HUMAN INTERLEUKIN 1

Purification to Homogeneity

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Interleukin 1 (IL-1)[1] is a protein factor secreted by macrophages that exerts a wide range of effects on cells of the immune system as well as a variety of other cell types (1–3). Recently, a number of biological responses have been attributed to factors that copurify with IL-1 and are thought likely to also be mediated by IL-1. Thus, it is probable that IL-1 mediates the activities previously referred to as lymphocyte-activating factor (4), endogenous or leukocytic pyrogen (5), B cell–activating factor (6), epidermal cell thymocyte-activating factor (7), leukocyte endogenous mediator (8), a bone resorption factor active in rheumatoid arthritis (9, 10), and a variety of other activities. Recent studies of human and murine IL-1 have characterized the partially purified molecule as having a molecular weight in the 11,000–16,000 range, and a pI of 4.5–5.5 for mouse (11), and ~6.8–7.2 for human IL-1 (12). We have been investigating simplified procedures for production and purification of IL-1 from human macrophages in order to produce quantities of protein sufficient for structural and biological characterization of the molecule.

Previously described methods for production and purification of IL-1 have generally suffered from low overall yields of IL-1 activity and insufficient purity of the final product. Our method takes advantage of mild, high yield procedures at every step, to obtain exellent recovery of active, homogeneous IL-1.

Materials and Methods

Thymocyte Proliferation Assay. IL-1 activity was measured in the CD-1 mouse thymocyte mitogenesis assay (13). Briefly, thymocytes (10^6 cells/well) from 10–12-wk-old CD-1 mice, obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA, were cultured in 150 μl Eagle's minimal essential medium supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mM glutamine, 0.2 mM gentamycin, 10 mM Hepes, pH 7.4 (supplemented MEM), containing 3% human serum and 10^{-5} M 2-mercaptoethanol. Cells in this medium were cultured in round-bottom microplate wells (Corning Glass Works, Corning, NY) in the presence of threefold serial dilutions of test samples for 72 h at 37°C in 5% CO_2. Thymidine incorporation into DNA was measured by the addition of 0.5 μCi [^{3}H]thymidine ([^{3}H]TdR) (2 Ci/mmol, New England Nuclear, Boston, MA) 4 h before the cultures were harvested with the aid of a multiple automated cell harvester (Cambridge Technology Inc., Cambridge, MA). IL-1 activity was calculated from the linear portion

[1] *Abbreviations used in this paper:* CTLL-2, IL-2-dependent continuous T lymphocyte line; IL-1, interleukin 1; MEM, Eagle's minimum essential medium; MES, 2-(*N*-morpholino)ethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; SPS, sulfopropyl Sephadex C-25; Staph A, *Staphylococcus aureus*; [^{3}H]TdR, [^{3}H]thymidine.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/85/3/0490/13 $1.00
Volume 161 March 1985 490–502

of the [^{3}H]TdR incorporation data by a method similar to the procedure used for determining IL-2 activity (14). IL-1 at a concentration of 1 U/ml induces 50% of the maximal proliferative response under the conditions described above.

The exact dilution at which a sample causes 50% of maximal [^{3}H]TdR incorporation was calculated by linear regression analysis of the raw cpm data using values for maximum cpm, minimum cpm, and the slope derived from the dilution curve for a standard sample of IL-1. With this dilution defined as 1 U/ml, the concentration of the original sample can be calculated. For example: if a test sample induces 50% maximal [^{3}H]TdR incorporation at a dilution of 1:15, then 1 U of IL-1 is found in 150 μl (assay volume)/15 (dilution) = 10 μl of sample. Therefore, the sample would contain (1,000 μl/ml)/(10 μl/U) = 100 U/ml.

Other IL-1 Assays. IL-1 activity at different stages of purification was also determined using two other IL-1 assays: (*a*) The IL-1 comitogenesis assay, described by Mizel et al. (15), measures the proliferation of murine thymocytes in response to IL-1 and a submitogenic dose of phytohemagglutinin. Proliferation is measured via uptake of [^{3}H]TdR. (*b*) The IL-1 conversion assay, described by Conlon (16), uses the ability of IL-1 to convert an IL-2 nonproducer murine tumor cell line, LBRM-33-1A5, to an IL-2 producer, which, in turn, stimulates mitogenesis in the IL-2-dependent continuous T lymphocyte line (CTLL-2).

Other Lymphokine Assays. Fractions at different stages of IL-1 purification were tested in the following lymphokine assays: (*a*) IL-2 activity was determined in a standard microassay based on the IL-2-dependent proliferation of CTLL-2 (14). (*b*) The murine colony-stimulating factor assay is a semisolid clonal cell culture method measuring committed hematopoietic progenitor cells. Mouse marrow is cultured for granulocyte-macrophage colony–forming units (17, 18). (*c*) γ-interferon activity was determined using an IMRX Interferon-Gamma Radioimmunoassay Kit (Centocor, Inc., Malvern, PA).

Endotoxin Assay. Bacterial endotoxin was determined using a Quantitative Chromogenic Lal (QCL-1000) Kit from Whittaker M. A. Bioproducts, Walkersville, MD. The method uses a modified limulus amebocyte lysate and a synthetic color-producing substrate to detect endotoxin chromogenically. All IL-1 samples to be tested for pyrogenicity were first assayed by this procedure to assure that they did not contain pyrogenic amounts of bacterial contamination.

Pyrogenicity. Biomed Research Laboratories, Seattle, WA tested the purified IL-1 for pyrogenicity in rabbits according to the U. S. Pharmacopeia "Pyrogen Test" protocol.

Protein Assay. Protein content was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin as standard (19).

Polyacrylamide Gel Electrophoresis (PAGE). The discontinuous Tris-glycine system of Laemmli (20) was used for 0.75-mm-thick sodium dodecyl sulfate (SDS) slab gels using a 10–20% gradient with a 3% stacker. The gels were run at constant current (30 mA) and stained by a silver nitrate method (21). Those samples containing a high salt concentration were initially dialyzed against 0.001% SDS in 0.1 mM NH_4HCO_3, dried down under vacuum, and reconstituted in Laemmli reducing buffer. Molecular weight was determined using protein standards; phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (21,000), and α-lactalbumin (14,400).

Two-dimensional PAGE. Concentrated samples were lyophilized and resuspended in 20 μl SDS solubilization buffer (10% vol/vol glycerol, 2% wt/vol SDS, 2% vol/vol 2-mercaptoethanol, 1% wt/vol cyclohexylaminoethane, in H_2O), after the method of Anderson and Anderson (22), as modified by Dunbar (23). Samples were then heated at 100°C for 10 min, applied to prefocused first-dimension gels, and focused for 20 h at 600 V (constant). After focusing, gels were scanned directly by a pH gradient gel scanner (GS127; Hoefer Scientific Instruments, San Francisco, CA) according to the manufacturer's instructions. After direct pH scanning, gels were rinsed in an equilibration buffer of 50% (vol/vol) Laemmli buffer, 9.3% (vol/vol) glycerol, 1% (wt/vol) SDS, and 0.8% (vol/vol) 2-mercaptoethanol, in H_2O, for 2 min, placed on top of the second dimension gel, and covered by low-melting-temperature agarose. Second-dimensional electrophoresis

(10–20% linear gradient of acrylamide) was run at constant current (40 mA/gel) until the dye front reached the bottom of the gel. Gels were stained by a color silver nitrate method according to Sammons et al. (24).

Production of Human IL-1. Leukocyte concentrates (350–400 ml), obtained from 30 U of blood (Portland Red Cross, Portland, OR) were mixed with Ca^{++} and Mg^{++}–free phosphate-buffered saline (PBS), layered onto Histopaque (Sigma Chemical Company, St. Louis, MO) and centrifuged at 600 *g* for 30 min at room temperature. The interface layer, at a density of 1.077 g/ml, containing the mononuclear cells, was washed with PBS and centrifuged at 400 *g* for 10 min at room temperature. After two further washes and centrifugations at 200 *g* for 10 min, the cells were cultured at 2×10^6 cells/ml in supplemented MEM containing 0.01 mg/ml Staph A (heat-killed, formalin-fixed *Staphylococcus aureus*, Igsorb; The Enzyme Center, Inc., Malden, MA) in a spinner flask for 24 h at 37°C in 5% CO_2. The cells were spun at 7,000 *g* for 30 min at room temperature, and the supernatant (6–8 liters) removed and stored in polypropylene bottles at −20°C until further use.

Purification of IL-1. All chromatography procedures were done at 4°C. Buffers were prepared in distilled water and sterile filtered before use. All chromatography fractions were assayed for IL-1 and protein concentration. Sample pH and conductivity were measured where appropriate. After each chromatography step, samples were analyzed by PAGE. The ion exchange gels were pretreated with 0.1% Triton-X and 10% fetal calf serum to reduce nonspecific absorption of IL-1 activity to the resin.

Sulfopropyl Sephadex (SPS) C-25. Culture supernatant (6–8 liters), adjusted by addition of 1 M sodium citrate buffer, pH 4, to a final concentration of 10 mM citrate, and reduced to pH 4 with concentrated hydrochloric acid, was applied at a rate of 4 ml/h to a 30 × 1.6 cm column of SPS C-25 (Pharmacia Fine Chemicals, Piscataway, NJ) that had been equilibrated in 10 mM citrate, 150 mM sodium chloride, pH 4. The column was washed with 10 column volumes of 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer, pH 5, and then eluted with 4 column volumes 10 mM Tris-HCl, pH 8.1, at 50 ml/h. Fractions of 5 ml were collected. IL-1 activity eluted with the rise in pH, enabling active fractions to be pooled after determination of pH levels (see Fig. 1). The pool was immediately applied to a DEAE-Sephacel Column.

DEAE-Sephacel. The SPS fractions containing IL-1 activity (45–55 ml) in 10 mM Tris-HCl, pH 8.6, were applied to a 10 × 2.5 cm column of DEAE-Sephacel (Pharmacia Fine Chemicals) that had been equilibrated in 10 mM Tris-HCl, pH 8.1 at 20 ml/h. The column was washed with five column volumes of the starting buffer and then eluted with a linear gradient (four column volumes) ranging from 0 to 400 mM NaCl in 10 mM Tris-HCl, pH 8.1. Fractions of 4 ml were collected, assayed for biological activity and stored at 4°C until further purified (Fig. 2).

Procion Red Agarose. The DEAE pool of IL-1-active fractions (20–28 ml) was diluted 1:4 in 10 mM Tris-HCl buffer, pH 8.1, to reduce the ionic strength to <40 mM and applied to a 10 × 1.6 cm column of Procion Red agarose (Bethesda Research Laboratories, Gaithersburg, MD) that had been equilibrated in 10 mM Tris-HCl buffer, pH 8.1, at 20 ml/h. The column was washed with five column volumes of the starting buffer, then eluted with a linear gradient (15 column volumes) ranging from 0 to 1 M NaCl in 10 mM Tris-HCl buffer, pH 8.1. Fractions of 8 ml were collected, assayed for biological activity, and stored at 4°C.

Concentration of Purified IL-1. Fractions of homogeneous IL-1 from the Procion Red column were diluted 10-fold, adjusted to pH 4.0 with1 N HCl, and applied to a 0.5 ml bed-volume SPS C-25 column that had been equilibrated in 0.01 M sodium citrate, 0.05 M NaCl, pH 4.0. After sample application, the column was washed with 10 ml of the equilibration buffer, followed by 20 ml of deionized water. IL-1 was eluted from the column with 5 mM Tris-HCl, pH 8.0. Fractions were stored at 4°C until further use.

Amino Acid Analysis. Samples were hydrolyzed in vacuo with constant boiling HCl (redistilled from concentrated HCl; Kodak, Rochester, NY) for 24 h. After hydrolysis, samples were evaporated to dryness under vacuum and resuspended in 0.2 N sodium citrate, pH 2.2. Samples were injected onto a single-column amino acid analyzer (model

4150-Alpha; LKB Instruments, Inc., Cambridge, England) using ninhydrin detection. Peak areas were calculated using an LKB model 2220 recording integrator.

Results

Table I shows the effects of various stimulants and culture conditions on IL-1 production. We chose Staph A (0.1% wt/vol) as the stimulant due to the more rapid appearance of IL-1 activity in the supernatant. Although three- to fivefold more IL-1 was produced in the presence of serum, there was 100-fold less protein in the serum-free supernatants, making further purification simpler.

The culture supernatant was applied to an SPS column at pH 4, conditions where 100% of the activity bound the column. In the presence of physiological levels of NaCl, IL-1 had only a weak affinity for the column. However, washing with 10 mM MES buffer, pH 5, reduced the salt concentration and eluted some of the contaminating protein without any elution of IL-1 activity. A subsequent application of 10 mM Tris-HCl buffer at pH 8.1 resulted in a pH rise after three column volumes, and simultaneous elution of the IL-1 peak (Fig. 1). This first step in the purification concentrated the IL-1 activity 140-fold, and removed 80% of the contaminating proteins.

Since IL-1 was eluted off the SPS column in the equilibration buffer of the DEAE column, the SPS pool could be loaded directly onto the DEAE column, thus avoiding any loss of activity by dialysis. IL-1 activity eluted from the DEAE column in a sharp peak at 0.08–0.12 M NaCl. Protein elution began at 0.1 M salt, with a broad peak eluting between 0.15 and 0.25 M (Fig. 2). This step removed >90% of the remaining contaminating proteins. PAGE of fractions

TABLE I
Effects of Various Stimulants and Culture Conditions on IL-1 Production by Human Mononuclear Cells

Stimulant	Amount	Human serum	Time	IL-1 activity
	μg/ml	*%*	*h*	*U/ml*
Staph A*	10	—	24	945
Staph A	20	—	24	586
Staph A	50	—	24	529
Staph A	10	—	48	709
LPS‡	1	—	24	765
LPS	1	—	48	866
Staph A	10	5	24	1,785
LPS	1	5	24	3,306
None		—	24	14
None		—	48	17
None		5	24	21

* Heat-killed, formalin-fixed, *Staphylococcus aureus* (Igsorb, The Enzyme Center, Inc.)

‡ Lipopolysaccharides prepared by trichloroacetic acid extraction from *Eschericha coli* serotype 026:B6 (Sigma Chemical Company).

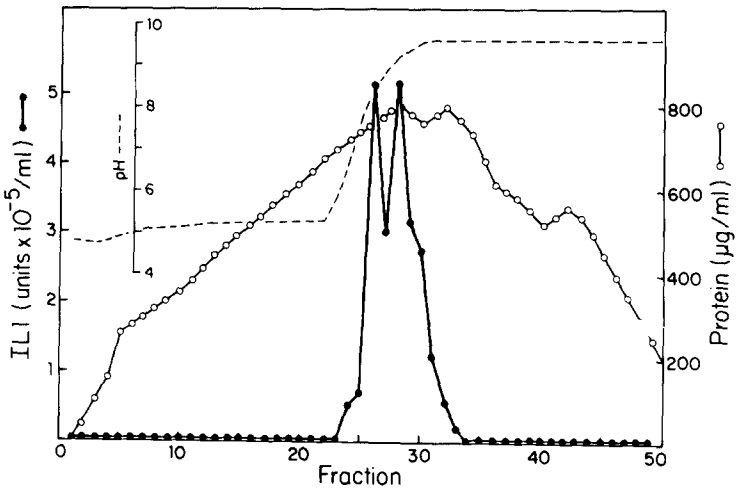

FIGURE 1. SPS chromatography of crude supernatant. After washing the column with 600 ml of 10 mM MES buffer, pH 5.0, IL-1 was eluted with 10 mM Tris-HCl, pH 8.1. Elution of IL-1 activity is indicated by the closed circles and protein elution by the open circles. The pH rise is indicated by the dashed line. Fractions 24–32 were pooled for DEAE-Sephacel chromatography.

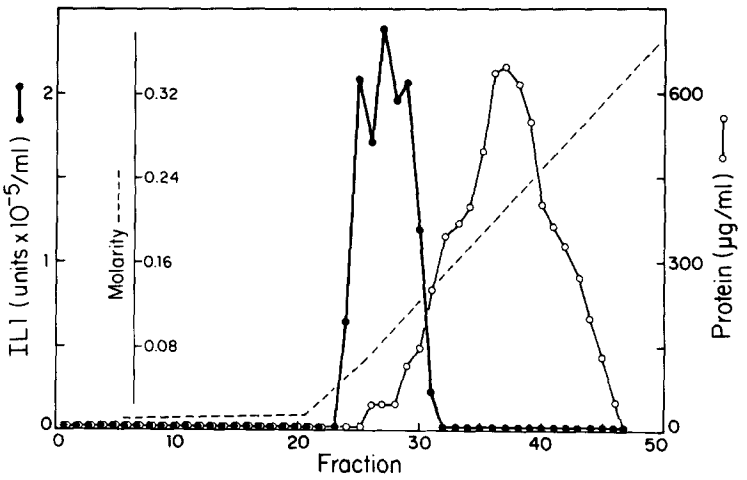

FIGURE 2. DEAE-Sephacel chromatography of SPS pool of IL-1 active fractions (see Fig. 1). Elution of IL-1 activity is indicated by the closed circles and protein elution by the open circles. A linear gradient ranging from 0 to 400 mM NaCl in 10 mM Tris buffer, pH 8.1, was used. The salt gradient is indicated by the dashed line. Fractions 24–29 were pooled for Procion Red agarose chromatography.

with IL-1 activity revealed some high molecular weight contaminants, as well as three major bands with molecular weights of approximately 17,500, 15,000 and 12,000 (Fig. 3).

We found it necessary to lower the ionic strength of the DEAE pool for IL-1 to bind strongly to the Procion Red column. IL-1 activity eluted from the Procion Red column in a sharp peak with 0.50–0.55 M NaCl (Fig. 4). PAGE of IL-1 active fractions demonstrates that the Procion Red step purifies IL-1 to

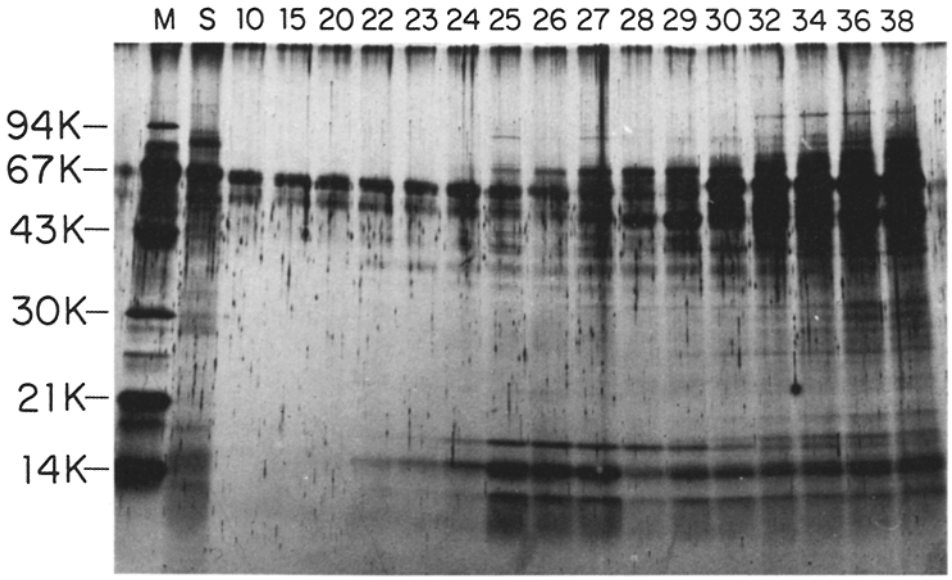

FIGURE 3. Analysis of DEAE-Sephacel–purified IL-1 by PAGE and silver staining. *M*, molecular weight standards; *S*, starting material. Numbers above lanes correspond to fractions collected during the chromatography depicted in Fig. 2.

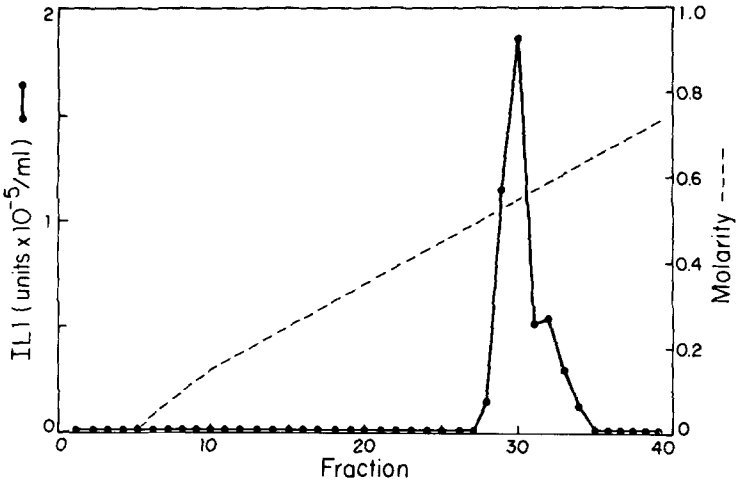

FIGURE 4. Procion Red agarose chromatography of DEAE-Sephacel pool of IL-1 active fractions (see Fig. 2). Elution of IL-1 activity with a linear gradient ranging from 0 to 1 M NaCl in 10 mM Tris buffer, pH 8.1, is shown. The salt gradient is indicated by the dashed line.

homogeneity. The IL-1 active fractions showed one protein band of ~17,500 mol wt. The high molecular weight bands were eluted in the flow-through of the column, and the two lower molecular weight bands eluted with a higher salt concentration (Fig. 5).

Table II shows the degree of IL-1 purification achieved with the various steps. The homogeneous protein was obtained with an overall purity of >99% at a

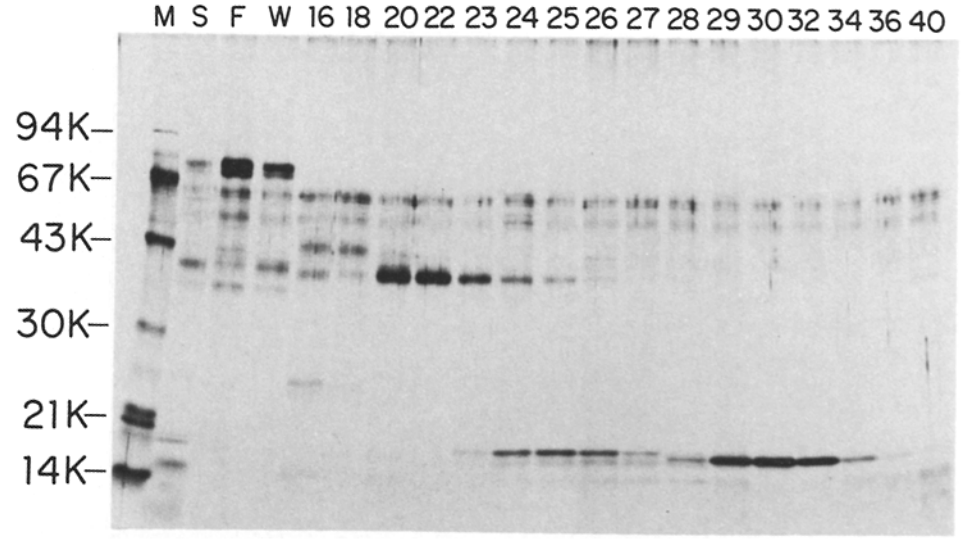

FIGURE 5. Analysis of Procion Red agarose–purified IL-1 by PAGE and silver staining. *M*, molecular weight standards; *S*, starting material; *F*, nonadsorbed (flow-through) material; *W*, 10 mM Tris-HCl, pH 8.1, wash. Numbers above lanes correspond to fractions collected during the chromatogram depicted in Fig. 4. Bands at approximately 67,000 and 55,000 are present in all lanes, and were seen in gels where no samples were run; they have been reported to be human skin proteins contaminating reagents and gels, but not present in samples (28). The IL-1 band at fractions 30–32 is free of detectable contaminating proteins. Fraction 30 corresponds to the peak of IL-1 activity eluting from the Procion Red column.

TABLE II
Purification IL-1

Treatment of starting material	Total activity ($U \times 10^6$)	Yield	Total protein	Specific activity
		%	*μg*	*U/mg*
Culture supernatant	6.6	100	311,000	2.12×10^4
SPS	3.7	56	53,000	7.0×10^4
DEAE-Sephacel	2.58	39	3,000	8.6×10^5
Procion Red agarose	2.1	31	6.5	3.2×10^8

Results represent averages for 10 separate IL-1 preparations.

31% yield from the starting supernatants. Table III lists the results obtained when a preparation of IL-1 was followed through the four purification steps with the three available IL-1 assays, as well as assays for the activities of other lymphokines. Each of the IL-1 assays shows approximately the same degree of specific activity increase, consistent with the fact that they all are measuring the same active molecule. No detectable IL-2 or colony-stimulating factors were observed at any step. Small amounts of γ-interferon were detected in the crude supernatant, but were removed by subsequent steps of the purification.

Table IV lists the results of amino acid analysis performed on 71 pmol of purified IL-1. Although we detected trace amounts of cystine, we have assigned cysteine in IL-1 an integer value of zero because the amount detected represents

TABLE III
Biological Activity of Purified IL-1

Treatment of starting material	IL-1 direct mitogenesis assay (U/mg protein)	IL-1 comitgenesis assay (U/mg protein)	IL-1 conversion assay (U/mg protein)	γ-Interferon assay (U/ml)
Culture supernatant	1.4×10^4	4.4×10^5	2.7×10^6	6
SPS	6.4×10^4	4.45×10^4	3.3×10^7	—*
DEAE-Sephacel	8.5×10^5	1.71×10^6	9.7×10^8	—
Procion Red agarose	7.5×10^7	4.37×10^7	5.7×10^{10}	—

IL-2 activity was undetectable in assay (<10 U/ml). Less than one colony-forming unit of colony-stimulating factor activity was detected in assays counting granulocyte macrophages.
* <0.1 U/ml.

TABLE IV
Amino Acid Analysis of Human IL-1

Amino acid	No. of residues per 17.500 mol wt
Asp	14.4 (14)
Thr	7.8 (8)
Ser	11.0 (11)
Glu	22.4 (22)
Pro	5.9 (6)
Gly	11.1 (11)
Ala	7.9 (8)
Cys	0.2 (0)
Val	10.6 (11)
Met	4.1 (4)
Ile	5.8 (6)
Leu	10.8 (11)
Tyr	3.5 (4)
Phe	10.7 (11)
His	3.1 (3)
Lys	13.1 (13)
Arg	3.2 (3)
Total	(146)

Results of 24-h hydrolysis of 71 pmol of human IL-1, as described in Materials and Methods. Numbers in parentheses represent the best integer-fit to the data. Tryptophan content was not determined.

only 0.2 residues per molecule. No peaks were eluted in the positions expected for glucosamine or galactosamine. When purified IL-1 was subjected to two-dimensional PAGE, we consistently obtained a series of spots of exactly the same molecular weight (17,500), regardless of which lot of starting supernatants was used. The most intense spots (Fig. 6, *a* and *b*), indicated pI of 5.9–6.0 and 5.2–5.3. We also observed two less intensely staining spots at the same molecular weight as *a* and *b*, with pI of 4.9 and 5.1. Although a black-and-white photograph is used for Fig. 6, a color, silver-staining method (24) yielded the exact same

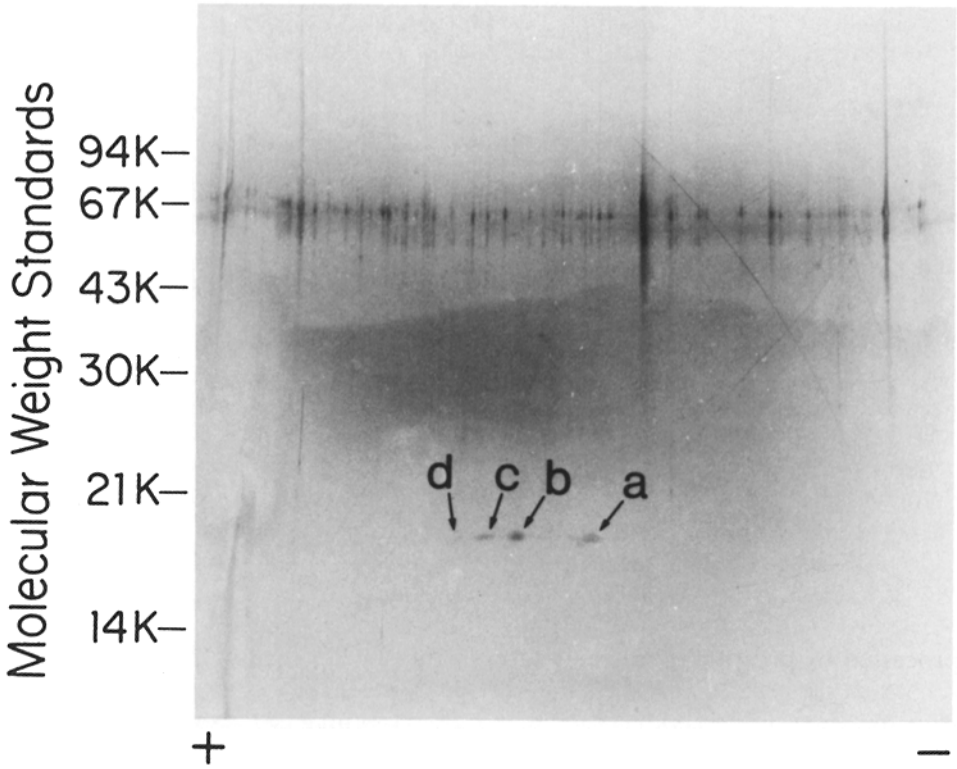

FIGURE 6. Two-dimensional PAGE of purified human IL-1. Letters indicate the individual pI spots for IL-1: *a*, pI between 5.9 and 6.0; *b*, pI between 5.2 and 5.3; *c*, pI 5.1; *d*, pI 4.9. Numbers on left side of gel represent migration points for molecular weight standards in the second dimension. (+, −) Anode and cathode for focusing in the first dimension. The vertical streaks and the bands across the gel at 67,000 and 55,000 mol wt are artifacts seen in similar gels run without sample application.

TABLE V
Pyrogenicity of Human IL-1

IL-1 dose	Endotoxin	Temperature rise*
ng/kg	*ng/kg*	*°C*
—‡	—	0.03 ± 0.03
—§	400	1.30 ± 0.25
80	0.25	0.36 ± 0.09
390	0.32	0.89 ± 0.05
780	0.64	0.74 ± 0.22

* Results represent mean and standard error of the mean for three rabbits. A rise in temperature of <0.6°C is considered negative for pyrogenicity.
‡ Negative control; sterile saline was injected, no limulus-positive material was detectable (<3 pg/kg).
§ Positive lipopolysaccharide control.

color for each spot, indicating that the same parent protein is most likely staining in all cases.

Table V shows results of pyrogenicity studies using material purified to homogeneity. Three rabbits (2.4–3.0 kg) were used for each dose, and in every

case the level of endotoxin present in the sample, as determined by endotoxin assay, was <1 ng/ml. This amount is less than the amount of endotoxin required to cause a significant elevation in body temperature. IL-1 doses of 390 and 780 ng/kg were pyrogenic, but doses of 80 ng/kg were not. In the positive animals, temperatures rose within 30 min of injection and remained elevated throughout the 3-h test period.

Discussion

This procedure represents the first high yield method for production and purification of human IL-1. One important contributing factor is the use of a serum-free culture medium during stimulation of IL-1 synthesis by the macrophages. Togawa et al. (25) showed that, in the presence of serum, IL-1 exhibits multiple apparent molecular weights, possibly due to a reversible association with a high molecular weight serum component. In contrast, IL-1 prepared by our procedure ran as a single molecular weight species on gel filtration (not shown). It is likely then, that elimination of serum has improved our yields by allowing IL-1 to be purified as a single species, rather than a mixture of forms resulting from association-dissociation with another molecule. An alternative explanation for the observations (25, 27) of a high molecular weight form of IL-1 is the possibility that IL-1 may be synthesized in a larger precursor form that is processed by proteolytic fragmentation to the smaller form. If this is the case, then it is likely that our IL-1 preparations contain only the processed form, probably due to the absence of serum protease inhibitors in our medium. This is also consistent with our inability to regenerate high molecular weight IL-1 by incubating our purified material with human serum albumin or with whole serum (data not shown).

The observed molecular weight of 17,500 is somewhat heavier than the 14,000 determined for mouse IL-1 (26), and is also greater than that previously reported (12, 25, 27) for human IL-1, which has been given a variety of molecular weights by other researchers, most being in the 11,000–16,000 range. The reasons for these discrepancies are not obvious, but in most cases the authors were relying on methods, such as gel filtration, which are not readily able to distinguish between proteins whose weights vary by <4,000–5,000. Because our yields of IL-1 are high, and because we have never observed multiple molecular weight species in any of our procedures, we feel that is unlikely that we have lost any significant amount of lower molecular weight IL-1, so we must conclude that the lower estimates of the molecular weight of human IL-1 are in error.

Table IV shows the amino acid composition of human IL-1. It is interesting to compare our findings to the partial composition of mouse IL-1 determined by Mizel and Mizel (26). Although lack of data for some amino acids in mouse IL-1 makes a complete comparison impossible, our composition shows a similar high content of aspartic acid, glutamic acid, leucine, and lysine. On the other hand, human IL-1 seems to have relatively lesser amounts of serine and alanine, and a total of six prolines, whereas no proline was reported for mouse IL-1. We found no cysteine residues, in agreement with our observations that human IL-1 activity was not altered by reducing agents and did not bind with high affinity to sulfhydryl-specific resins such as organomercurial Sepharose (data not

shown). In agreement with our finding that human IL-1 migrates as a single molecular weight species in PAGE, we observed no glucosamine or galactosamine on amino acid analysis. One or both of these would be expected if human IL-1 has attached carbohydrate moieties. Therefore, it is unlikely that human IL-1 is a glycoprotein.

Two-dimensional gel analysis of homogeneous IL-1 consistently yielded a series of spots, all migrating at exactly the same molecular weight (17,500) (Fig. 6) and staining the same color with a silver stain method (24). These spots were always in the same positions and in the same relative proportions, regardless of which lots of starting supernatants were used. ~70–80% of the total IL-1 is represented by the most intense spot at pH 5.9–6.0. The other spots may represent deamidation products, either present in vivo, or resulting from the purification procedure, which exposed IL-1 to pH 4.0 for several hours. It is interesting to note that a similar charge heterogeneity was observed for mouse IL-1. Our range of pI values for human IL-1 is somewhat lower than that observed by other investigators. However, we are analyzing purified, homogeneous material, whereas others have analyzed samples containing other proteins, which might influence the isoelectric point for IL-1 by forming complexes.

IL-1 obtained by this procedure retains a high level of biological activity which is stable for long periods in the final buffer, 10 mM Tris-HCl, pH 8.1. The pure material has a specific activity of 3.2×10^8 U/mg of protein by the direct mitogenesis assay. This specific activity is higher than any previously reported, and is similar to activities reported for other lymphokines. Based upon quantitative data obtained by amino acid analysis of IL-1 samples, we find that 1 U/ml of IL-1 (50% of maximum thymocyte response) occurs at a final concentration of 1.8×10^{-13} M. Another activity that has been associated with IL-1 in the past is pyrogenicity. Our preparations of pure IL-1 are pyrogenic at doses of 390 and 780 ng/kg, but not at a dose of 80 ng/kg. In contrast to the findings of Lachman (12), who found a short duration (60 min) of fever-producing activity, our material caused a longer-lasting pyrogenic effect that continued to the end of the 3-h test period. Our findings confirm that at least a part of the pyrogenic activity ascribed to the macrophage product, endogenous pyrogen, is due to the observed pyrogenicity in pure IL-1. However, our results do not rule out the possibility that other pyrogenic products may have been separated from IL-1 during our purification procedure. In this light, it is notable that pure IL-1 caused a lower fever than comparable amounts of lipopolysaccharide (LPS) (Table V). It seems likely that if IL-1 were the sole mediator of fever, it would be active at lower doses, and to a higher degree of pyrogenicity than that seen for our samples. Further experiments will be necessary to clarify the role of IL-1 in the generation of fever, and to identify or rule out pyrogens other than IL-1.

Summary

We have purified human interleukin 1 (IL-1) to homogeneity by a simplified procedure that results in excellent yields of pure material that retains a high level of biological activity. IL-1, secreted by human peripheral blood macro-

phages that have been stimulated with *Staphylococcus aureus,* was purified by ion exchange chromatography and affinity chromatography on Procion Red agarose. The pure protein has a specific activity of 3.2×10^8 U/mg in the thymocyte mitogenesis assay, and is pyrogenic. No molecular weight heterogeneity was observed, in contrast to findings for mouse IL-1 and earlier reports of human IL-1. Purified IL-1, as analyzed by two-dimensional electrophoresis/electrofocusing gels, exhibited a series of charged species with isoelectric points ranging from 6.0 to 4.9, all with a molecular weight of ~17,500. Amino acid analysis indicated an abundance of acidic residues, in agreement with the low isoelectric points. There is little or no cysteine in the molecule. No evidence was found for the presence of carbohydrate moieties. The overall yield for this procedure was ~31% of the activity contained in the initial culture supernatant.

The authors wish to thank Mr. Steve Hartman for excellent technical assistance, Drs. Steven Gillis, Kenneth Grabstein, and Jacques Bertoglio for helpful discussions, and Ms. Judy Byce for the preparation of the manuscript.

Received for publication 2 July 1984 and in revised form 13 November 1984.

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