PURIFICATION AND PARTIAL BIOCHEMICAL CHARACTERIZATION OF NORMAL HUMAN INTERLEUKIN 1

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Interleukin 1 (IL-1),[1] formerly lymphocyte activating factor, was originally described in 1972 by Gery et al. (1) as a product of human monocytes capable of stimulating the proliferation of murine thymocytes. Subsequent studies have shown that monocyte-macrophages from other species also produce a polypeptide mediator capable of stimulating murine thymocyte proliferation (2, 3). These and subsequent studies suggest that IL-1 may be an important signal in T (4) and B lymphocyte activation (5). Furthermore, in addition to its immunologic properties, IL-1 may be an important mediator in chronic inflammation. This is based on studies showing that IL-1 or molecules similar to IL-1 function as endogenous pyrogens (6), stimulate the production of collagenase by fibroblasts (7) and by rheumatoid synovial cells (8), and stimulate the release of acute phase reactants by liver (9). Molecules very similar or identical to IL-1 also stimulate fibroblast proliferation and therefore may contribute to the fibrosis often observed in chronic inflammatory diseases (10, 11). Although the results of these studies are provocative and suggest that IL-1 may be an important mediator in a variety of physiologic and pathophysiologic processes, the conclusions are tentative because partially purified IL-1 was used in these studies. Moreover, some of the IL-1 preparations were prepared in the presence of phorbol esters (12) or carrier ampholytes (13), each of which has been demonstrated to be active in diverse in vitro biological assay systems (14–16).

Some progress has been made in the purification and biochemical characterization of human IL-1 since its discovery in 1972. Studies show that it is a polypeptide having an approximate molecular weight of 15,000 (17) and a major isoelectric point (pI) of 6.8 (13). Minor charged species possessing isoelectric points of 5.2, 5.4, and 6.0 have also been described (13), though the basis for this charge heterogeneity is unclear. Detailed biochemical characterization of human IL-1 has been hampered, however, by the lack of a rapid, efficient purification procedure. Although highly enriched preparations of human IL-1 have been prepared using either ultrafiltration and isoelectric focusing (IEF)

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DEAE, diethyl aminoethyl; HPLC, high performance liquid chromatography; IEF, isoelectric focusing; IL-1, interleukin 1; IL-2, interleukin 2; PAGE, polyacrylamide gel electrophoresis; pI, isoelectric point; RPC, reverse phase chromatography; SDS, sodium dodecyl sulfate.

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techniques (18) or a polyspecific rabbit antiserum containing antibodies to IL-1 (19), there is not yet convincing data that these procedures are capable of producing homogeneously pure material suitable for amino acid analysis, spectral analysis, or ligand binding studies. Although murine IL-1 has been purified to homogeneity (12), there is evidence that human IL-1 differs significantly from murine IL-1 with respect to its biochemical and antigenic properties. Human IL-1 has a much higher pI than does murine IL-1 (4.9–5.1) and is inactivated by α-chymotrypsin (17), whereas murine IL-1 is resistant to inactivation by α-chymotrypsin (20). Moreover, a heterologous antiserum raised against murine IL-1 cross-reacted with human IL-1 only weakly (21).

The purification of human IL-1 has been difficult because (*a*) there is no cell line capable of producing relatively large amounts of this material under serum-free culture conditions (22), and because (*b*) most conventional purification procedures yield poor recovery of activity (12, 13, 17, 18). In this study, peripheral blood mononuclear cells were obtained in large quantities from normal donors by leukopheresis and cultured under serum-free conditions in the presence of purified phytohemagglutinin in order to produce culture supernatants containing a high specific activity of IL-1. IL-1 was purified from concentrated culture supernatant using a combination of anion exchange and size exclusion high performance liquid chromatography (HPLC). The separations performed by HPLC anion exchange chromatography were novel in that isocratic, instead of gradient, conditions were employed (see reference 23 for review). The culture conditions and purification procedure presented here yield a highly purified preparation of human IL-1 consisting of a single charged species, and free of contaminating phorbol esters and carrier ampholytes.

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Materials and Methods

Mononuclear Cell Cultures. Peripheral blood mononuclear cells were obtained by leukopheresis of normal volunteers using an IBM 2997 blood cell separator. The resulting cell suspension (1×10^{10} mononuclear cells in 200 ml) was supplemented with 300 U of heparin and diluted with an equal volume of culture medium consisting of RPMI-1640 that contained 20 mM Hepes, 4 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The mononuclear cell fraction was then obtained using Ficoll Paque (Pharmacia, Piscataway, NJ) discontinuous density gradients. The interface cells were diluted with an equal volume of culture medium and centrifuged at 4°C for 10 min at 250 *g*. The cell pellets were resuspended in 50 ml of culture medium and counted. After centrifugation, the cells were resuspended in culture medium with or without 1% fetal calf serum [No. 309, Gibco, Grand Island, NY] at a concentration of 5×10^6/ml. 100-ml volumes of cell suspension were placed in upright or horizontal 75-cm^2 flasks (Corning No. 25110, Corning, NY), supplemented with purified phytohemagglutinin (Burroughs Wellcome, Research Triangle, NC) at a final concentration of 1 μg/ml, and incubated for 4 d at 37°C in a humidified atmosphere of 5% CO_2/air. In preliminary experiments, aliquots of culture supernatants were carefully removed at daily intervals during the 4-d culture period in order to measure thymocyte proliferation activity and IL-2 activity as detailed below.

Supernatant Preparation. The culture supernatants were clarified by centrifugation at 3,000 *g* for 30 min at 4°C, supplemented with polyethylene glycol to minimize nonspecific adsorption (mol wt ≅ 6,000; 30 μg/ml, final concentration) and sodium azide (0.04%, final concentration), and then filtered through a 0.45-micron Metricel filter (Gilson, Ann Arbor, MI). For preparative purposes, the supernatant was then concentrated approximately 20-fold by a Pellicon cassette ultrafiltration device (Millipore, Bedford, MA)

equipped with 0.5 ft^2 of a PT membrane having a nominal molecular weight exclusion limit of 10,000. The retentate was subsequently concentrated an additional 50-fold by means of a stirred cell ultrafiltration device (Amicon, Danvers, MA) equipped with an Amicon YM-10 membrane. The crude concentrate was dialyzed against 5 mM Na_iPO_4 (pH 7.25) and filtered through a Millipore 0.22-μm Millex-GV filter.

Anion Exchange HPLC. 200-μl aliquots of the crude concentrate (~6 mg protein) were chromatographed isocratically on a preparative Synchropak AX300 column (250 × 10 mm; Synchrom, Linden, IN) equilibrated in 5 mM Na_iPO_4 buffer, pH 7.25, at a flow rate of 3 ml/min. This and subsequent chromatography steps in the purification protocol were performed at ambient temperature by a programmable Hewlett-Packard 1084B liquid chromatograph equipped with injector, peak integrator, variable wavelength detector, and effluent collection device. The active fractions from several consecutive runs were pooled and concentrated ~200-fold using an Amicon model 8 MC ultrafiltration device equipped with a YM-10 membrane. Aliquots (150 μl) of this concentrate were then chromatographed as above on an analytical size (250 × 4.1 mm) Synchropak AX300 column at a flow rate of 1 ml/min. Fractions were collected in tubes containing polyethylene glycol (final concentration 100 μg/ml) and the active fractions were concentrated as above. After 10 consecutive isocratic runs, each of the AX300 columns was washed with a gradient of increasing ionic strength (5–500 mM $Na_i PO_4$, pH 7.25) in order to remove bound proteins.

Size Exclusion High Performance Liquid Chromatography. Aliquots (150 μl) of the concentrated material obtained after rechromatography on Synchropak AX300 were chromatographed on a 600 × 7.5 mm TSK-G 2000 SW HPLC size exclusion column (LKB, Gaithersburg, MD) equilibrated in 100 mM Na_iPO_4 buffer, pH 6.95, at a flow rate of 1 ml/min. The column was calibrated with the following molecular weight standards: BSA, 67,000; ovalbumin, 43,000; soybean trypsin inhibitor, 21,500; ribonuclease A, 13,700; and aprotinin, 6,500.

Analytical SDS Gradient PAGE. Gradient gels (10–30% T with 2.6% C) having dimensions of 0.07 × 8.5 × 8.5 cm were prepared using a Pharmacia GSC-8 gel casting apparatus and a Pharmacia gradient maker. Samples and the molecular weight standards were dissolved in Laemmli sample buffer containing 1 mM dithiothreitol and heated to 90°C for 3 min. Electrophoresis was performed using the discontinuous buffer system of Laemmli at constant voltage (150 V) for 3 h at 10°C in a Pharmacia GE-4 electrophoresis apparatus. The molecular weight standards used to calibrate the gels were as follows: phosphorylase b, 94,000; BSA, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 20,100; α-lactalbumin, 14,400; and cytochrome *c*, 12,500. The gels were fixed and stained with silver as previously described (24).

Analytical IEF. Analytical IEF was performed on 5% T polyacrylamide gels having a thickness of 0.5 mm and containing carrier ampholytes with a pH range of 3.5–9.5 (UltroPag; LKB). The anolyte was 1 M H_3PO_4 and the catholyte was 1 M NaOH. The gels were run at constant power (25 W) until a maximum of 2,000 V was reached, at which point they were run at constant voltage. The runs lasted 1 h and were performed at 10°C on a Pharmacia FBE-3000 flatbed electrophoresis apparatus. Afterwards, the gels were fixed in a solution containing 11.5% trichloroacetic acid/3.4% sulfosalicylic acid for 1 h and in a solution containing 25% ethanol/8% acetic acid (vol/vol) for 5 min. The gels were then stained with Coomassie Brilliant Blue R250 using standard techniques. Calibration of the gels was performed with the following pI standards: lentil lectin: 8.65, 8.45, and 8.15 species; horse myoglobin: 7.35 and 6.85 species; human carbonic anhydrase B, 6.55; bovine carbonic anhydrase B, 5.85; β-lactoglobulin A, 5.20; soybean trypsin inhibitor, 4.55; and amyloglucosidase, 3.50.

Samples of IL-1 analyzed by analytical IEF were first concentrated and desalted by ultrafiltration using a YM-10 membrane. Aliquots (10 μl) of the sample and standard mixture were then applied to the surface of the gel with a template. Bioactivity was determined after electrophoresis by slicing a lane into 1.5-mm sections and eluting the sections in culture medium containing 10% fetal calf serum at 4°C for 12 h before assay.

Bioassays. IL-1 bioactivity was determined by its capacity to stimulate thymocyte

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proliferation in the presence of phytohemagglutinin, as previously described (25). Units of IL-1 activity were calculated, as previously described (26), by making reference to aliquots of a partially purified preparation of IL-1 that had been arbitrarily assigned an activity of 100 U/ml. The dose response lines of the samples and that of the standard preparation were parallel and they were compared at 50% of the response given by a 1:10 dilution of the standard preparation. Because thymocyte proliferation may also indicate the presence of interleukin 2 (IL-2), crude culture supernatants and purified preparations of IL-1 were also tested for IL-2 activity by using a standard microassay (26) based on the IL-2–dependent proliferation of a murine T cell line (HT-2).

Protein Determinations. During the chromatographic separations, absorbance was monitored at 210 nm and the integration values obtained by the peak integrator were used to determine the amount of protein in a given peak (27). In those cases where incomplete resolution from neighboring peaks prevented accurate integration, the region of the chromatogram corresponding to the active fractions was cut and weighed. The relationship between paper weight and integration units was determined by performing the same operation on well-resolved peaks. The number of integration units shown for the crude concentrate (Table II) was obtained by summing all the integration values obtained during the isocratic runs on the preparative Synchropak AX300 column, plus all those obtained when the column was eluted with a gradient of increasing ionic strength to remove bound protein (5–500 mM Na_iPO_4, pH 7.25). Integration units were corrected for column flow rate (27) and the number of area units obtained during blank runs.

The following method was used to convert integration units to protein weight. Known amounts of bovine serum albumin (BSA) (1–50 μg) were chromatographed on the HPLC size exclusion column at a flow rate of 1 ml/min while monitoring absorbance at 210 nm. By plotting the number of integration units as a function of protein weight, a linear relationship was obtained. The slope of this line (89.1×10^6 integration U/mg protein) was then used to calculate the amount of protein in the crude concentrate (see above) and the amount of protein associated with IL-1 activity after each step of the purification protocol (Table II).

For the purpose of measuring protein concentration in crude culture supernatants (Table II), the Coomassie Brilliant Blue method of Spector (28) was employed using BSA as a standard.

Results

Thymocyte proliferation activity was generated in culture supernatants by stimulating peripheral blood mononuclear cells with purified phytohemagglutinin for 4 d. Although thymocyte proliferation activity may reflect the presence of IL-2 as well as IL-1, IL-2, as specifically measured on HT-2 cells (see Materials and Methods), peaked on day 1 and thereafter decreased dramatically. By day 4, 1% of peak IL-2 activity remained (data not shown). These observations are in agreement with previous studies, which have shown that IL-2 is rapidly absorbed in culture by activated T lymphocytes (29, 30). Thymocyte proliferation activity, on the other hand, continued to rise or remained stable from days 1–4. It was therefore concluded that the thymocyte proliferation activity found in 4-day-old culture supernatants was largely due to IL-1.

Attempts to obtain a high specific activity of IL-1 by culturing the mononuclear cells in the absence of exogenous serum proteins were unsuccessful until the cells were cultured at high settled cell density. As shown in Table I, the generation of thymocyte proliferation (IL-1) activity was largely serum dependent in low density cultures (horizontal flasks). Culture in upright vessels nearly eliminated the serum requirement for IL-1 generation with a resultant steep increase in specific biological activity. The culture supernatants used for the purification of

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TABLE I

*Effect of Culture Conditions on the Generation of Thymocyte Proliferation Activity by Mononuclear Cells**

Culture orientation	FCS (1%)	Activity	Sp act[‡]
		U/ml	*U/mg*
Vertical	−	54	1,800
	+	59	62
Horizontal	−	7	233
	+	35	39

* The results are those of a representative experiment in which the supernatants from replicate flasks were pooled after 4 d of culture and assayed in the thymocyte proliferation assay.

‡ Protein was determined by reaction with Coomassie Blue as previously described (28).

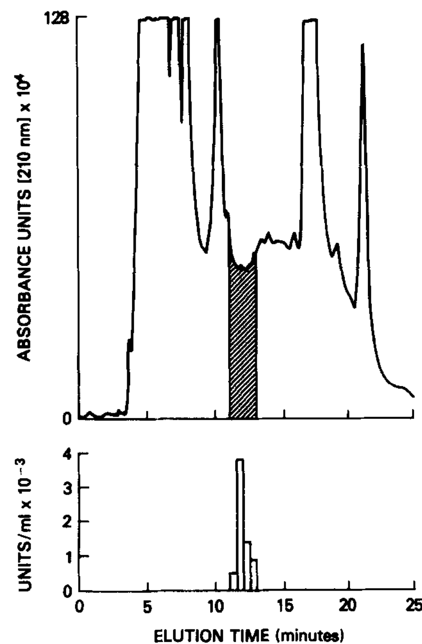

FIGURE 1. High performance anion exchange chromatography of concentrated culture supernatant. 200-μl aliquots of 1,000-fold concentrated culture supernatant were chromatographed isocratically on a preparative (250 × 10 mm) Synchropak AX300 column equilibrated in 5 mM Na_iPO_4 buffer, pH 7.25, at a flow rate of 3 ml per min. The absorbance of the effluent at 210 nm is shown in the upper panel. Fractions were collected at 0.5 min intervals and assayed for IL-1 activity (U/ml) in the thymocyte proliferation assay (lower panel). The hatched area in the upper panel represents those fractions in consecutive runs that were pooled, concentrated, and further purified.

IL-1 were consequently prepared in upright flasks in the absence of exogenous serum.

When concentrated culture supernatant was chromatographed isocratically on a preparative HPLC DEAE anion exchange column (Synchropak AX300) equilibrated in 5 mM Na_iPO_4 buffer, pH 7.25 (Fig. 1), a significant portion of the

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TABLE II
*Purification of Normal Human IL-1**

Step	Total activity‡ in units × 10^{-3}	Total protein§	Sp act	Times purified	Percent yield
		mg	*U/mg*		
1. Crude concentrated culture supernatant	406	147	2.7×10^3	—	100
2. Preparative HPLC anion exchange chromatography	146	0.52	2.8×10^5	102	36
3. Analytical HPLC anion exchange chromatography	138	0.021	6.5×10^6	2,380	34
4. HPLC size exclusion chromatography	122	0.016	7.6×10^6	2,761	30

* The results are those of a representative run in which 5,000 ml of crude culture supernatant were processed.
‡ Activity was measured in the thymocyte proliferation assay as described in Materials and Methods.
§ Protein was measured by calibrating the peak integrator with known amounts of BSA as described in Materials and Methods.

applied activity (~40%, Table II) failed to bind tightly to the column. Similar observations have been made with conventional diethyl aminoethyl (DEAE) columns (11, 17, 31). In contrast to conventional DEAE substituted resins, however, the IL-1 activity was separated from most of the other nonbinding proteins as it eluted from the column. Not shown in Fig. 1 is the gradient portion of the run, which demonstrated that approximately two-thirds of the total protein and 60% of the thymocyte proliferation activity bound tightly to the column and could be eluted with a gradient of increasing ionic strength. The bound activity was mainly due to the various minor charged species of IL-1 (data not shown) and no further attempt at their purification was made. In these studies, the liquid chromatograph was programmed to perform 10 consecutive isocratic runs before regenerating the column with a gradient of increasing ionic strength. A highly reproducible elution pattern permitted the automated collection of IL-1 containing fractions in narrow bands (hatched areas in Figs. 1 and 2) without any significant loss of this material or excessive contamination with nearby protein peaks.

The active fractions represented by the hatched area in Fig. 1 were concentrated by ultrafiltration and chromatographed on an analytical AX300 column using the same conditions as for the preparative size column, except that the flow rate was reduced to 1 ml/min. This column step was necessary (*a*) to remove the trailing portions of peaks, which eluted in advance of IL-1 on the preparative column and (*b*) to remove late eluting material from previous consecutive runs. Fig. 2 shows that, upon chromatography on the analytical AX300 column, the IL-1 activity eluted as a single sharp peak and coincided with a peak of A_{210} absorbing material. SDS-PAGE of the active and flanking fractions showed that a ~15,000-mol wt band was the dominant species in the most active fraction and

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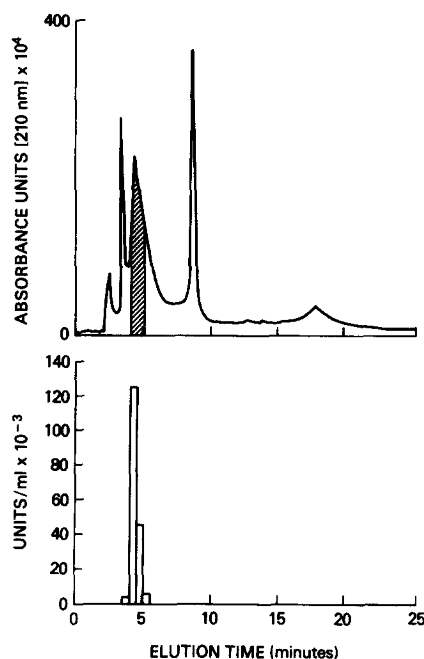

FIGURE 2. Rechromatography of partially purified human IL-1 on Synchropak AX300. Partially purified IL-1 obtained after passage on a preparative Synchropak AX300 column was concentrated by ultrafiltration and chromatographed isocratically on an analytical (250 × 4.1 mm) Synchropak AX300 column equilibrated in 5 mM Na_iPO_4 buffer, pH 7.25, at a flow rate of 1 ml/min. The absorbance of the column effluent at 210 nm is shown in the upper panel. Fractions were collected at 0.5 min intervals and assayed for IL-1 activity (U/ml; lower panel). The hatched area in the upper panel represents those fractions that were pooled, concentrated, and further purified by high performance size exclusion chromatography.

that its staining intensity in this and flanking fractions correlated well with the amount of IL-1 activity present (data not shown).

Since the fractions flanking the hatched area in Fig. 2 appeared on SDS-PAGE to contain proteins of 25,000 mol wt or greater, the fractions represented by the hatched area in Fig. 2 were concentrated by ultrafiltration and chromatographed on an HPLC size exclusion column (TSK-G 2000 SW). As shown in Fig. 3, the IL-1 activity eluted with a sharp symmetrical peak of A_{210} absorbing material. The molecular weight of this material, as determined by calibration of the column with known molecular weight standards (Fig. 4, panel *B*), was ~12,000. Rechromatography of this material, i.e., the material eluting between 20.5 and 22.5 min, yielded a single peak when monitored at either 210 or 280 nm (data not shown). When the fractions containing thymocyte proliferation activity were tested for IL-2 activity, none was found.

The purity of this IL-1 preparation was further demonstrated in two ways. First, the four IL-1-containing fractions shown in Fig. 3 were pooled and analyzed by SDS gradient PAGE under reducing conditions as described in Materials and Methods. Electrophoresis followed by silver staining revealed only a single band (Fig. 5). The molecular weight of this material, as determined by calibration of the gel with known molecular weight standards, was 15,000 (Fig.

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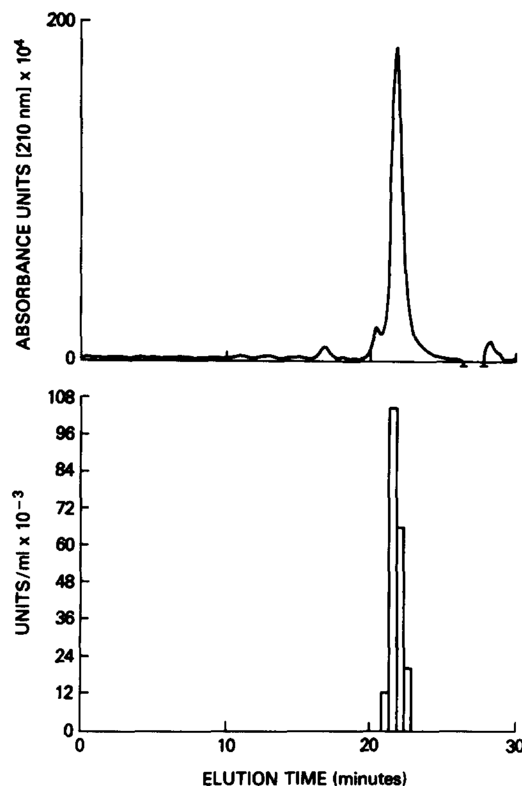

FIGURE 3. High performance size exclusion chromatography of partially purified human IL-1. Partially purified IL-1 obtained after chromatography on an analytical Synchropak AX300 column was chromatographed on a 600 × 7.5 mm TSK-G 2000 SW size exclusion column equilibrated in 100 mM Na_iPO_4, pH 6.95, at a flow rate of 1 ml per min. The absorbance of the column effluent at 210 nm is shown in the upper panel. Fractions were collected at 0.5 min intervals and assayed for IL-1 activity (U/ml; lower panel). The four fractions shown in the lower panel (vertical bars) eluted between 20.5 and 22.5 min. The negative deflection at the end of the chromatogram is due to passage of the sample buffer through the detector.

4, left panel). Second, the isolated material was examined by analytical IEF on ultrathin polyacrylamide gels. After focusing, adjacent lanes were either stained with Coomassie Brilliant Blue R-250 or sliced, eluted, and assayed for IL-1 activity. As shown in Fig. 6, a single band having a pI of 6.8 was observed. Furthermore, this band was coincident with a single sharp peak of IL-1 activity.

Table II summarizes the purification of the major charged species of human IL-1. The results from one representative run are given. The integration values obtained by the peak integrator when monitoring absorbance at 210 nm were used to determine the amount of protein obtained after each purification step (see Materials and Methods). As shown, the protocol results in a 30% yield of activity and approximately a 2,700-fold purification of IL-1. A specific activity of 7.6×10^6 U per mg protein was calculated (Table II). Most of the losses of IL-1 activity resulted from the loss of minor charged species during the first AX300 step.

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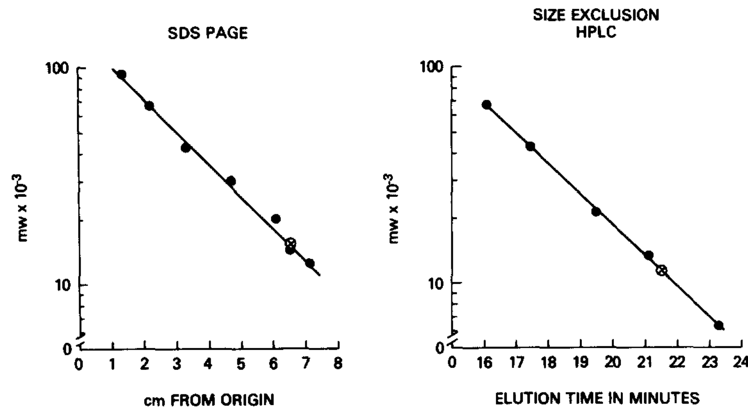

FIGURE 4. Molecular weight determination of purified human IL-1 by SDS gradient PAGE and high performance size exclusion chromatography. The log of the molecular weights of standard proteins (see Materials and Methods) was plotted as a function of their migration distance on SDS gradient PAGE or their elution time on size exclusion chromatography. The molecular weight of IL-1 was determined by plotting the observed migration distance and elution time (X) of the purified material on the calibration line obtained from the polyacrylamide gel and size exclusion column, respectively.

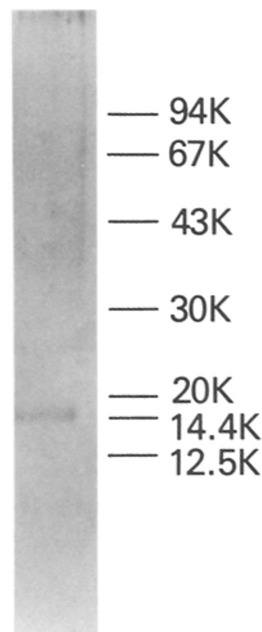

FIGURE 5. SDS gradient PAGE of purified human IL-1. The active fractions in Fig. 3 (elution time 20.5–22.5 min) were pooled and an aliquot was analyzed by SDS gradient PAGE as detailed in Materials and Methods. The gel was then fixed and stained with silver. The positions of the standard proteins used to calibrate the gel are shown at right.

Purified human IL-1 was tested at various concentrations in the murine thymocyte proliferation assay (Fig. 7). The concentration of IL-1 was determined by measuring absorbance at 210 nm (see Materials and Methods) and the molarity was calculated using a molecular weight of 15,000. Based on these calculations,

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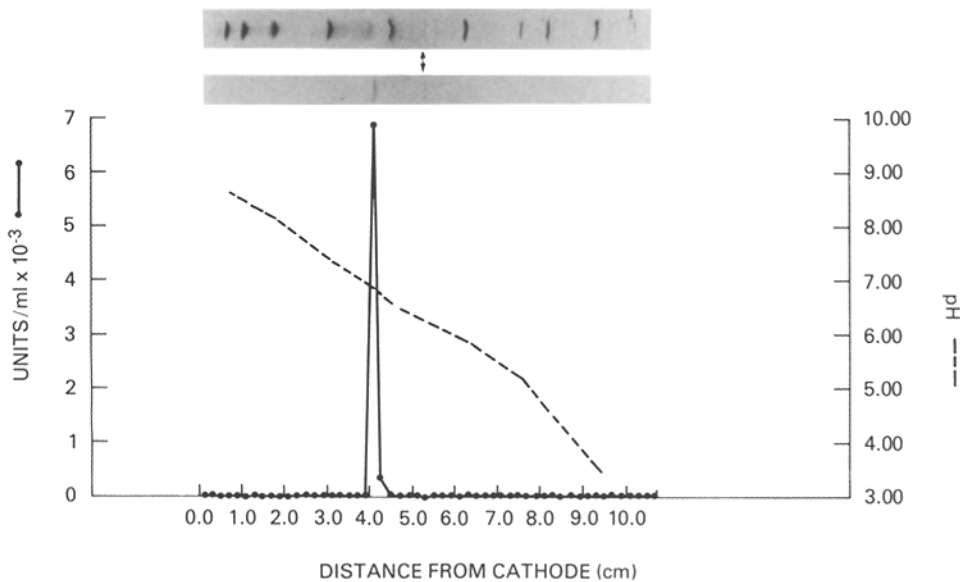

FIGURE 6. Analytical IEF of purified human IL-1. After removing an aliquot for analysis on SDS gradient PAGE, the remaining isolated material (Fig. 3; elution time 20.5–22.5 min) was desalted, concentrated, and applied to two adjacent lanes of an IEF gel (pH 3.5–9.5) by means of an applicator template (see Materials and Methods). Proteins of known pI were applied to separate lanes in order to calibrate the pH gradient of the gel. After focusing, the lanes bearing standard proteins (upper gel slice) and one of the lanes bearing IL-1 (lower gel slice) were fixed and stained with Coomassie Brilliant Blue R-250 (see Materials and Methods). The remaining gel slice bearing IL-1 was sliced, eluted, and assayed for IL-1 bioactivity (U/ml). The arrow indicates a staining artifact seen in both the standard protein and IL-1-bearing lanes resulting from scoring of the gel surface by the applicator template.

saturable stimulaton of thymocyte proliferation was observed with a half-maximal stimulation at 10^{-10} M IL-1. Maximal stimulation was obtained at 10^{-8} M and detectable activity was still observed at 10^{-12} M IL-1.

Discussion

The current study describes a rapid, efficient protocol for the purification of normal human IL-1. The success of the protocol was dependent in part on an ability to achieve a high specific activity of IL-1 by culturing the mononuclear cells under "serum-free" conditions. As had been reported by others, it was initially difficult to produce IL-1 in the absence of serum or exogenous proteins (32). However, by culturing the cells in upright flasks and thereby achieving a high settled cell density, this difficulty was circumvented (Table I). Phytohemagglutinin was chosen as an inducing agent because it could be obtained in highly purified form, was readily separated from IL-1 on the basis of charge and molecular weight, and because it is known to recruit T cells that augment the production of IL-1 by monocyte-macrophages (25, 33). The ability of other stimulants (e.g. silica, lipopolysaccharide) to substitute for purified phytohemagglutinin in this protocol remains to be studied.

My initial efforts to purify human IL-1 involved two different approaches,

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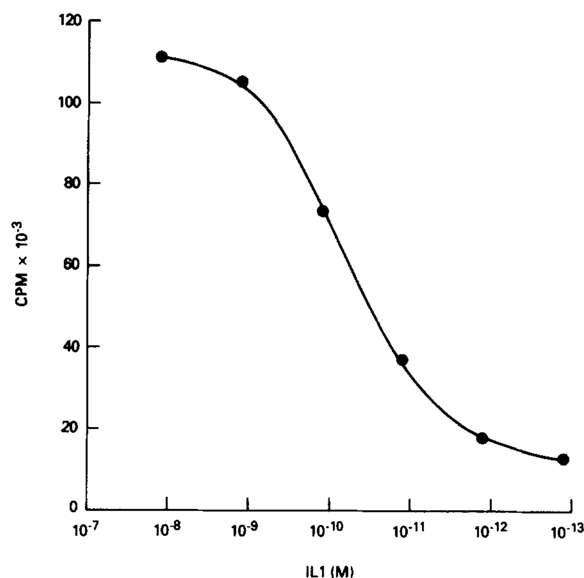

FIGURE 7. Dose response analysis of purified human IL-1 in the murine thymocyte proliferation assay. The amount of IL-1 protein used in this experiment was determined by measuring absorbance at 210 nm as described in Materials and Methods. Molarity was calculated using a molecular weight of 15,000. The points represent the means of triplicate determinations.

neither of which was ultimately satisfactory. The first of these used preparative IEF. This method offered several advantages including high capacity, high resolution, and sample concentration. Moreover, since the majority of proteins have pI's in the range of 4 to 6 (34), sharp separation of the pI 6.8 species of IL-1 from most of the contaminating proteins was achieved. Others have used IEF as a technique for purifying human IL-1 on the basis of these advantages (13, 18). However, a major drawback to using IEF for the purification of a small protein such as IL-1 is contamination of the sample with carrier ampholytes. Although most of these can be removed by simple dialysis, it was impossible to completely remove them from IL-1 by a combination of exhaustive dialysis and two sequential passages over an HPLC size exclusion column (J. Schmidt and C. Oliver, unpublished observations). Despite manufacturers' assurances to the contrary, high molecular weight species of carrier ampholytes have been documented (35, 36). Given the activity of carrier ampholytes in some in vitro biological (14) and biochemical (37) assays, their strong UV absorption, and their recognized ability to interfere in most assays used for protein determination (36), it was decided to use carrier ampholytes and IEF only as analytical tools. The second approach involved the use of reverse phase high performance liquid chromatography (RPC), a technique that has been highly successful in the purification of other trace mediators such as IL-2 (38), transformation growth factors (39), and immune interferon (40). Despite using a variety of buffer systems and bonded phases under various conditions of pH and temperature, it was not possible to obtain sufficient recovery of activity to make this approach

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feasible. Nevertheless, preliminary results suggest that RPC may be a powerful tool for the purification of inactive IL-1 for structural analysis.

The purification protocol presented illustrates the power of anion exchange HPLC as a tool for the purification of a trace, labile lymphokine, such as IL-1. Moreover, these results introduce the use of isocratic separations in high performance anion exchange liquid chromatography as an effective means of separating otherwise poorly resolved protein species. Experience with conventional DEAE-substituted media suggested that anion exchange chromatography (11, 17, 31) was not a satisfactory method for the purification of human IL-1 because a large portion of the IL-1 activity failed to bind to these media and little resolution among nonbinding proteins was obtained. Likewise, initial experiments with a number of different HPLC anion exchange columns showed that a significant portion of the applied IL-1 activity did not bind. However, by chromatographing the sample in low ionic strength buffer at a pH slightly above the major charged species of IL-1, excellent separation of IL-1 from the majority of nonbinding contaminants was obtained. Furthermore, these conditions allowed most contaminating proteins and the minor charged species of IL-1 to be removed as a result of tight binding to the column. Subsequent chromatography of the partially purified material on an analytical HPLC anion exchange column and HPLC size exclusion column was sufficient to yield highly purified material as judged by SDS gradient PAGE and analytical IEF.

This approach to the purification of IL-1 is advantageous because it results in the isolation of a single charged species. Analysis of mixtures of IL-1–charged species might be misleading, since the various species may differ considerably with respect to their biochemical properties and specific biological activity. Modification of the purification protocol should make it possible to isolate other charged species of IL-1 so that their biochemical and biological properties can be compared. Although calculations of yield may be favorably or adversely influenced by the presence of inhibitors or comitogens in the starting material that are subsequently removed during purification, the overall yield of bioactivity compares quite favorably with yields previously reported by others for the purification of murine IL-1 (12) and the partial purification of human IL-1 (13, 17–19). Small differences in specific activity were observed (±10%) for the purified material but are attributed to imprecision encountered in measuring IL-1 activity in the thymocyte proliferation assay. In the thymocyte proliferation assay, IL-1 exhibited saturable stimulation at a concentration of 10^{-8} M and half-maximal stimulation at a concentration of 10^{-10} M. These data are consistent with those recently reported for purified murine IL-1 (12) and suggest that IL-1 action is mediated by saturable, high affinity receptors.

Partial biochemical characterization of purified IL-1 showed that it has an isoelectric point of 6.8, in good agreement with values obtained previously by preparative focusing techniques (10, 11, 13). The molecular weight of the material, as determined by SDS–PAGE, is 15,000, also in agreement with values obtained previously by conventional gel filtration chromatography on Sephadex (17, 31). The somewhat lower value obtained by HPLC size exclusion chromatography is most likely due to a weak interaction with the column packing material. This is based on the observations (*a*) that IL-1 is retarded on this type

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of size exclusion media as compared to Sephacryl (11), and (*b*) that ethylene glycol is required to obtain good recoveries and to prevent band broadening on TSK-G 3000 SW preparative size exclusion columns (unpublished observations).

Because the elution time observed for the high pI species of IL-1 on each of the three HPLC columns was highly reproducible, chromatography was readily automated. Furthermore, the speed of HPLC separations now make it possible to prepare microgram quantities of IL-1 on a regular basis (15–30 μg/wk). This amount, though small by conventional protein chemistry standards, has been shown to be adequate for receptor binding studies (41) and many protein analytical techniques including amino acid analysis (42), peptide analysis (43), and sequence analysis (44). Thus, it should now be possible to study the composition of human IL-1 in detail, compare it to IL-1 or IL-1–like molecules derived from other sources, such as keratinocytes (45), and initiate studies designed to identify receptor sites for IL-1 on a variety of lymphoid and nonlymphoid cells and tissues. Moreover, because the material was purified without the use of phorbol esters or carrier ampholytes, this material should prove useful in confirming the various bioactivities ascribed to IL-1.

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Summary

A protocol for the rapid, efficient purification of the major charged species of human interleukin 1 (IL-1) has been developed using high performance anion exchange and size exclusion chromatography. The isolated material is pure as determined by sodium dodecyl sulfate (SDS) gradient polyacrylamide gel electrophoresis (PAGE) and analytical isoelectric focusing (IEF). The molecular weight of the purified material is 15,000 and the isoelectric point (pI) is 6.8, values that are in good agreement with those previously reported for human IL-1. 10^{-10} M concentrations of the purified material give half-maximal stimulation in the thymocyte proliferation assay. Amounts of IL-1 sufficient for receptor studies and detailed biochemical analysis can now be produced on a regular basis.

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