Myelomonocytic Cell Line THP-1. THP-1 (human myelomonocytic cell line) (Tsuchiya et al., 1980) was obtained from the American Type Culture Collection (ATCC, Rockville, MD). This cell line was freed of contaminant *Mycoplasma fermentans* by Dr. A. Nordin (F. S. Key Medical Center, NIA, NIH, Baltimore, MD). THP-1 cells were grown and expanded by spinner cultures in RPMI 1640 (Microbiological Associates, Walkersville, MD) with 10% fetal calf serum (FCS) (Hyclone, Logan, VT), 200 μg/mL L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5% CO_2 in humidified air. IL 1 was produced by incubating 2×10^6 cells/mL in RPMI 1640 with only 1% FCS and with 1 μg/mL lipopolysaccharide (LPS; *Escherichia coli*, 055:B55, Difco, Detroit, MI), 50 μg/mL silica (0.014 μm, Sigma, St. Louis, MO), and 2 mM hydroxyurea (HU; Sigma). After 48-h incubation, cells and supernatants were separated by centrifugation at 400*g* at 4 °C for 7 min; cell pellets were washed twice with Dulbecco's phosphate-buffered saline (D-PBS), and both supernatants and cells were frozen at −80 °C before processing. To extract IL 1 activity from cells, 9 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS; Pierce, Rockford, IL) in D-PBS was added to cells at 4 °C for 1 h with stirring and dialyzed against 10 000 volumes of D-PBS for 24 h at 4 °C. Insoluble material was removed by centrifugation at 20000*g* at 4 °C for 30 min.

Assay of Thymocyte Proliferative Response to IL 1. Single-cell suspensions of thymocytes from C3H/HeJ mice (female, 6 to 10 weeks old) were cultured at 1.5×10^6 cells/200 μL per well in RPMI 1640 medium supplemented with 5% v/v FCS, 2.5×10^{-5} M 2-mercaptoethanol, 0.5 μg/mL concanavalin A (Calbiochem, La Jolla, CA), 100 units/mL penicillin (Gibco), 100 μg/mL streptomycin (Gibco), 50 μg/mL gentamycin (Gibco), and 200 μg/mL L-glutamine (Gibco), in the presence of serially diluted test samples for 72 h. Cultures were pulsed with 0.5 μCi of [^{3}H]thymidine (2 Ci/mmol, New England Nuclear, Boston, MA) per well for the final 16 h of incubation and harvested, and the radioactivity was incorporated in DNA determined in a liquid scintillation counter (Beckman, Berkeley, CA). A unit of activity was defined as the reciprocal of the dilution at which 50% of the maximum response was obtained (Matsushima, 1985a).

Gel Chromatography. The 4.0-mL samples were applied to a 2.6 × 90 cm column of Sephacryl S-200 (Pharmacia, Sweden) equilibrated with D-PBS supplemented with 0.01% polyethylene glycol (PEG, M_r 8000, Sigma) at 4 °C. The flow rate was adjusted to 15.0 mL/h, and 3.0-mL fractions were collected. The column was calibrated with bovine serum albumin (M_r 68 000), ovalbumin (M_r 43 000), chymotrypsinogen A (M_r 25 000), and cytochrome *c* (M_r 12 500).

HPLC. Every HPLC manipulation was performed at room temperature. An Altex Model 110 (Altex, Berkeley, CA) HPLC system was used for the purification of IL 1-β from culture supernatants and a LKB 2150 (LKB, Sweden) HPLC system was used for the purification of IL 1-α and IL 1-β from THP-1 cell extracts.

(*A*) *Cation-Exchange HPLC.* The samples were dialyzed against 0.05 M acetate buffer, pH 5.5 at 4 °C, and applied to a 6.0 mm × 15 cm IEX-535 CM column (Toyo Soda, Japan) equilibrated with the same buffer at room temperature. The starting buffer was 0.05 M acetate buffer, pH 5.5, and the limiting buffer was 0.05 M acetate buffer–0.5 M NaCl, pH 5.5. The flow rate was 1.0 mL/min, and 2.0-mL fractions were collected.

(*B*) *Anion-Exchange HPLC.* The samples were dialyzed against 0.02 M Tris-HCl buffer, pH 8.0 at 4 °C, and applied to a 250 × 4.1 mm SynChropak AX 300 column (SynChrom, Inc., Linden, IN) equilibrated with the same buffer at room temperature. The starting buffer was 0.02 M Tris-HCl buffer, pH 8.0, and the limiting buffer was 0.02 M Tris-HCl buffer–0.5 M NaCl, pH 8.0. The flow rate was 1.0 mL/min, and 2.0-mL fractions were collected.

(*C*) *Hydroxyapatite HPLC.* The samples were dialyzed at 4 °C against 0.01 M sodium phosphate buffer (NaPB) at 4 °C, pH 7.0, 0.3 mM $CaCl_2$, and applied to a 100 × 7.8 mm hydroxyapatite (HPHT) column (Bio-Rad, Richmond, CA) equilibrated with the same buffer at room temperature. The starting buffer was 0.01 M NaPB, pH 7.0–0.3 mM $CaCl_2$, and the limiting buffer was 0.3 M NaPB, pH 7.0–0.01 mM $CaCl_2$. The flow rate was 1.0 mL/min, and 2.0-mL fractions were collected.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). Electrophoresis was carried out with a vertical slab gel of 0.75-mm thickness, 12.5% (w/v) acrylamide, in a discontinuous Tris–glycine buffer system as described by Laemli (1970). Samples were applied in 25% glycerol in this glycine buffer. As molecular weight standards, phosphorylase *b* (M_r 94 000), albumin (M_r 67 000), ovalbumin (M_r 43 000), carbonic anhydrase (M_r 30 000), trypsin inhibitor (M_r 20 100), and α-lactalbumin (M_r 14 400) from Pharmacia were used. After electrophoresis at 10 mA for 6 h, the gel was stained with a Bio-Rad silver staining kit or as originally described (Merrill et al., 1979).

Amino Acid Composition Analyses of Human IL 1. After being desalted into 5% (v/v) acetic acid in distilled water, an aliquot of purified human IL 1 was lyophilized and then hydrolyzed with 6 N HCl containing 0.1% phenol, at 110 °C, for 24 h and analyzed on a Durrum D-500 amino acid analyzer (Copeland et al., 1980).

N-Terminal Sequence Analyses of Human IL 1. Purified protein was subjected to gas-phase automated Edman degradation (Thewick et al., 1981) in an Applied Biosystems sequenator, Model 470A, as described (Yoshinaka et al., 1985). Conversion to the phenylthiohydantoin (PTH) derivative of each amino acid was accomplished with 25% (v/v) trifluoroacetic acid. PTH amino acids were then characterized and quantitated on a Waters phenylalkyl reverse-phase column (Henderson et al., 1980).

Determination of Protein Concentration in Column Eluates. Protein concentration in column eluates was monitored at 280 nm by a spectrophotometer. Protein concentration of pooled fractions from each purification step was measured by a Bio-Rad dye protein assay with bovine serum albumin as a standard.

Results

Induction of IL 1 Activity from THP-1 Cells. Several combinations of stimulants, including LPS, silica (Dinarello, 1984), and hydroxyurea (Luger et al., 1981), were examined for their capacity to induce the production of IL 1 activity by THP-1 cells. A total of 2×10^6 THP-1 cells in 1 mL of RPMI 1640 with 1% FCS was cultured for 48 h in the presence of 1 μg/mL LPS, 50 μg/mL silica, and/or 2 mM HU. As shown in Table I, either medium alone or LPS alone did not induce detectable levels of supernatant IL 1 activity (thymocyte

Table I: Agents Inducing Production of IL 1 Activity by THP-1 Cells

	medium alone	LPS (1 μg/mL)	silica (50 μg/mL)	LPS + silica	HU (2 mM)	LPS + silica + HU
supernatant (units/mL)[a]	<1.0	<1.0	9.0	21.4	<1.0	124
cell extract (units/2 × 10^6 cells)[a]	<1.0	<1.0	<1.0	<1.0	<1.0	55

[a] A total of 2 × 10^6 cells/mL of THF-1 cells was cultured in RPMI 1640–1% FCS in the presence of LPS, silica, and/or HU for 48 h. Culture supernatants and cell pellets were collected by centrifugation. Cellular IL 1 was extracted from cells with 9 mM CHAPS. Prior to thymocyte comitogenic assay, culture supernatants and cell extracts were dialyzed against RPMI 1640 medium and filter-sterilized.

Table II: Purification Scheme for Human IL 1 from THP-1

		total protein (μg)	total IL 1 act. (units)[a]	% recovery of IL 1 act.	sp act. (units/mg)
A	concentrated and dialyzed supernatants[e]	1 870 000[b]	153 000	100	82
	DEAE-Sephacel	181 000[b]	113 000	74.2	626
	Sephacryl S-200	4 190[b]	139 000	90.9	33 200
	HPLC CM	90[b]	87 500	57.2	972 000
	HPLC hydroxyapatite	3.0[c]	68 800	45.0	22 900 000
B	cell extracts[f]	836 000[b]	4 960 000	100	5 940
	Sephacryl S-200	36 000[b]	810 000	16	22 500
B-i	HPLC CM (1st)	269[b]	769 000	15.4	2 830 000
	HPLC hydroxyapatite	NT[d]	642 000	12.9	NT
	HPLC CM (2nd)	7.3[c]	277 000	5.6	37 900 000
B-ii	HPLC CM (1st)	NT	74 000	1.5	NT
	HPLC AX 300	NT	84 000	1.7	NT
	HPLC hydroxyapatite	0.79[c]	42 000	0.85	53 200 000

[a] One unit is the reciprocal of dilution at which 50% of maximal proliferative response of thymocytes to standard IL 1 was obtained. [b] Protein concentration was determined by Bio-Rad dye protein assay with bovine serum albumin as standard. [c] Protein concentration was determined by amino acid composition analysis. [d] NT = not tested. [e] Four liters of culture supernatants was concentrated and dialyzed by a hollow fiber system. [f] Cell extracts were prepared from about 1.8 × 10^{11} cells with 9 mM CHAPS in D-PBS at 4 °C.

comitogenic activity), silica alone induced low levels of IL 1 activity, and LPS and silica together induced moderate levels of IL 1 activity in the supernatants. HU alone did not induce detectable levels of IL 1 activity, but LPS, silica, and HU together exhibited greater synergy in inducing IL 1 activity in the supernatants. Furthermore, considerable IL 1 activity was also detected in the cell extracts of THP-1 cells that were stimulated with LPS, silica, and hydroxyurea.

Purification of IL 1 from THP-1 Cell Culture Supernatants. As summarized in Table IIA, 4 L of pooled culture supernatants containing 153 000 units of IL 1 activity was used as a starting material. Culture supernatants were sequentially concentrated and dialyzed by a hollow fiber system (molecular weight cutoff 10 000) and treated with DEAE-Sephacel at 0.02 M NaPB, pH 7.4, for 30 min with gentle shaking, and the nonabsorbed fraction was concentrated to 4.0 mL for application on a Sephacryl S-200 gel filtration column. IL 1 activity, which was eluted from S-200 in the range of 10–30 kDa, was pooled, concentrated to 4.0 mL, and dialyzed against 0.05 M acetate buffer, pH 5.5. The dialyzed sample was applied to a CM-HPLC column equilibrated with the same buffer. IL 1 activity eluted only at ~0.05 M NaCl predominantly in a single absorbance peak. This eluting position was almost identical with that of human IL 1-β derived from normal monocytes (Matsushima et al., 1985a). At this stage, the specific activity was 9.7 × 10^5 units/mg. But, since the specific activity of IL 1-β, which was purified from human monocyte culture supernatants, was 4.3 × 10^7 units/mg (Matsushima et al., 1985a), the IL 1 containing CM-HPLC fraction was dialyzed against 0.01 M NaPB, pH 7.0, and further applied to a HPLC hydroxyapatite column. Two peaks at the break-through fractions and the fractions corresponding to ~0.05 M NaPB were detected by absorbance at 280 nm, and the IL 1 activity was completely coeluted with the latter peak. The purity of this IL 1 preparation was confirmed by SDS–PAGE. As shown in Figure 1a, only one 19-kDa band was detected with silver staining, and the specific activity was 2.3 × 10^7 units/mg.

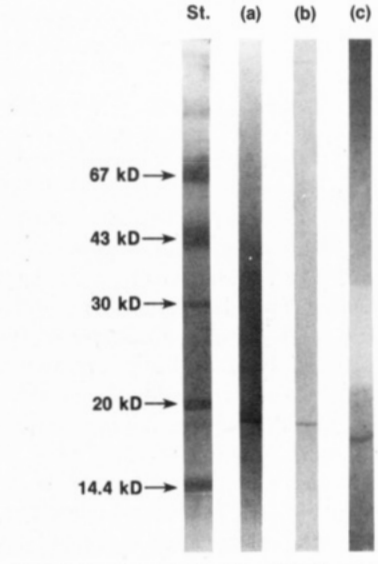

FIGURE 1: SDS–PAGE (12.5%) of purified human IL-1. A total of 20 μL of untreated purified IL-1 from the HPLC hydroxyapatite (right lane) fraction was applied to a 12.5% polyacrylamide gel. After electrophoresis at 10 mA for 6 h, the gel was stained with silver staining. Molecular weight standards (St) were applied in the left lane. (a) Purified neutral IL 1 from THP-1 supernatants; (b) purified neutral IL 1 from THP-1 cell extracts; (c) purified acidic IL 1 from THP-1 cell extracts.

Purification of Two Forms of IL 1 from Cell Extracts. Since high levels of IL 1 activity were present in the cell extracts, this extracted IL 1 activity was also purified. As summarized in Table IIB-i, cell pellets containing 1.8 × 10^{11} cells were extracted with 500 mL of 9 mM CHAPS in D-PBS and dialyzed against D-PBS, and the insoluble material was removed by centrifugation. The supernatant containing 4 960 000 units of IL 1 was concentrated and applied to a Sephacryl S-200 gel filtration column. IL 1 activity was eluted from 10 to 30 kDa, pooled, dialyzed against 0.05 M acetate buffer, pH 5.5, and applied to a HPLC CM column. As shown

Table III: Amino Acid Composition of Interleukin 1 (mol %)

	purified IL 1 (p*I* = 7.0) from THP-1 supernatants	purified neutral (p*I* = 7.0) IL 1 from THP-1 cell extracts	purified neutral (p*I* = 7.0) IL 1 from PBMo supernatants[b]	deduced from cloned neutral human (p*I* = 7.0) IL 1[c]	purified acidic IL 1 from THP-1 cell extracts (p*I* = 4.9)	deduced from cloned acidic IL 1[d]
Asp	10.8	11.8	10.6	11.1	11.3	12.6
Thr	3.8	3.8	4.1	3.9	6.1	7.5
Ser	8.7	9.2	8.9	9.2	8.7	6.3
Glu	15.9	15.8	14.6	15.0	12.7	10.1
Pro	4.7	5.1	4.9	5.2	3.5	4.4
Gly	11.3	8.7	13.8	5.2	14.6	3.1
Ala	3.8	4.5	4.1	3.3	7.6	8.8
Val	6.9	6.5	5.7	7.2	4.1	4.4
Met	2.9	1.6	2.4	3.9	1.2	1.9
Ile	3.2	3.0	3.3	3.3	4.6	8.2
Leu	8.8	9.9	8.9	9.8	7.7	9.4
Tyr	3.1	2.2	2.4	2.0	3.4	4.4
Phe	5.1	5.5	4.9	5.2	4.5	6.2
His	0	1.0	0.8	0.7	2.0	1.9
Lys	8.3	9.3	8.1	9.8	5.2	6.9
Arg	2.7	2.1	2.4	2.6	2.7	1.9
Cys	ND[a]	ND	ND	1.3	ND	0.6
Trp	ND	ND	ND	0.7	ND	1.3

[a] ND = not determined. [b] IL 1, p*I* = 7.0, M_r 17 000, was purified to homogeneity as reported previously (Matsushima, 1985a). [c] Deduced amino acid composition (117th to 269th amino acid = C-terminal 153 residues) from neutral IL 1 cDNA (Auron et al., 1984). [d] Deduced amino acid composition (113th to 271th amino acid = C-terminal 159 residues) from acidic IL 1 cDNA (March et al., 1985).

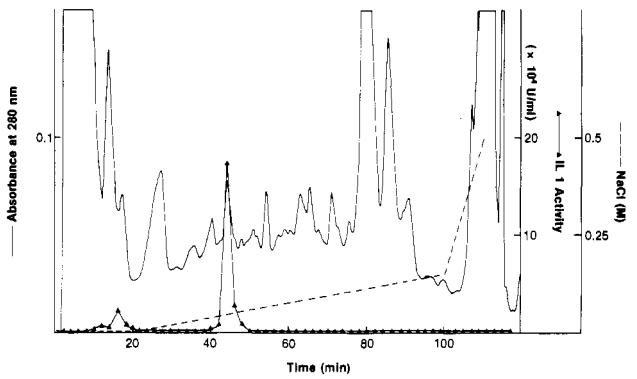

FIGURE 2: CM HPLC of acidic and neutral IL 1 from THP-1 cell extracts. A sample from Sephacryl S-200 gel filtration was applied to a 6.0 mm × 15 cm column of IEX-535CM. The starting buffer was 0.05 M acetate buffer, pH 5.5, and the limiting buffer was 0.05 M acetate buffer, pH 5.5–0.5 M NaCl. The flow rate was 1.0 mL/min, and 2.0-mL fractions were collected. After chromatography, the units of IL 1 activity in each fraction were determined by a thymocyte proliferation assay.

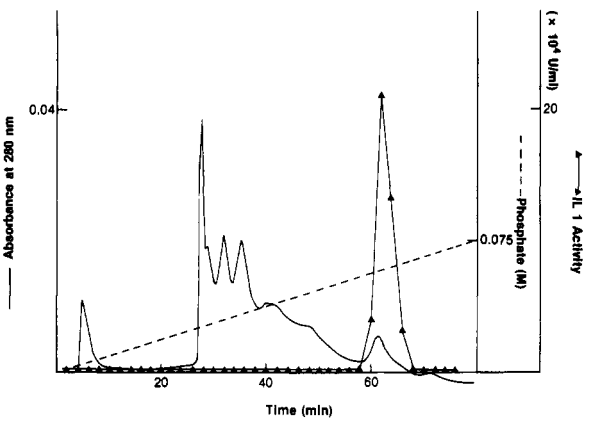

FIGURE 3: Hydroxyapatite HPLC of neutral IL 1 (fractions 22–24 in CM HPLC) from THP-1 cell extracts. The sample from HPLC cation exchange was applied to a 100 × 7.8 mm column of hydroxyapatite. The starting buffer was 0.01 M NaPB, pH 7.0–0.3 mM $CaCl_2$. The flow rate was 1.0 mL/min, and 2.0-mL fractions were collected. After chromatography, the units of IL 1 activity in each fraction were determined by thymocyte comitogenic assay.

in Figure 2, IL 1 activity appeared at break-through fractions (8–20 min) and fractions at ~0.05 M NaCl (42–46 min), suggesting the existence of acidic and basic IL 1 activities. First, the major basic IL 1 activity was concentrated, dialyzed against 0.01 M NaPB, pH 7.0, and applied to a HPLC hydroxyapatite column. As shown in Figure 3, IL 1 activity appeared at ~0.05 M NaPB (60–66 min) as a single absorbance peak. The purity of this IL 1 preparation was examined by SDS–PAGE and two bands (19 and 16 kDa) were detected. Consequently, IL 1 containing hydroxyapatite fractions were rechromatographed on CM HPLC under the same conditions with the previous CM HPLC, and this yielded only one major peak at ~0.05 M NaCl (42–46 min), which was completely identical with IL 1 activity. As shown in Figure 1b, only one band was detected at 19 kDa by SDS–PAGE with silver staining, and the specific activity was 3.8×10^7 units/mg. Second, the minor acidic IL 1 activity that passed through a CM HPLC column (Figure 2) was concentrated, dialyzed against 0.02 M Tris-HCl, pH 8.0, and applied to an AX 300 anion exchange HPLC column. The IL 1 activity was only eluted at ~0.2 M NaCl. IL 1 containing fractions were pooled, concentrated, and applied further to a HPLC hydroxyapatite column. As shown in Figure 4, IL 1 activity was eluted at ~0.025 M NaPB corresponding to one of the three absorbance peaks. The purity of this IL 1 preparation was confirmed by SDS–PAGE, and as shown in Figure 1c, only one specific 18-kDa band was detected by the original silver staining method (Merrill et al., 1979). The specific activity was 5.3×10^7 units/mg (Table IIB-ii). The isoelectric point of the purified acidic form of IL 1 was 4.9 as determined by agarose isoelectrofocusing (data not shown).

Amino Acid Composition and N-Terminal Sequence Analyses of Both Forms of Purified IL 1. As shown in Table III, the amino acid composition of the purified neutral form of IL 1 from both culture supernatants and cell extracts of THP-1 cells was essentially identical with that of human IL 1-β purified from normal peripheral blood monocytes. The amino acid composition was also identical, except for Gly, with that predicted from the amino acid sequence deduced from the cDNA sequence for human IL 1-β (Auron et al., 1984), assuming the amino terminal of the 17-kDa IL 1 starts from ^{117}Ala-^{118}Pro as shown below. However, the amino acid

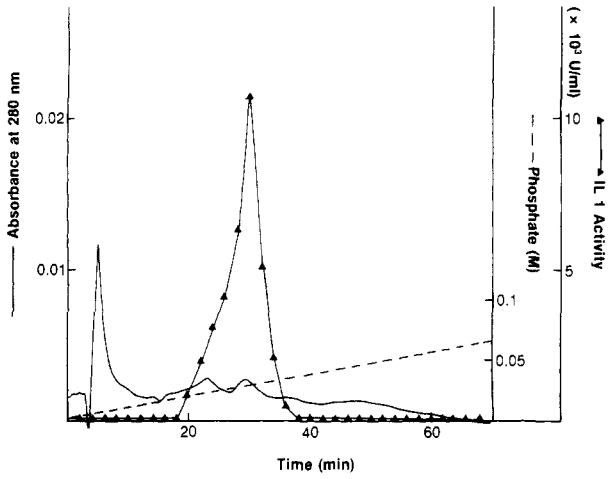

FIGURE 4: Hydroxyapatite HPLC of acidic IL 1 from THP-1 cell extracts under the same conditions as described in Figure 3.

composition of the acidic-form IL 1 from cell extracts differed from that of human IL 1-β in Thr, Ala, Val, His, and Lys content, but was similar to that of the C-terminal portion (159 residues) of IL 1-α (March et al., 1985) except for Gly and Ile.

As shown in Figure 5, the amino-terminal sequence analyses of the purified neutral form of IL 1 from both the supernatants and cell extracts of THP-1 cells showed complete identity with that of purified IL 1-β from human monocytes and a part of the deduced amino acid sequence of the human IL 1-β cDNA sequence. Asn at position 7 is not glycosylated even though it is a potential site because of the Asn-X-Thr sequence. The amount of acidic IL 1 recovered from the cell extracts was insufficient to determine the amino-terminal sequence.

DISCUSSION

We have developed an effective induction protocol for the production of high levels of human IL 1 activity by human myelomonocytic THP-1 cell line cells. LPS, silica, and HU by themselves did not effectively induce IL 1 production, but these three stimulants in combination had a considerable synergistic effect on the IL 1 production by THP-1 cells. The level of IL 1 production becomes almost equivalent to that produced by the same number of normal human monocytes stimulated with LPS and silica ($\sim$100 units/10^6 cells). Since hydroxyurea is known as a G_1/S-phase cell cycle blocker (Steinerb, 1969), the augmentation of IL 1 production of THP-1 cells by hydroxyurea suggests that IL 1 production mainly occurs in the late G_1 phase of cell cycle. In our experience, the same concentration (2 mM) of hydroxyurea had no effect on the production of IL 1 by normal human monocytes, which do not usually proliferate in vitro.

We successfully purified biochemically identical neutral (pI = 7.0) human IL 1-β moieties from both culture supernatants and cell extracts of stimulated THP-1 cells. The final recovery of IL 1-β activity from THP-1 cell culture supernatants was 45% with a specific activity 2.3 × 10^7 units/mg, whereas the recovery from THP-1 cell extracts was only 5.6% with specific activity of 3.8 × 10^7 units/mg. In purifying IL 1-β from cell extracts, the major loss of IL 1 activity occurred during concentration, dialysis, and Sephacryl S-200 gel filtration, probably due to degradation of IL 1-β by proteases present in the cell extracts. Simultaneously, we also purified biochemically distinct acidic IL 1 (pI = 4.9) with 0.85% recovery and 5.3 × 10^7 units/mg of biological activity from cell extracts. We were not able to recover acidic IL 1 from THP-1 supernatants, which was removed by the first step of DEAE-Sephacel fractionation, because of high levels of serum protein contamination in the fractions containing acidic IL 1. The purified neutral IL 1 from culture supernatants and cell extracts showed identity in molecular weight, amino acid composition, and N-terminal sequence analyses to peripheral monocyte-derived IL 1-β and to the 17-kDa C-terminal portion of IL 1-β deduced from the nucleotide sequence of cDNA, confirming that IL 1 in the supernatants is directly derived from cells. Recently, March et al. have reported the existence of two distinct IL 1 cDNA (March et al., 1985). The neutral form of IL 1-β contributes >90% of IL 1 activity produced by normal human monocytes or THP-1 cells. Nevertheless, we were able to purify a minor biochemically distinct acidic IL 1, which was similar in predicted isoelectric point, molecular weight, and deduced amino acid composition of 17-kDa C-terminal portion to IL 1-α (March et al., 1985), from THP-1 cell extracts. This result confirms the existence of at least two "natural" forms of human IL 1 in the THP-1 monocytic cell line cells.

Recently, we have proposed that IL 1 is a cytosolic nonsecretory protein, on the basis of the intracellular distribution of IL 1 activity in LPS-activated monocytes or THP-1 cells. IL 1 activity in cytosol fraction of freshly isolated human monocytes has a molecular mass of 23 kDa as estimated by HPLC gel filtration (Matsushima et al., 1985c). Our successful purification of IL 1 from cell extracts of THP-1 cells

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                    (115)          (120)          (125)          (130)      (135)

a).....Ala·Tyr·Val·His·Asp·Ala·Pro·Val·Arg·Ser·Leu·Asn·Cys·Thr·Leu·Arg·Asp·Ser·Gln·Gln·Lys·Ser·
       Leu·Val·Met.....

b)                        Ala·Pro - X - X - X - Leu·Asn - X - Thr·Leu - X - X - X - Gln·Gln.....

c)                        Ala·Pro - X - X - X - Leu·Asn - X - Thr·Leu - X - X - X - Gln·Gln.....

d)                        Ala·Pro·Val·Arg·Ser·Leu·Asn·X·Thr·Leu·Arg·Asp·Ser·Gln·Gln·Lys·
       Ser·Leu - X - Met.....

e).....Ala·Cys·Ser·Ile·Ser/Ala·Pro·Ala·Arg·Ser·Pro·Ser·Pro·Ser·Thr·Gln·Pro·Trp·Gln·His·Val·
       Asn·Ala·Ile·Gln.....

f).....Leu·Val·Thr·Asn·Ser/Ala·Pro·Thr·Ser·Ser·Ser·Thr·Lys·Lys·Thr·Gln·Leu·Gln·Leu·Gln·His·Leu·
       Leu·Leu·Asp.....
```

FIGURE 5: Comparison of amino-terminal sequences of human neutral IL 1 purified from different sources with those of human GM-CSF and human IL 2: (a) amino acid sequence of IL 1 deduced from IL 1-β cDNA (Auron et al., 1984); (b) amino-terminal sequence of neutral IL 1 purified from human peripheral blood monocytes (Matsushima et al., 1985a); (c) N-terminal sequence of IL 1-β from THP-1 supernatants; (d) N-terminal sequence of IL 1-β from THP-1 cell extracts; (e) amino acid sequence of human GM-CSF (Wong et al., 1985); (f) amino acid sequence of human interleukin 2 (Taniguchi et al., 1983).

confirmed that a considerable proportion of IL 1 remains in the cell without being released. The discrepancy between the 17-kDa molecular mass of the purified IL 1 from THP-1 cell extracts and that of 23-kDa IL 1 activity in the cytosol of LPS-stimulated human monocytes may be due to differences in isolation procedures. The cytosol from peripheral monocytes was obtained from cells by a Dounce homogenization procedure immediately after culture rather than by freeze-thawing. Furthermore, the homogenization was performed at 4 °C in the presence of 2-ME, EDTA, EGTA, and PMSF. Presumably, this provided less opportunity for enzymatic cleavage to occur during the IL 1 extraction procedure. No enzymes have yet been identified that process or cleave primarily translated 30-kDa human IL 1 to the biologically active 23- or 17-kDa forms (Auron et al., 1984). The purification of IL 1-β with the same molecular mass from both cell extracts and culture supernatants suggests that enzymes, which are involved in the processing or cleavage of IL 1 to become a biologically active 17-kDa molecule, exist in or are released from cells rather than derived from medium containing fetal calf serum. Furthermore, as shown in Figure 5, the processing enzyme that cleaves the amino terminal of ^{117}Ala-^{118}Pro is probably a relatively ubiquitous enzyme, which may be also involved in the processing of GM-CSF (Wong et al., 1985) and IL 2 (Taniguchi et al., 1983) with similar amino-terminal amino acids.

In conclusion, we have established the purification of two distinct forms of human IL 1 from myelomonocytic leukemic cell line (THP-1) cells. The neutral 17-kDa IL 1-β from both culture supernatants and cell extracts of THP-1 showed identity with IL 1-β from normal human peripheral blood monocytes. This is also true of the cell-associated acidic 17-kDa IL 1-α although information is as yet incomplete. Therefore, it is valid to use THP-1 cells as a source of natural human IL 1.

Acknowledgments

We thank Drs. Masaaki Taguchi, Keith Elliott, Howard Young, and Dan Longo for discussion and review of the manuscript. We also thank the Fermentation Group of NCI-Frederick for supplying a large amount of culture supernatants and cell pellets of THP-1 cells.

Registry No. HU, 127-07-1; silica, 7631-86-9.

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