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Purification of Human Interleukin 1

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Interleukin I (IL-1) is a lymphocyte stimulant released by human monocytes cultured for 18–24 hours in tissue culture medium containing 5% serum and the non-specific immunostimulant lipopolysaccharide (LPS). Human IL-1 is found in the conditioned medium in a low molecular weight (∿ 13,000) and a high molecular weight (∿85,000) form. The high MW activity may result from the formation of a complex between IL-1 and serum constituents. During the course of purification, the low MW IL-1 activity is often recovered in a high MW form. Hollow fiber diafiltration and membrane ultrafiltration has been found to rapidly separate low MW IL-1 from all measurable protein with a yield of 4% of the original activity. The IL-1 which converts to the high MW form during the purification is recoverable, 21% of the original activity, but contains small amounts of serum proteins. Isoelectric focusing (IEF) of the low MW IL-1 resulted in a very highly purified sample which was analyzed by polyacrylamide gel electrophoresis (PAGE). Utilizing a new staining procedure which detects less than 1 ng of protein per band, the IEF-purified IL-1 revealed trace quantities (< 1 ng) of a slowly migrating protein similar to immunoglobulin and no other bands. There were no bands which corresponded with the known electrophoretic mobility of IL-1. Since the samples applied to the gel contained significant biological activity, this result implies that human IL-1 is biologically active in picogram quantities.

Key words: lymphocyte activating factor (LAF), Interleukin I, purification of human IL-1, hollow fiber diafiltration, isoelectric focusing, polyacrylamide gel, electrophoresis, human monocytes, endotoxin stimulation, IL-1 release, thymocyte mitogenic activity

Lymphocytes and macrophages in culture release a number of soluble substances (factors) which may affect lymphoid cells undergoing an immune response [1]. The term "lymphokine" has been used to describe lymphocyte-derived factors, and the term "monokine" has been proposed for macrophage- or monocyte-derived factors. Since the description of the first lymphokine, MIF (migration, inhibitory factor for macrophages) [2], the number of reported lymphokines and monokines has nearly defied categorization [3]. Interleukin 1 (IL-1, previously known as Lymphocyte Activating Factor – LAF), is perhaps the most well studied monocyte-derived factor [4–7]. IL-1 is

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found in the culture medium of human adherent mononuclear cells (monocytes) [8], mouse peritoneal exudate cells [4], mouse macrophage cell lines [9], and human monocytic leukemia cells [10]. Purification of IL-1 has been directed toward human peripheral blood-monocyte derived IL-1, and IL-1 derived from the $P388D_1$ macrophage cell line [9, 11, 12]. Human monocyte IL-1 has been partially purified by sequential gel filtration-ion exchange chromatography [5] and by hollow fiber ultrafiltration and isoelectric focusing [6]. The present report will describe our current procedure for large scale fractionation of IL-1 and evaluation of the purified activity.

MATERIALS AND METHODS

IL-1 Production

IL-1 was prepared on a large scale in a manner similar to the procedure of Gery et al [8]. Heparinized (50 U/ml) whole blood was mixed with one quarter volume of pyrogen-free Plasmagel (HTI Corp., Buffalo, NY), and the red blood cells were allowed to sediment for 30 min in a 37° water bath. The white blood cells were sedimented at 300g for 20 min, and the cells washed twice with Minimum Essential Medium-Earle's salts (MEM) supplemented with 2mM glutamine, 10 mM HEPES, penicillin (100 units/ml), and streptomycin (100 μg/ml) (Grand Island Biochemical Co., NY). Condition medium containing IL-1 was prepared by culturing the white blood cells in MEM at 0.5×10^6 cells/ml with 5% allogeneic (Grand Island Biochemical) or autologous human serum 10^{-5}, M 2-mercaptoethanol (Fisher Scientific Co., Medford, MA), and where indicated 20 μg/ml of E coli lipopolysaccharide W (LPS, 0.55:B5 or 0.27:B8, Difco Laboratories, Detroit, MI). Cell suspensions were cultured in sterile petri dishes (100 ml per 150 × 25 mm dish, Bioquest, Cockeysville, MD) at 37° in a humidified atmosphere of 5% CO_2 or spinner culture flasks in a 37° warm room for 24 hours. Cultures containing MEM and 5% serum without cells and LPS were incubated as controls. The cells were removed from the conditioned medium by centrifugation at 1000g for 20 min, and the medium could be stored for several months at −20°.

IL-1 Assay

To prepare thymocytes for the IL-1 assay, mouse thymuses (CD-1 mice, female 8–10 weeks old, Charles River Breeding Laboratories, Wilming, MA) were removed asceptically and homogenized in 10 ml MEM using teflon and glass hand homogenizer.

The suspension of cells was allowed to settle for 10 min; the thymocytes were separated from settled debris with a sterile Pasteur pipette; and the cell count adjusted to 10×10^6 cells/ml in MEM containing 5% normal human serum from a single donor and 10^{-5} M 2-mercaptoethanol. One tenth ml of the cell suspension was dispensed into each well of sterile tissue culture plates (IS-MRC-96-TC, Linbro Company, Hamden, CT). IL-1-containing samples were assayed in triplicate by adding 0.01 ml of sample to the wells. The plates were incubated for 72 hours in a humidified atmosphere of 5% CO_2 with the addition of 0.2 μCi of (^{3}H) thymidine (1.9 Ci/mmole, Schwartz/Mann, Orangeburg, NY) in 0.01 ml of 0.85% NaCl after 48 hours. The cells were then collected on glass fiber filters (Reeve Angel, Clifton, NJ) using an automatic cell harvester (Otter Hiller Company, Madison, WI). Liquid scintillation counting of the oven dried (30 min, 120°) filters was performed in a scintillation fluid containing 5 gm PPO and 0.5 gm POPOP/100 ml toluene (New England Nuclear, Boston, MA). All results are expressed as the average of triplicate cultures. A unit of IL-1 activity was determined based on the procedure of Gillis et al [13].

Hollow Fiber Diafiltration and Ultrafiltration

An Amicon hollow fiber filtration device (Model DC2, Amicon Corp., Lexington, MA) was used sequentially for diafiltration of the starting conditioned medium and removal of albumin by ultrafiltration. In the diafiltration step, the conditioned medium was extracted with HEPES (0.05M, pH 7.4)-NaCl (0.85%) buffer using a 50,000 MW cut-off hollow fiber cartridge (H1X50). The diafiltrate (ten times the volume of the original sample) was then ultrafiltered through the same 50,000 MW cartridge (after washing) to remove the small amounts of protein (~ 0.04 mg/ml) present in the diafiltrate. The ultrafiltrate, containing the low MW IL-1 activity, was then concentrated to ~45 ml by ultrafiltration using a stirred cell and a YM 10 (10,000 MW cut-off) membrane (Amicon Corp.).

Sephadex Gel Filtration Chromatography

Sephadex G100 (Pharmacia, Uppsala, Sweden) chromatography of 20-ml samples was carried out at 4° with a water-jacketed column (2.5 × 90 cm) using HEPES-C1 buffer. To concentrate samples of the original conditioned medium, the extracted conditioned medium, and the diafiltrate for Sephadex gel filtration chromotography, a 5,000 MW cut-off hollow fiber cartridge (H1P5) was used. The flow rate was 0.5 ml/min, and fractions of 11.4 ml were automatically collected with a refrigerated fraction collector (LKB Instruments, Rockville, MD).

Isoelectric Focusing

Isoelectric focusing (pH 8–4) was performed on a 40-ml sample using an LKB instrument (model 8100, 110 ml, capacity, Stockholm, Sweden) with a 5–50% sucrose gradient. Voltage was applied for 18–20 hours with an LKB constant power source (Model 2103, Stockholm, Sweden) using the settings of 1600 maximum voltage and 15 watts maximum power. Fractions of 1.5–2.0 ml were collected, measured for pH, and stored at 4°C. To remove Ampholine and sucrose prior to thymocyte assay, IEF fractions were dialyzed at 4° against HEPES-C1 buffer for 20 hours with one change of buffer. The dialyzed fractions received 0.05 ml of fetal calf serum (FCS) per ml of sample before sterile filtering (Disposable filters, 0.22 μm, Millipore, Bedford, MA). Samples could be sterile filtered without the addition of serum by using 0.2 μm Acrodisc membrane filters (Gelman, Ann Arbor, MI) instead of Millex filters.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was performed at 4° using 7% slab gels of 1-mm thickness with a pH 8.9 Tris-glycine buffer and a 2.5% stacking gel according to the procedure of Davis [14]. Isoelectric focusing fractions found to contain IL-1 activity (pH 6.8–7.2) were dialyzed against the running buffer for 20 hours at 4°, mixed with one-tenth volume of 0.1% bromophenol blue containing 10% sucrose, and PAGE was performed on 25 μl of each fraction. Slab gels were stained with $AgNO_3$ according to the procedure of Switzer et al [16].

RESULTS

Hollow Fiber Diafiltration and Ultrafiltration of IL-1 Conditioned Medium

IL-1 containing conditioned medium (1000 ml) from spinner culture was extracted with 10 liters of HEPES-C1 buffer (as explained in Materials and Methods). This procedure

allows all medium components of MW less than 50,000 daltons to collect with the diafiltrate. For the purpose of Sephadex chromatography, the original conditioned medium, extracted medium, and the diafiltrate were rapidly concentrated to 20 ml with a hollow fiber cartridge of 5,000 MW exclusion (H1P5). The concentrated samples were subjected to Sephadex G100 chromatography and the collected fractions compared with the original conditioned medium for IL-1 activity (Fig. 1). The results clearly indicate (Fig. 1C) that the < 50,000 MW diafiltrate contains only low MW IL-1 activity and is not contaminated with any of the high MW IL-1 activity observed in the unfractionated medium (Fig. 1A) or the medium from which the low MW activity has been partially extracted (Fig. 1B).

The hollow fiber diafiltrate (< 50,000 MW) contained small amounts of protein (~40 μg/ml), of which most was serum albumin. The reason small amounts of albumin and other high MW proteins are found in the < 50,000 MW fraction is that hollow fiber cartridges have an *average* MW cut-off, and thus allow small amounts of components with a MW greater than the listed cut-off to pass through the pores of the fibers. The residual protein was removed by ultrafiltration of the diafiltrate through the hollow fiber cartridge (50,000 MW exclusion) which was used in the original step. This procedure concentrated the protein in the > 50,000 MW retentate (50 ml) but also retained considerable IL-1 activity (Table I). The ultrafiltrate (9,950 ml), when concentrated to 43 ml using a stirred cell and YM 10 membrane, contained only about 20% of the IL-1 activity found in the diafiltrate. The ultrafiltration step of the diafiltrate is the poorest yield step in the purification procedure, but reveals a very interesting property of IL-1. The IL-1 activity was

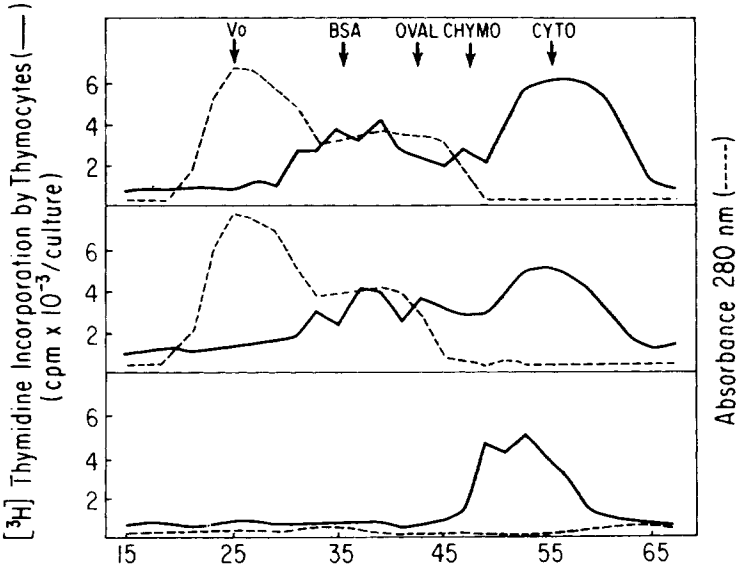

Fig. 1. Sephadex G100 chromatography of IL-1 conditioned medium and hollow fiber-separated, high- and low-MW IL-1 fractions. A) Unfractioned conditioned medium; B) the > 50,000 MW fraction; and C) the < 50,000 MW fractions of conditioned medium were prepared by hollow fiber diafiltration and concentration as described in Materials and Methods. Column fractions were sterile filtered and assayed for IL-1 activity in the thymocyte (^{3}H) thymidine uptake assay.

TABLE I. Separation of Low-Molecular-Weight IL-1 Activity From Conditioned Medium by Hollow Fiber Diafiltration and Ultrafiltration

				(^{3}H) Thymidine incorporation by thymocytes (cpm/culture)			
Purification step[a]	MW range	Volume	Undiluted	1:2	1:4	1:8	1:6
Conditioned medium	–	1,000	25,219[b]	21,428	15,739	7,565	
Extracted medium	>50,000	1,000	19,010	14,898	9,028		
Diafiltrate	<50,000	10,000	3,821	24			
Concentrated diafiltrate[c]	>50,000	50	25,784	25,283	25,580	19,470	15,485
Ultrafiltrate[c]	<50,000	9,950	8,660	1,102	1,062	372	
Concentrated ultrafiltrate	10–50,000	43	18,200	19,489	13,042	8,688	
Control medium without white blood cells			2,584				
Medium			1,980				

[a]The hollow fiber purification procedure is described in Results.

[b]Background values for control cultures not containing white blood cells have been subtracted.

[c]As explained in the text, the IL-1 activity which was able to pass through the 50,000-MW hollow fiber cartridge during diafiltration was only partially able to pass through the same hollow fiber cartridge during ultrafiltration.

able to pass through the hollow fiber cartridge during diafiltration (Table I and Fig. 1) but when ultrafiltered through the same cartridge, ∼80% of the IL-1 activity remained concentrated in the > 50,000 MW fractions. This phenomenon has been partially explained by the research of Togwa et al [15], who found that low MW IL-1 would form a high-MW complex when concentrated in the presence of serum proteins. We believe that as the protein concentration increases in the > 50,000 MW fraction, the ability of IL-1 to pass through the hollow fiber cartridge is greatly reduced. The hollow fiber ultrafiltration step greatly reduces the yield of IL-1 activity, but substantially improves the purity. The ultrafiltrate, when concentrated to 43 ml in a stirred well with a YM 10 membrane, was found to contain insufficient protein to be measured by any conventional technique.

The H1P5 hollow fiber cartridge (5,000 MW cut-off) used to concentrate the hollow fiber fractions for Sephadex G100 chromatography was found to irreversibly bind the high MW IL-1, and thus explained why the chromatogram of the concentrated diafiltrate (Fig. 1C) revealed only the uncomplexed, low MW IL-1 activity.

Isoelectric Focusing of Hollow-Fiber-Purified, Low-MW IL-1

The concentrated ultrafiltrate was subjected to pH 8–4 sucrose gradient isoelectric focusing, and the resulting fractions were assayed for IL-1 activity (Fig. 2). The center of the peak of IL-1 activity is pH 7.1. In this and other isoelectric focusing experiments, the most active fractions were always found in the pH 6.8–7.2 region. A second much smaller peak of IL-1 activity is occasionally found in the pH 5.3–5.8 region. Isoelectric focusing of the concentrated diafiltrate, however, reveals a large peak of IL-1 activity in the pH 5.3–5.8 region as well as the peak of activity in the pH 7 region. These data indicate that the IL-1 activity found in the concentrated diafiltrate is present as both the pH 7 activity seen in the low-MW concentrated ultrafiltrate and as a pH 5 activity which we believe represents the high-MW complex of IL-1 with a serum protein. The isoelectric point of 5.3–5.8 may indicate that IL-1 has complexed to human serum albumin.

PAGE of Isoelectric Focusing Purified IL-1

The isoelectric focusing fractions from the concentrated ultrafiltrate which contained the IL-1 activity (pH 6.7–7.3 and 5.3–5.5) (Fig. 2A) were dialyzed overnight against the Tris-glycine running buffer and 30-μl samples containing 5 μl of tracking dye was applied to each well of a polyacrylamide slab gel. Staining of the gel with $AgNO_3$ was performed as described [16]. The sensitivity of the staining procedure was confirmed by detection of bovine serum albumin samples containing 1 ng of protein (Fig. 3M). The concentrated ultrafiltrate, prior to isoelectric focusing, contained approximately 2.0 μg of protein per ml based upon the intensity of the visible band of albumin (Fig. 3C). This technique for determining protein concentration is strictly an estimate and is limited by the inability of the staining procedure to detect bands containing less than 1 ng of protein per mm of gel, and the possibility that some proteins may not be detected by this procedure.

The fractions of IEF-purified IL-1 from the pH 6.7–7.3 region (Fig. 3D–H) revealed a very faint band (less than 1 ng of protein) with an R_f of 0.1 in the first four fractions (Fig. 3D–G) and a more definite band in the final fraction (Fig. 3H). The fractions

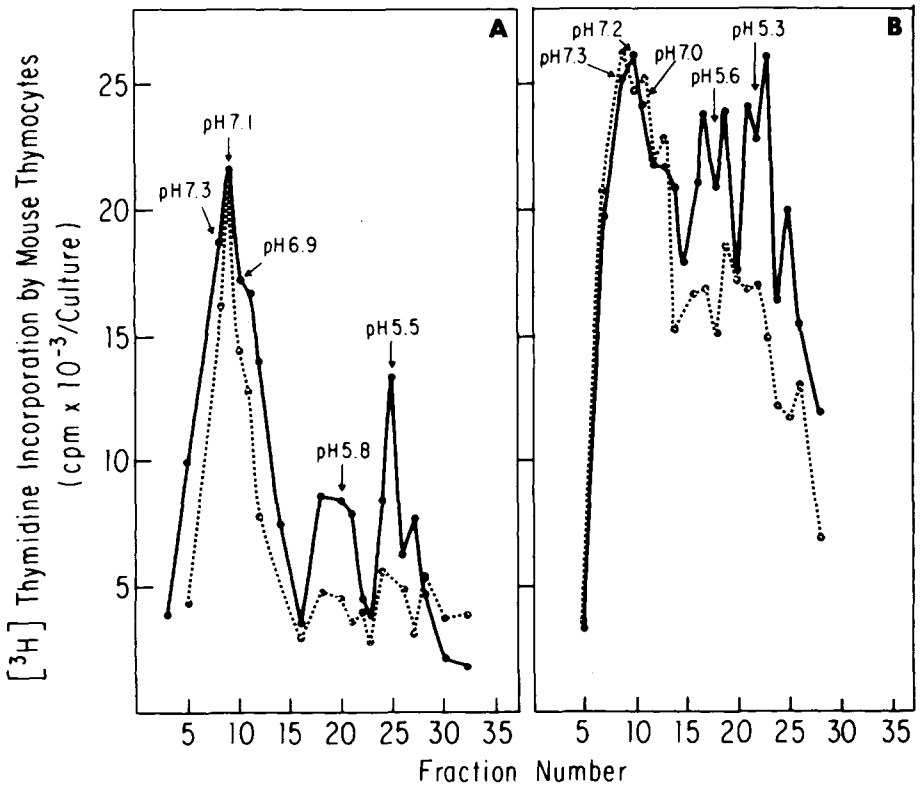

Fig. 2. Sucrose gradient isoelectric focusing of IL-1 activity in a pH 8 to 4 Ampholine gradient. The isoelectric focusing fractions of the concentrated ultrafiltrate (A) and the concentrated diafiltrate (B) from one liter of conditioned medium (Table I) were dialyzed overnight to remove Ampholine and sucrose prior to the thymocyte (^{3}H) thymidine incorporation assay. The dialyzed fractions were assayed undiluted (——) and diluted one to ten (-------). The thymidine incorporation by unstimulated thymocytes was approximately 1,000 cpm/culture.

from the pH 5.5–5.3 region (Fig. 3 I, K) revealed small quantities of albumin and an extremely faint band at R_f 0.1. The R_f and isoelectric point of these faint bands would indicate that they are immunoglobulin. The 0.1 R_f bands do not correspond with the electrophoretic mobility of IL-1 (0.36 vs dye) in this gel system (data not shown) or the R_f or IL-1 relative to albumin (0.65 R_f). In addition, the intensity of the staining does not correspond with the relative biological activity since the samples in wells E, F, and G (Fig. 3) contained the most IL-1 activity. The conclusions reached from this very critical experiment are that 1) the IEF-purified IL-1 activity (pH 6.7–7.3) is very highly purified and contains only trace quantities of protein (<1 ng/25 μl), and 2) no detectable bands of proteins were evident which corresponded with the electrophoretic mobility of IL-1. Selective staining of similarly prepared gels with lipid and carbohydrate specific stains is currently being performed.

DISCUSSION

Separation of IL-1 activity from conditioned medium by hollow fiber ultrafiltration is based on the ability of low-MW IL-1 to pass through the pores of hollow fiber

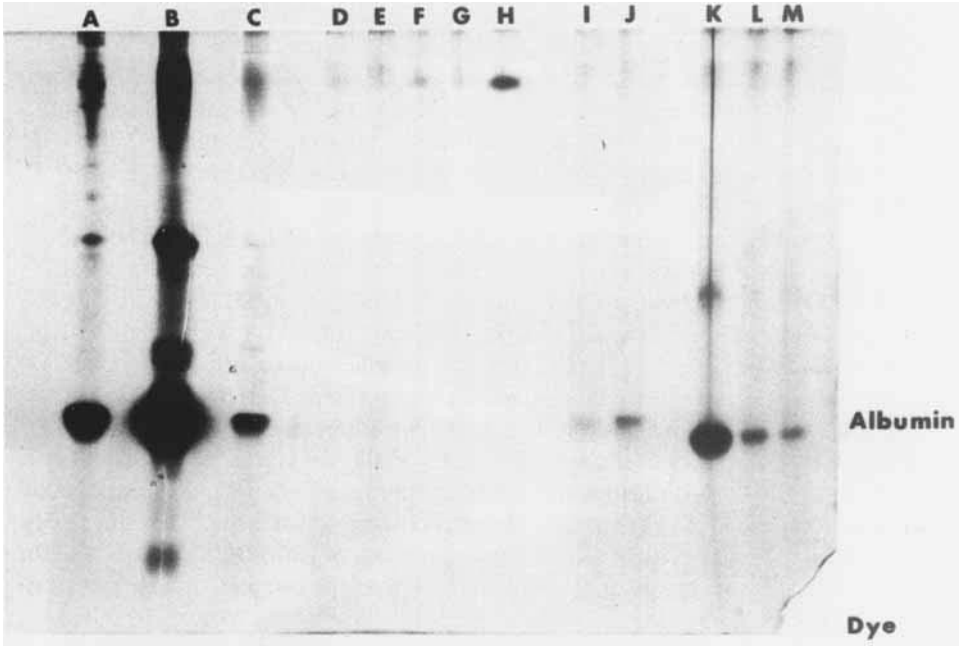

Fig. 3. Polyacrylamide gel electrophoresis of isoelectric focusing purified IL-1. Electrophoresis of the 7% slab gel with a 2.5% stacking gel in non-denaturing buffer was performed as described in Materials and Methods. The gel was stained for the presence of protein using the Ag staining procedure of Switzer et al [16]. Each well contained 25 μl of sample and 5 μl 0.1% bromophenol blue. The samples were: A) unfractionated IL-1 containing medium diluted 100-fold; B) concentrated diafiltrate (Table I); C) concentrated ultrafiltrate ; D–H) isoelectric focusing fractions pH 7.4–6.8 (Fig. 3A); I, J) isoelectric focusing fractions pH 5.8 and pH 5.5 (Fig. 3A); crystalline bovine serum albumin – 100 ng (K), 10 ng (L), and 1 ng (M).

devices while passage of serum proteins is retarded. The hollow fiber procedure originally described [6] has been improved in efficiency and speed of operation by the availability of hollow fiber equipment designed for large scale purification procedures. A major problem with all types of ultrafiltration is that, as the solution becomes concentrated, the ability of the molecule of interest to pass through the pores of the membrane is reduced. We have circumvented this difficulty by the technique of diafiltration. This procedure allows for separation of the $<50,000$ MW components from the conditioned medium without increasing the protein concentration of the starting sample. IL-1 activity was found to be in both the $>50,000$ MW fraction (the extracted conditioned medium) and the diafiltrate ($<50,000$ MW). This result is consistent with the observation of high MW ($\sim 85,000$ daltons) peak of IL-1 activity during Sephadex G100 chromatography [5, 15]. In these earlier studies, Sephadex chromatography was performed after the conditioned medium had been concentrated by ultrafiltration: and thus the possibility existed that the high MW IL-1 activity could result from binding of low-MW IL-1 to a carrier protein such as albumin. In the present study, high-MW IL-1 activity is found following diafiltration, when the serum concentration is not increased. If a high-MW complex exists, it appears to be a stable complex, since the original conditioned medium is extracted with ten volumes of buffer during diafiltration. Another possibility is that IL-1 is diafiltered more slowly than would be expected for a $\sim 14,000$ MW molecule, and that the remaining IL-1 activity in the extracted medium simply reflects incomplete diafiltration. Sephadex G100 chromatography of the unfractionated conditioned medium and hollow fiber fractions (Fig. 1) clearly indicates that the $<50,000$ MW diafiltrate contains low-MW IL-1 activity (Fig. 1C) and that diafiltration with ten volumes of buffer was not sufficient to remove the high-MW IL-1 activity from the conditioned medium (Fig. 1B). In addition, the appearance of low-MW IL-1 during Sephadex chromatography of the $>50,000$ MW extracted medium could be due to dissociation of the IL-1 protein complex during chromatography.

When the diafiltrate is concentrated using a stirred cell with a YM 10 membrane, a high MW complex of IL-1 is formed. The evidence for this complex formation is that 80% of the IL-1 activity which previously passed through the 50,000 MW hollow fiber cartridge is now retained. Formation of a high-MW complex of IL-1 with serum protein has been previously reported [15]. The IL-1 activity which does not pass through 50,000 MW hollow fiber cartridge, the concentrated diafiltrate (Table I), is not discarded. This activity is recoverable following isoelectric focusing, but purity of this activity is not nearly at the level of purity of the activity which was able to pass through the hollow fiber. We are currently investigating techniques which can reduce complex formation of IL-1 with serum proteins, and thus shift the balance toward recovery of the most highly purified activity. We have tested 10 mM 2-mercaptoethanol and 0.01% Triton-X 100 for this purpose, and found that neither reduced the level of the complex. Another interesting point to remember is that this complex is readily absorbed to PM ultrafiltration membranes manufactured by the Amicon Corporation. We found that the complex is readily absorbed to a 5,000-MW hollow fiber cartridge containing a PM membrane (Results) and also believe the frequently mentioned instability of IL-1 during concentration may be due to absorption of this complex to ultrafiltration membranes.

Isoelectric focusing of the concentrated ultrafiltrate is not only a purification step, but demonstrated a slight charge heterogeneity for low-MW IL-1 (Fig. 2A). IL-1 activity is always found between pH 6.8 and 7.2, and occasionally small peaks of IL-1 activity in the pH 5.5–6.0 region are present. The pH 5.3–5.8 peaks have never been greater

TABLE II. Summary of IL-1 Purification

Purification step	Volume (ml)	Protein (mg)	Specific activity (units/mg)[a]	Purification	Yield (%)
Conditioned medium	1,000	3,800[b]	1.4	1	100
Concentrated ultrafiltrate	43	0.1[c]	2,110	1,465	4
Isoelectric focusing of concentrated ultrafiltrate	9	if <0.0001[d]	>768,000	>500,000	1.5

[a]See text and Table I for a complete explanation of the purification procedure and calculation of units IL-1 activity.

[b]Determined by the microbiuret procedure.

[c]The protein concentrations in these solutions are too low to be estimated by conventional techniques. The values given are estimated from the intensity of stained bands compared with known concentrations of standard proteins.

[d]The sensitivity of the silver-nitrate staining procedure is 1 ng of protein per 2-mm band.

than one third the area of the pH 6.8–7.2 peak, and in several purifications the small peaks were not detected. We believe the pH 5.3–5.8 peaks may be the high-MW IL-1 complex, since they were present in much greater quantity in the fraction of activity which was unable to pass through the 50,000 MW hollow fiber cartridge. The recovery of IL-1 activity following IEF is about 1.5% of the original activity or 3% of the desired low MW IL-1, since the original medium contains about equal amounts of high and low MW IL-1. Isoelectric focusing is not a high yield procedure (Table II), but offers a significant increase in purity (Fig. 3). The final degree of purification of IL-1 can only be estimated, since the IEF fraction contains too small an amount of protein to be measured by conventional techniques (Table II). When IEF-purified human IL-1 was applied to non-denaturing polyacrylamide gels, biological activity could be eluted from the gels as a single peak [6]. An identically run gel did not reveal any protein bands when stained with Coomassie brilliant blue. This result indicated that IL-1 was not a protein or that IL-1 was not present in sufficient quantity to visibly bind the protein stain. Currently, staining or polyacrylamide gels with Ag^+, a technique which detects as little as 1 ng of protein per band, has not revealed any protein bands in the IEF-purified IL-1 samples which correspond to IL-1. The polyacrylamide gels clearly indicated that the purity of the IEF sample is excellent since only trace quantities of proteins are present in the sample, and none of these in a concentration of greater than 1 ng/25 μl of sample. These results also directly indicate that a quantity of IL-1 which is sufficent to stimulate a strong response in mouse thymocyte cultures* does not reveal a distinct band of at least 1 ng of protein which correlates with the known electrophoretic mobility of IL-1 [6, 17, 18]. Possible explanations for this finding are that IL-1 is present in only picogram quantities, that IL-1 may not have stained by this technique, or that IL-1 is only partially composed of protein.

Inhibition of IL-1 release by monocytes when cultured in the presence of cycloheximide is not direct evidence that IL-1 is a protein. Recent experiments have demonstrated that IL-1 is enzymatically inactivated by trypsin and chymotrypsin and chemically

*Each thymocyte culture of 0.1 ml is stimulated with 10 μl of sample. Each well of the gel contained 25 μl of IEF-purified IL-1. The IEF-purified IL-1 applied to the gel (Fig. 3D–H) contained 20 units of activity/ml in fractions E, F, and G and 10 units/ml in fractions D and H.

inactivated by cleavage with cyanogen bromide. Radioiodination of IEF-purified IL-1 has been performed using ^{125}I-labeled Bolton-Hunter reagent [19]. PAGE of the iodinated sample did not reveal a radioactive peak which corresponded with the electrophoretic mobility of IL-1 (data not shown).

IL-1 from the P388D$_1$ and J774.1 mouse macrophage cell lines has been partially purified using a procedure similar to the one described in this paper [12]. The results indicate that culture medium from the LPS-stimulated macrophage cell lines also contain a high ($\sim$60,000–80,000) MW and a low ($\sim$ 17,000) MW IL-1 activity. Similar to the human activity, the high MW IL-1 is stable during diafiltration. The major isoelectric point, pH 5.0–5.4, of the low-MW mouse macrophage cell line IL-1 differs significantly from the isoelectric point of pH 6.9–7.1 for human IL-1. In addition the isoelectric point of the human IL-1 is sharp, indicating lack of charge heterogeneity, while the isoelectric point of the mouse macrophage cell line IL-1 is broad [12].

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