Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7429–7433, October 1986
Immunology

Isolation and characterization of a primitive interleukin-1-like protein from an invertebrate, *Asterias forbesi*

(Echinodermata/evolution/primitive phagocyte)

GREGORY BECK AND GAIL S. HABICHT*

Department of Pathology, Health Sciences Center, State University of New York, Stony Brook, NY 11794

Communicated by Lewis Thomas, June 9, 1986

ABSTRACT **Interleukin 1 (IL-1) is the major immunoregulatory molecule produced by macrophages and other related cells. It acts as the molecular orchestrator of nonspecific host defense mechanisms against multifarious insults. In this study we report the characterization of an IL-1-like protein from the starfish *Asterias forbesi*. IL-1-like activity was found in the coelomocytes of this invertebrate and was also isolated and purified from the coelomic fluid. The coelomocytes were capable of phagocytosing latex particles and were adherent to plastic tissue culture dishes. Starfish IL-1 stimulated murine thymocyte proliferation directly and to a greater degree in the presence of submitogenic concentrations of concanavalin A. Starfish IL-1 also stimulated fibroblast proliferation and fibroblast protein synthesis. It was found to have a M_r of 29,500 and pI values of 7.5, 5.9, and 4.8. Starfish IL-1 activity was inhibited by an antibody to human IL-1. These characteristics suggest starfish IL-1 to be very similar to murine and human IL-1. The similarities to vertebrate IL-1 considered along with the evolutionary position of the starfish suggest that IL-1 is an important, ancient, and functionally conserved molecule.**

Interleukin 1 (IL-1), a cytokine released by a variety of cells (e.g., macrophages, Langerhans cells, Kupffer cells, and astrocytes), is a major immunoregulatory protein. The immunological effects of IL-1 include thymocyte proliferation or lymphocyte activation factor (LAF) activity, lymphokine production, and receptor expression on lymphocytes (1). Equally important are the effects of IL-1 as a mediator of nonspecific host defense mechanisms. Fever induction, stimulation of acute-phase reactants, neutrophilia, stimulation of fibroblasts and synovial cells, and chemotaxis of neutrophils are just a small sample of the manifold activities of IL-1 (2, 3). Microorganisms, microbial products, antigens, lectins, and phagocytic challenge are among the diverse agents that induce IL-1 release (2).

Biochemical characterization of IL-1 from a number of vertebrate species has revealed basic similarities in the structure and properties of this cytokine (2). The M_r is usually in the 12,000–20,000 range although large M_r forms of between 35,000 and 70,000 are found. Isoelectric focusing studies have shown charge heterogeneity often with pI values in the 5.0–5.6 and 6.8–7.5 ranges. The protein nature of IL-1 has been confirmed by studies using pronase and proteinase K to destroy IL-1 activity (4). Heating IL-1 at 70°C for 30 min greatly diminishes the activity. Recently, human and murine IL-1 cDNAs have been cloned and the amino acid sequences derived share 62% homology (5, 6).

The tremendous importance of this protein to host immunologic and defensive systems along with the striking similarities of its properties shared by so many different species suggested to us that IL-1 may be a protein that has been conserved through evolution. Pioneering work by Metchnikoff demonstrated that echinoderms possess large mononuclear phagocytic cells that participate in host defense (7). He likened these cells to mammalian macrophages. We asked whether part of their defensive armament might include an IL-1-like molecule. We therefore examined the coelomic fluid and coelomocytes of the starfish *Asterias forbesi* for the presence of a similar molecule or homologue. Other proteins extensively studied and found to be of major importance to mammals have also been found in invertebrates, including α_2-macroglobulin in the horseshoe crab *Limulus polyhemus* and many proteases and protease inhibitors from other lower invertebrates (8, 9). This report then deals with the isolation and characterization of a primitive IL-1-like protein that shares many of the physical and biological properties of vertebrate IL-1.

MATERIALS AND METHODS

Materials. Pyrogen-free water and pyrogen-free saline were obtained from Travenol (Deerfield, IL). *Escherichia coli* 0111:B4 lipopolysaccharide W (LPS) was obtained from Difco. Sterile pyrogen-free syringes and needles were obtained from Becton Dickinson. All plastic ware was obtained from Falcon. All tissue culture media were obtained from Flow Laboratories. All other reagents were of analytical grade or better and were obtained from Sigma or Fisher.

Collection of Starfish. The starfish *Asterias forbesi* were obtained from the waters of Long Island Sound at Port Jefferson, NY, and were kept in aerated sea water at 18°C while in the laboratory. All animals were used within 24 hr of collection.

Isolation of Coelomocytes and Coelomic Fluid. Coelomocytes were obtained from the coelom of the starfish by cutting off the tip of the arm with scissors and draining the fluid into sterile beakers. It was found that recovery of coelomocytes was increased by first swelling the starfish in a hypotonic solution of 50% sea water. The coelomic fluid was put at 4°C for ≈15 min and then centrifuged at 2000 × *g* for 10 min at 4°C to separate the cells (10). The coelomic fluid was then centrifuged at 20,000 × *g* for 20 min at 4°C to remove particulate matter and stored at −20°C until used. The coelomocytes were washed two more times in starfish medium (SFM; powdered minimal essential medium plus sea water diluted with pyrogen-free water; filter sterilized, pH 7.6), a nutritive solution with the ionic strength of sea water (804 mosM). In one series of experiments the coelomocytes were plated into 60-mm tissue culture dishes at a cell density of 5×10^6 cells per ml in SFM and incubated at 37°C in a 5% CO_2 in air incubator. Various dilutions of LPS were added and the supernatants were harvested 24 hr later, centrifuged at 3000 × *g* for 10 min at 4°C, and frozen at −20°C until used.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL-1, interleukin 1; LPS, lipopolysaccharide; LAF, lymphocyte activation factor.

*To whom reprint requests should be addressed.

In other experiments, the washed coelomocytes were resuspended in a small amount of pyrogen-free water and freeze-thawed at −20°C for three cycles. The resulting mixture was then centrifuged at 20,000 × *g* for 20 min at 4°C to remove particulate matter, and the supernatant was frozen at −20°C until used.

Preparation of Human IL-1. IL-1 was produced as described in detail by stimulating U937 cells with the spirochete *Borrelia burgdorferi* (11). In brief, cell-free supernatants containing IL-1 were subjected to ultrafiltration, dialysis, and column chromatography on ACA 54 (LKB, Bromma, Sweden). Sodium dodecyl sulfate/polyacrylamide gel electrophoresis ($NaDodSO_4$/PAGE) analysis revealed the human IL-1 to have a M_r of 13,500 and isoelectric focusing revealed two peaks at pI 7.25 and pI 4.4–5.0 (11).

IL-1 Assays. *Thymocyte proliferation assay.* Samples were assayed for IL-1 activity with thymocytes from 4- to 8-week-old BALB/c mice, as described (11). One unit of IL-1 per ml induced 50% of the maximal thymocyte proliferative response, as described by Mizel (12). An IgG fraction of rabbit anti-human IL-1 antibody was kindly provided by Robert C. Newton of DuPont (Glenolden, PA). The antibody was produced by injection of purified α and β human monocyte-derived IL-1 into rabbits. This antibody (at a 1:640 or 1:320 dilution) is capable of ≈50% inhibition of 1 unit of human IL-1 α or β activity, respectively, when assayed in the thymocyte proliferation assay. It also inhibited the activity of IL-1 in a fibroblast proliferation assay and in a chondrocyte stimulation assay but had no effect in IL-2 assays. Starfish fractions were incubated for 30 min at room temperature with and without antibody before addition of thymocytes.

Fibroblast proliferation assay. The stimulation of L929 fibroblasts was used as another assay for IL-1. Fibroblast proliferation was measured as described (11), except that the cells were pulsed with a mixture of 1 μCi of [^{3}H]thymidine and 0.25 μCi of [^{14}C]leucine (1 Ci = 37 GBq). For all assays, significance of differences was assessed by Student's *t* test.

Purification of Starfish IL-1. Samples of coelomic fluid were concentrated by ultrafiltration using a YCO-50 filter (Amicon). Samples were then applied to a Sephadex G-100 column and eluted with phosphate-buffered saline containing 10 μg of gentamycin per ml at a flow rate of 1.2 ml/min at 4°C. Column eluates containing peaks of thymocyte proliferation activity were pooled, concentrated, applied to a chromatofocusing column (Pharmacia), and eluted between pH 4 and pH 7.5 as per the manufacturer's instructions. All samples were brought to a pH of ≈7.2, then sterilized by Millipore filtration (0.22 μm; Millipore), and frozen at −20°C until used. Samples were assayed in the IL-1 assays as described above. Freeze-thawed coelomocyte preparations were chromatographed as above but were not concentrated before application to the G-100 column. $NaDodSO_4$/PAGE in 15% gels was used for molecular weight determinations (11). Samples were prepared by incubation with 1% $NaDodSO_4$ for 8 hr at 37°C as described by Mizel and Rosenstreich (13). Samples prepared in this way retain biological activity and can be used to determine the molecular weight of IL-1 (11, 13). Unstained lanes were cut into 10-mm sections and the slices were eluted and run in IL-1 assays as described (11).

Phagocytosis Assays. Coelomocytes prepared for tissue culture as described above were used to study the phagocytosis of latex particles. Latex particles (carboxylate modified, 0.907 μm; Dow Diagnostics, Indianapolis, IN) were added to the coelomocytes at a concentration of between 20 and 50 beads per cell. The dishes were placed into an incubator for 18 hr. The cells were then washed twice with SFM and the cells were examined by light microscopy. In some experiments the supernatants from these cultures were saved and used in IL-1 assays.

RESULTS

Characterization of Coelomocytes. Coelomocytes were collected, separated from coelomic fluid by centrifugation, washed, and suspended in SFM. After 24 hr in culture in a 37°C incubator or at room temperature, the cells were 100% viable when examined by light microscopy using trypan blue. The coelomocytes adhered to plastic tissue culture dishes and were capable of phagocytosing latex particles. After 7 hr in culture with latex particles at a concentration of 50 particles to each coelomocyte, large vacuoles were observed in the coelomocytes and on an average each coelomocyte had phagocytosed >15 latex particles.

Because of the similarities between coelomocytes and macrophages we attempted to stimulate the coelomocytes to release a LAF. Coelomocytes were cultured for 24 hr with LPS at concentrations ranging from 3 to 50 μg/ml or with latex particles at a ratio of 50:1. Both of these agents have been shown to stimulate macrophages to release IL-1 (2). These treatments failed to release any LAF activity (data not shown).

We next looked for intracellular LAF activity that may have not been released by the coelomocytes. Pooled coelomocytes were lysed by three cycles of freeze-thawing, and the resulting supernatant was tested for LAF activity. Again, no LAF activity was detected (data not shown). However, after the crude cell lysate was passed over a series of molecular sieve columns we were able to demonstrate LAF activity (Fig. 1). Activity at three M_r ranges, >70,000, 20,000–40,000, and slightly less than 10,000, was detected following chromatography on a Sephadex G-100 column. This preparation was a pooled fraction taken from the lower molecular weight area of a Sephacryl S-300 column. The S-300 column revealed that a high molecular weight (M_r >100,000) fraction had a powerful inhibitory effect on the thymocytes.

Characterization of Coelomic Fluid. The coelomic fluid collected from several starfish was pooled and concentrated by ultrafiltration. When assayed, the unconcentrated coelomic fluid showed no LAF activity. When this preparation was concentrated and chromatographed on Sephadex G-100 a major peak of LAF activity was observed (Fig. 2). This peak corresponds to a M_r of ≈30,000. This activity elution profile is in contrast to that seen with the cell lysates, which contained several peaks of LAF activity. The coelomic fluid also possessed a high molecular weight factor that was inhibitory to the thymocytes (Fig. 2). Whether this activity

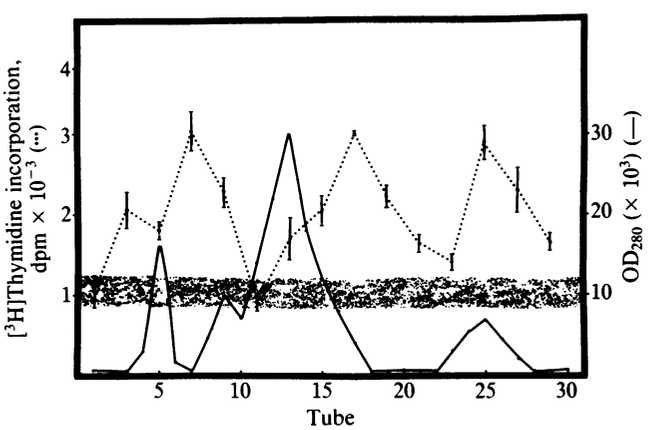

FIG. 1. Fractionation of intracellular LAF from coelomocytes. The lysate from starfish coelomocytes was chromatographed on Sephadex G-100. Column fractions were tested at a 1:16 dilution for their effect on thymocyte proliferation. The shaded area is the background stimulation of the thymocytes. [^{3}H]Thymidine data are shown as mean ± SEM.

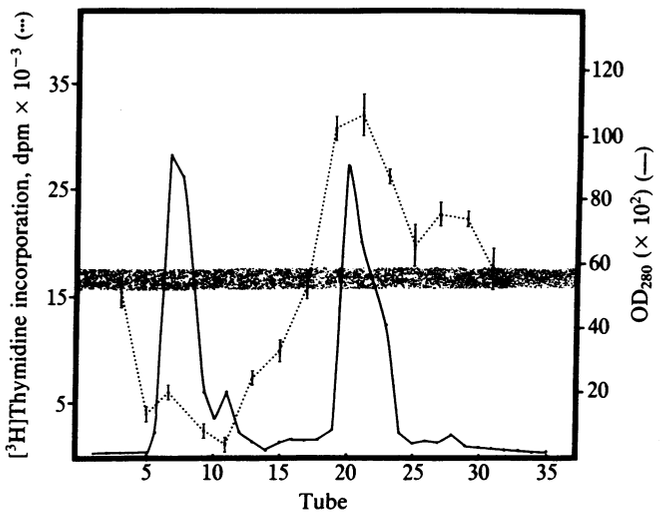

FIG. 2. Fractionation of coelomic fluid. Coelomic fluid was collected, pooled, concentrated, and chromatographed on Sephadex G-100. Column fractions were tested at a 1:16 dilution for their effect on thymocyte proliferation. The shaded area is the background stimulation of the thymocytes. [3H]Thymidine data are shown as mean ± SEM.

inhibits the thymocytes directly or is a LAF inhibitor is yet to be determined.

In a further attempt to characterize the starfish LAF, the major peak of LAF activity from the G-100 column was pooled, concentrated, and applied to a chromatofocusing column. A linear pH elution gradient from pH 7.5 to pH 4 revealed three major peaks of activity, which had pI values of 7.5, 5.9, and 4.8 (Fig. 3). These fractions were tested to obtain a relative index of activity for the various forms by assaying them at various dilutions. The pI 5.9 and 4.8 fractions were more active than the pI 7.5 fraction in the murine thymocyte proliferation assay (Table 1). These data are only an indication of biologic activity and not absolute to the amounts of material assayed.

To define the molecular weight and to determine the purity

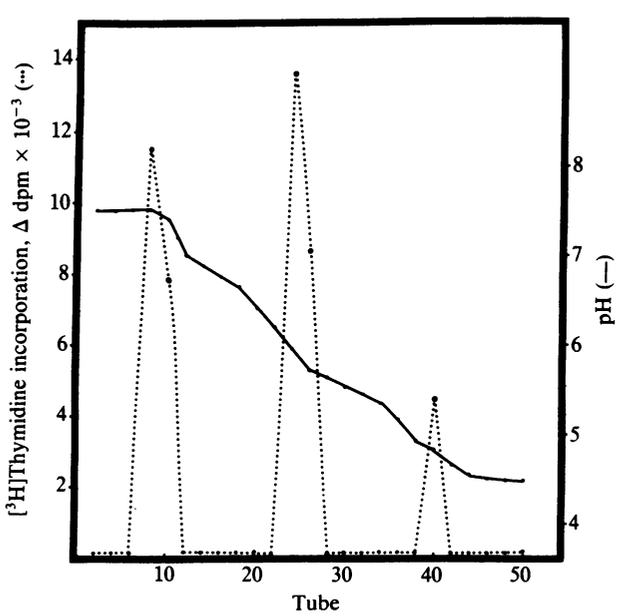

FIG. 3. Chromatofocusing of the pooled LAF activity from the Sephadex G-100 column fractionation of the coelomic fluid. The column was eluted between pH 7.5 and pH 4. The fractions were tested at a 1:32 dilution for their effect on thymocyte proliferation.

Table 1. Effect of starfish LAF on murine thymocyte proliferation

Sample	[3H]Thymidine incorporation, dpm
Control	12,671 ± 846
pI 7.5	16,511 ± 894
pI 5.9	39,903 ± 797*
pI 4.8	40,492 ± 3106*

pI fractions were tested at a 1:32 dilution. Thymocyte proliferation is expressed in terms of [3H]thymidine incorporation. Data are shown as mean ± SEM.
*$P < 0.001$ as compared to control.

of our preparations, NaDodSO4/PAGE was performed. As has been demonstrated with human and murine IL-1 (11), the starfish LAF activity could be eluted from the gel. As shown in Fig. 4, the major band of activity recovered from the coelomic fluid had a M_r of 29,500. Other proteins in the gel were in the M_r range of 60,000 and 100,000 and had no LAF activity. Though not apparent in this gel, there were other bands discernible in the M_r 15,000 and 20,000 areas of the gel. When the three chromatofocusing fractions were analyzed by NaDodSO4/PAGE, proteins stained in the M_r 29,500, 20,000, and 15,000 areas of the gel (data not shown).

To explore further the biological activity of the LAF isolated from the starfish, we next assayed the fractions for their ability to stimulate fibroblast proliferation and fibroblast protein synthesis. These results are presented in Table 2. As shown, the pI 7.5 and 4.8 fractions stimulated the replication of subconfluent fibroblasts, whereas the pI 5.9 fraction had no effect. However, all three fractions stimulated the incorporation of [14C]leucine into proteins.

Next, whole coelomic fluid and the purified LAF pI 5.9 and 4.8 chromatofocusing fractions were incubated with a polyclonal antibody to human IL-1 and then were tested in the thymocyte proliferation assay. As seen in Table 3, our control human IL-1 was significantly neutralized by this antibody. The coelomic fluid and purified isoelectric focusing fractions were also significantly neutralized.

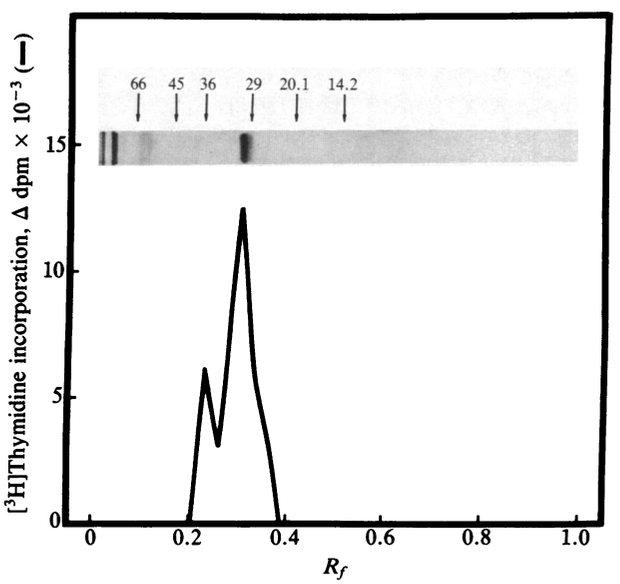

FIG. 4. NaDodSO4/PAGE of concentrated coelomic fluid. Coelomic fluid was run on a 15% slab gel. The gel was sliced into 10-mm pieces and the samples were eluted. The samples were assayed at a 1:16 dilution in the thymocyte proliferation assay. Molecular weight marker proteins are indicated as $M_r \times 10^{-3}$ along the top.

Table 2. Effect of starfish LAF on proliferation and protein synthesis of fibroblasts

Sample	[^{3}H]Thymidine incorporation		[^{14}C]Leucine incorporation	
	dpm	% stimulation	dpm	% stimulation
Control	53,670 ± 813		1086 ± 34	
pI 7.5	85,486 ± 1997	59 ± 4*	2355 ± 82	116 ± 10*
pI 5.9	56,898 ± 4037	6 ± 7†	2461 ± 247	126 ± 24*
pI 4.8	89,147 ± 4627	66 ± 9‡	2710 ± 49	149 ± 9*

Data are shown as mean ± SEM.
*$P < 0.001$ as compared to control.
†Not significant.
‡$P < 0.02$ as compared to control.

DISCUSSION

In this report we have shown that the starfish *Asterias forbesi* contains a primitive IL-1-like protein. Several lines of evidence point to the similarities between vertebrate IL-1 and this invertebrate protein. These include the observation that murine thymocyte proliferation is stimulated directly by starfish LAF and to a greater extent in the presence of submitogenic concentrations of concanavalin A (Table 1). Murine and human IL-1 have this property also (1). Starfish LAF is found in the starfish coelomocytes, which are closely related to the macrophage (14), the vertebrate cell that is responsible for the production and secretion of IL-1 (2). Intracellular IL-1 and several secreted forms of IL-1 have been characterized from murine and human cells (15, 16). Starfish LAF also shows molecular weight heterogeneity. We observed several different species of intracellular LAF, all active in the thymocyte proliferation assay and ranging in M_r from ≈10,000 to >70,000 (Fig. 1). High molecular weight species in murine and human systems have been isolated and extensively studied by several investigators (17, 18). Secreted LAF, isolated from the coelomic fluid, was found to have a M_r of 29,500 (Figs. 2 and 4), which is similar to the M_r 35,000 species of human IL-1 isolated and characterized by Ihrie and Wood (19). Chromatofocusing of the starfish LAF revealed three distinct species with pI values of 7.5, 5.9, and 4.8 (Fig. 3). Isoelectric focusing of human IL-1 reveals three predominant species at pI 7.2, 6.0, and 5.4 (1), whereas murine IL-1 has pI values of 7.2 and 5.2 (11). Other vertebrates, including rabbits and rats, have IL-1 species that fall into these pI ranges (20). Another characteristic of vertebrate IL-1 is its ability to stimulate fibroblast proliferation and protein synthesis (21). Starfish LAF was found to be capable of stimulating murine fibroblasts to proliferate concomitant with an increase in protein synthesis (Table 2). Finally, an antibody to human IL-1, raised in rabbits, was found to inhibit starfish LAF just as effectively as human IL-1 when their activities were measured in the thymocyte proliferation assay (Table 3). When these data are considered together, they support the conclusion that invertebrates do indeed possess a protein with the biochemical and biological properties of vertebrate IL-1. This protein can therefore be called a primitive IL-1.

Table 3. Effect of a polyclonal antibody to human IL-1 on starfish LAF-induced thymocyte proliferation

Sample	Dilution	Antibody dilution	% inhibition
Human IL-1	1:128	1:160	32.9 ± 4.2*
		1:320	34.8 ± 4.3*
Concentrated coelomic fluid	1:64	1:160	40.4 ± 3.1†
		1:320	35.7 ± 5.1*
Starfish IL-1 pI 5.9	1:32	1:160	40.6 ± 4.5*
		1:320	27.6 ± 6.4*
pI 4.8	1:32	1:160	19.7 ± 8.0‡
		1:320	20.3 ± 10.4

Data are shown as mean ± SD.
*$P < 0.01$ as compared to control.
†$P < 0.001$ as compared to control.
‡$P < 0.05$ as compared to control.

Starfish are members of the class Asteroidea in the phylum Echinodermata. The echinoderms are more closely related to vertebrates than to the other invertebrates in that the echinoderms and chordates belong to the group Deuterostomia, whereas the other invertebrates are in the group Protostomia (22). Even though the echinoderms are known for their radial symmetry it is believed this is a secondary development, perhaps due to their sessile life (23). The embryology of the echinoderms suggests that they are related to prochordates, since the larva is bilaterally symmetrical. Biochemically, the blood and chemistry of muscle action of the echinoderms are more similar to the vertebrates than to any of the other invertebrate phyla (23). Though it is considered highly unlikely that the vertebrates arose directly from the echinoderms, they appear to be related and perhaps shared a common ancestor (24). The Asteroidea (as well as many of the other invertebrates) have been on the earth and relatively unchanged since about the Cambrian period (≈600 million years B.P.) (25). Since echinoderms are ancient creatures and are close to the vertebrates, they are excellent for studying immunity intermediate between vertebrates and invertebrates.

Coelomocytes are the invertebate correlate of vertebrate leukocytes, specifically the macrophage. They both adhere to glass or plastic, they can phagocytize and encapsulate, and both are involved in host defense (26). But, coelomocytes have other functions not likened to those of macrophages. Coelomocytes play an important role in nutrition, clotting, oxygen transport, and differentiation (26). The discovery of an IL-1-like protein in the coelomocyte gives further evidence to the similarities of the coelomocyte and the macrophage. This IL-1-like protein was isolated intracellularly from coelomocytes and extracellularly in the coelomic fluid. This suggests that the IL-1 is secreted by the coelomocyte into the coelom of the starfish in response to as yet unknown stimuli. The specific type of coelomocyte that secretes the IL-1 is not known. The function of IL-1 in the starfish is also not known. It may have activities similar to those of vertebrate IL-1, including chemotaxis, induction of secreted products, and host defense (2). Prendergast and coworkers have isolated a protein of M_r 39,000 (seastar factor) from coelomocytes of starfish that produces many effects on vertebrate cells. These include inhibition of immune responses to T-dependent antigens, induction of a skin reaction similar to delayed hypersensitivity, macrophage migration inhibition, and induction of experimental corneal neoangiogenesis (10, 27, 28). This protein inhibits T-cell activity and has a higher molecular weight than the protein we isolated. Their seastar factor was purified from disrupted coelomocytes, whereas our protein is a secreted protein that is abundant in the coelomic fluid. The seastar factor has activities possessed by IL-1, and perhaps it is a large molecular weight precursor of the secreted IL-1. We found large molecular weight IL-1 activity intracellularly (Fig. 1). In any case, the relationship of this protein to the protein we isolated is yet to be determined.

In conclusion, we have shown that an IL-1-like protein is present in invertebrates. This protein has many of the properties of vertebrate IL-1 when tested using mammalian cells. It is interesting to note that the different molecular species of this IL-1 isolated by chromatofocusing have different biologic activities. This heterogeneity has also been

observed with mouse and human IL-1 (2). These activities may be of use in probing the structure and function of IL-1(s) and in the clinical use of IL-1. But of equal importance, the existence of IL-1 in starfish suggests that IL-1 is an ancient and functionally conserved protein.

We thank Robert F. O'Brien and Hyun-Soo Lee for their help in the collection of the starfish. We also thank Dr. Howard Fleit for critical review of the manuscript and Dr. Robert C. Newton for the gift of the rabbit anti-human IL-1 antibodies. This work was supported by Grants AG00801 and AM36028 (to G.S.H.) from the National Institutes of Health.

1. Luger, T. A. & Oppenheim, J. J. (1983) in *Advances in Inflammation Research*, ed. Weissmann, G. (Raven, New York), Vol. 5, pp. 1–25.
2. Dinarello, C. A. (1984) *Rev. Inf. Dis.* **6**, 51–95.
3. Habicht, G. S. & Beck, G. (1985) in *The Physiologic, Metabolic, and Immunologic Actions of Interleukin-1*, eds. Kluger, M. J., Oppenheim, J. J. & Powanda, M. C. (Liss, New York), pp. 13–24.
4. Mizel, S. B. (1979) *J. Immunol.* **122**, 2167–2178.
5. Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A., Wolff, S. M. & Dinarello, C. A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7907–7911.
6. Lomedico, P. T., Gubler, U., Hellman, C. P., Dukovich, M., Giri, J. G., Pan, Y. E., Collier, K., Raina, S., Chua, A. O. & Mizel, S. B. (1984) *Nature (London)* **312**, 458–462.
7. Metchnikoff, E. (1893) *Lectures on the Comparative Pathology of Inflammation*: English Translation (Dover, New York); reprinted (1968) translation from the French.
8. Quigley, J. P. & Armstrong, P. B. (1983) *J. Biol. Chem.* **258**, 7903–7907.
9. Tschesche, H. & Dietl, T. (1976) *Methods Enzymol.* **45B**, 772–781.
10. Prendergast, R. A. & Suzuki, M. (1970) *Nature (London)* **227**, 277–279.
11. Habicht, G. S., Beck, G., Benach, J. L., Coleman, J. L. & Leichthling, K. D. (1985) *J. Immunol.* **134**, 3147–3154.
12. Mizel, S. B. (1980) *Mol. Immunol.* **17**, 571–582.
13. Mizel, S. B. & Rosenstreich, D. L. (1979) *J. Immunol.* **122**, 2173–2179.
14. Tam, M. R., Reddy, A. L., Karp, R. D. & Hildemann, W. H. (1976) in *Comparative Immunology*, ed. Marchalonis, J. J. (Blackwell Scientific, Oxford), pp. 98–119.
15. Schmidt, J. A., Mizel, S. B., Cohen, D. & Green, I. (1982) *J. Immunol.* **128**, 2177–2182.
16. Rosenwasser, L. J. & Dinarello, C. A. (1981) *Cell. Immunol.* **63**, 134–142.
17. McKernan, L. N. & Largen, M. T. (1983) *Cell. Immunol.* **80**, 84–96.
18. Dinarello, C. A., Goldin, P. W. & Wolff, S. M. (1974) *J. Exp. Med.* **139**, 1369–1381.
19. Ihrie, E. J. & Wood, D. D. (1985) *Lymphokine Res.* **4**, 169–181.
20. Kampschmidt, R. F. (1984) *J. Leukocyte Biol.* **36**, 341–355.
21. Postlethwaite, A. E., Lachman, L. B. & Kang, A. H. (1984) *Arthritis Rheum.* **27**, 995–1001.
22. Hyman, L. H. (1955) *The Invertebrates: Echinodermata, the Coelomate Bilateria* (McGraw-Hill, New York), Vol. 4.
23. Rosser, S. (1976) in *Comparative Immunology*, ed. Marchalonis, J. J. (Blackwell Scientific, Oxford), pp. 1–19.
24. Tasch, P. (1973) *Paleobiology of the Invertebrates: Data Retrieval from the Fossil Record* (Wiley, New York).
25. Twenhofel, W. H. & Shrock, R. R. (1935) *Invertebrate Paleontology* (McGraw-Hill, New York).
26. Jenkin, C. R. (1976) in *Comparative Immunology*, ed. Marchalonis, J. J. (Blackwell Scientific, Oxford), pp. 80–97.
27. Prendergast, R. A., Cole, G. A. & Henney, C. S. (1974) *Ann. N.Y. Acad. Sci.* **234**, 7–20.
28. Willenburg, D. O. & Prendergast, R. A. (1974) *J. Exp. Med.* **139**, 820–833.