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# Production of Interleukin 1 by SK Hepatoma Tumor Cells

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ABSTRACT: SK hepatoma cells and SK hepatoma conditioned media contain an 18 000-dalton factor which is pyrogenic, stimulates collagenase and prostaglandin production in skin and synovial fibroblasts, induces bone resorption, and stimulates the proliferation of murine thymocytes. These results are consistent with the finding that this tumor cell line produces interleukin 1 [Doyle, M. V., Brindley, L., Kawasaki, E., & Larrick, J. (1985) *Biochem. Biophys. Res. Commun. 130*, 768-773] since all these activities have been associated with this cytokine. Greater than 80% of the cellular activity has a molecular weight of 30 000, while in contrast, greater than 80% of the activity in the tumor-conditioned media has a molecular weight of 18 000. When active material from the cells is incubated with trypsin, this high molecular weight material is completely converted into an active 18 000 molecular weight species. The isoelectric point of all active material is always between p*I* 4.0 and 5.1, regardless of molecular weight. All of these results are consistent with the hypothesis that active, high molecular weight interleukin 1$\alpha$ is first synthesized and stored by the tumor cell. This cytokine is then cleaved by a trypsin-like protease to an active, lower molecular weight species which can be secreted into the media.

Interleukin 1 (IL-1)[1] is a small polypeptide hormone produced by activated macrophages (Gery et al., 1972). While it was first identified by its ability to stimulate thymocyte proliferation via induction of interleukin 2 release, it is now known to possess a much wider array of activities. Recently, cDNA clones for two distinct IL-1 species, IL-1$\alpha$ and IL-1$\beta$, were isolated from lipopolysaccharide-stimulated macrophage RNA, and the corresponding amino acid sequences of both forms were determined (March et al., 1985). From these studies, it was shown that both forms of the cytokine are first synthesized by the cell as 30 000-dalton precursors, which then undergo proteolytic cleavage to an 18 000-dalton form.

Many pathogenic molecules which were previously thought to be unique species are now believed to be some form of IL-1. For example, in rheumatoid arthritis, mononuclear cells have been shown to produce mononuclear cell factor (MCF) which induces the synovial cells lining the joint capsule to produce collagenase, the enzyme responsible for the irreversible destruction of the joint (Dayer et al., 1977a,b, 1980). Recently, it was shown that IL-1 has the same biological activities as those found in MCF (Mizel et al., 1981). Conversely, when MCF is added to thymocytes, uptake of tritiated thymidine is stimulated, an activitiy characteristic of IL-1. In addition, the molecular weights, isoelectric points, and sensitivity to

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[1] Abbreviations: IL-1, interleukin 1; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; FBS, fetal bovine serum; $PGE_2$, prostaglandin $E_2$; SDS, sodium dodecyl sulfate; LAF, lymphocyte activating factor; MCF, mononuclear cell factor; EDTA, ethylenediaminetetraacetic acid; Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride.

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phenylglyoxal are similar for the two activities. All of these results suggest that MCF is a cytokine whose structure is similar to or identical with that of IL-1 (Mizel et al., 1981).

The role of IL-1 in neoplasia has not been investigated. The ability of tumors to grow and invade the host depends critically on the induction of vessel growth from the host to the tumor and on the generation of proteolytic activity as a means of invasion (Folkman et al., 1971; Folkman & Haudenschild, 1980; Lobb et al., 1985). Both processes involve the breakdown of the host's extracellular matrix, an event which is the hallmark of arthritis, and which implies that a common mediator, such as IL-1, may be present in both disease states. In this work, we show that an 18000-dalton fraction from SK hepatoma conditioned media contains the same activities as those attributed to IL-1. In addition, we show that this secreted material is derived from a high molecular weight precursor ($M_r$ 30000) which is probably the $\alpha$-form of the cytokine.

## Materials and Methods

*Materials.* Recombinant human IL-1$\alpha$ and rabbit anti-human IL-1 were obtained from Genzyme Corp.

*Synovial Cell Culture.* Approximately 2.0 g of synovial tissue was obtained from a knee synovectomy and was stored overnight at 0 °C in 250 mL of McCoys 5A (modified) medium containing 200 $\mu$g/mL gentamicin. The next day, the tissue was cut into small pieces and incubated for 1 h with 4 mg/mL clostridial collagenase in serum-free DMEM and then with 0.25% trypsin for 30 min. The cells were spun down at 400*g* for 10 min and treated again with 0.25% trypsin/EDTA (Gibco 1X) for 30 min. The cells were mixed every 10 min by drawing through a 25-mL sterile pipet and collected by centrifugation. After the cells were washed with PBS/DMEM (1:1) containing 10% FBS, they were resuspended at $1 \times 10^6$ cells/mL in DMEM containing 10% FBS and 100 $\mu$g/mL gentamicin. The cells were incubated overnight at 37 °C under 5% $CO_2$, and the next day the nonadherent cells were aspirated off. The adherent cells were washed with PBS/DMEM (1:1) containing 10% FBS, followed by DMEM containing only 10% FBS. For assay studies, cells were transferred from T flasks and plated in 16-mm-diameter wells.

*SK Hepatoma Cells.* SK hepatoma cells were purchased from American Type Culture Collection (HTB-52) and maintained in a 500-mL spinner flask in DMEM + 10% FBS. The cells were harvested 3 times per week by centrifugation.

*Human Skin Fibroblast Cells.* Cells were purchased from American Type Culture Collection (CRL 1224) and grown in 75 $cm^2$ T flasks using DMEM containing 10% FBS. For assay studies, cells were plated in 16-mm-diameter wells.

*$PGE_2$/Collagenase Assay.* Samples to be assayed were diluted with fresh culture media (DMEM + 10% FBS) to a volume of 5 mL, sterile filtered, and applied either to confluent human synovial cells or to skin fibroblast cells grown in 16-mm wells. For each sample, 1 mL was added to each of four wells. After 18 h at 37 °C, the medium was removed, diluted appropriately, and assayed for $PGE_2$ content by using an $^{125}I$ RIA kit from New England Nuclear. The data were reported as nanograms of $PGE_2$ produced per milliliter of sample per 18 h.

The same procedure was followed for the measurement of collagenase activity; only samples were removed at 1–3-day intervals, and fresh sample was applied to the cells. Aliquots were then assayed by using the soluble collagen substrate method described elsewhere (Moore & Spilburg, 1986).

*Verification of Collagenase Activity.* Samples which contained collagenase activity were passed over an affinity column prepared as described elsewhere (Moore & Spilburg, 1986). At pH 7.5, the enzyme binds to the resin and can be eluted at pH 9.0. The purified enzyme was incubated with skin collagen, and cleavage products were visualized on 7.5% polyacrylamide gels containing SDS (Laemmli, 1970).

*Thymocyte Proliferation Assay.* Samples were assayed according to Matsushima et al. (1985), with $P388D_1$ cell supernatant used as the positive control. Briefly, concanavalin A and test samples (100 $\mu$L) were added with 2-fold dilution to 96-well plates. Thymocytes ($1.5 \times 10^6$ cells/well) from 8-week-old CD-1 mice were plated into each well. After 48 h, the cultures were pulsed with 0.2 $\mu$Ci of [$^3$H]thymidine, and 24 h later, individual wells were harvested into filters, and radioactivity was measured by scintillation counting.

*Bone Resorption Assay.* Calvaria from mice embryos were cultivated in serum-free medium 199 in roller tubes (two calvaria for 1.2 mL of medium) as described elsewhere (Morgan et al., 1955; Lenaers-Claeys & Vaes, 1979). Lyophilized samples were dissolved directly into the culture medium, and the mixture was incubated with the mouse calvaria in the roller tube. As a positive control, purified parathyroid hormone (138 USP units/mg) was added to culture media to induce bone resorption.

Bone resorption was evaluated by three methods. First, a semiquantitative procedure was used in which the size and number of resorption lacunae were graded from zero to five, as described by Vaes (1965). Second, the accumulated $Ca^{2+}$ in the culture medium was measured spectrophotometrically by complex formation with *o*-cresolphthalein complexone. Third, at the end of each experiment, the calvaria were hydrolyzed, and the hydroxyproline content was measured according to Bergman and Loxley (1963).

*Pyrogen Testing.* All samples were first tested in the limulus amebocyte lysate assay and shown to contain less than 0.12 ng/mL endotoxin. In a typical experiment, three rabbits were injected with sample, and the temperature increase was followed as a function of time. All animal testing was performed by Scientific Associates (St. Louis, MO) following the procedure outlined in the U.S. Pharmacopeia XX.

*Partial Purification of IL-1.* While spent media can be used as the starting material for IL-1 isolation, the tumor cells themselves are a superior starting source since contaminating serum proteins can be removed by simply washing the cells with isotonic salt. In addition, the cells contain more activity than the tumor-conditioned media. In detail, SK hepatoma cells ($2 \times 10^5$ cells/mL) were harvested several times each week from a 500-mL spinner flask by centrifugation of the media at 900*g* for 20 min. The cells were resuspended in 50 mL of PBS and recentrifuged, and the supernatant was discarded. After this step was repeated, the washed cells were suspended in 25 mL histidine hydrochloride, pH 5.9, and frozen. The cell suspension (3–5 mL) was thawed and centrifuged for 30 min at 30000*g* to obtain the supernatant which was then lyophilized. This freezing and thawing step released almost all of the IL-1 activity from the cells.

To characterize the cytokine activity in tumor-conditioned media, the medium was made 60% of saturation of ammonium sulfate at 4 °C. The precipitate was dissolved in water and dialyzed against cold water in 3500 molecular weight cutoff dialysis tubing, and the clear solution was lyophilized.

## Results

*Collagenase and $PGE_2$ Stimulation.* When cultured in normal media, confluent human synovial cells or skin cells do not produce detectable amounts of either collagenase or prostaglandin. However, when the 18000-dalton fraction (see

Table I: Stimulation of Collagenase and $PGE_2$ in Synovial Cells and Skin Cells by SK Hepatoma Derived IL-1

| collagenase (dpm/mL) | | | $PGE_2$ (ng/mL) | | |
|---|---|---|---|---|---|
| time (days) | synovial | skin | time (h) | synovial | skin |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.2 | 0 | 2682 | 4 | 15 | |
| 1 | 0 | 3692 | 8 | 65 | |
| 3 | 633 | 4259 | 24 | 594 | 0 |
| 6 | 1049 | | 48 | | 5.2 |
| 10 | 3105 | | 96 | 589 | |
| 13 | 2394 | | 114 | | 4.0 |
| 17 | 3574 | | | | |

Table II: Inhibition of Synovial Cell $PGE_2$ Production by IL-1 Antibody

| | $PGE_2$ [ng $mL^{-1}$ $(18\ h)^{-1}$] | |
|---|---|---|
| sample | −antibody | +antibody (4 units/mL) |
| SK hepatoma cell (18 000-dalton fraction) | 58 | 13 |
| IL-1 (3 units/mL) | 45 | 10 |

below) from either SK hepatoma conditioned media or the tumor cells themselves is placed on these cells, both collagenase and prostaglandin increase at a rate which is characteristic of each cell type. As shown in Table I, collagenase activity appears only 4 h after media replacement over skin fibroblasts, and the activity reaches a constant level after 24 h. For synovial cells, the same pattern is observed; only the time required is much longer. After 3 days of culture in SK hepatoma conditioned media, activity is first observed, and this activity reaches its maximum, constant value after 10 days in culture. As shown in Table I, prostaglandin production is also induced for both cell types, with a constant level achieved after either 24 h (synovial) or 48 h (skin) of exposure to SK hepatoma conditioned media or cell extract. In addition, the prostaglandin and collagenase activities remain high and constant with long-term exposure to the tumor cell extract. There is no evidence that either cell product diminishes with time. Both prostaglandin and collagenase activities can also be blocked by adding either 0.1 μg/mL actinomycin D or 3 μg/mL cycloheximide to the SK hepatoma media (data not shown). Furthermore, addition of an IL-1 antibody also diminishes the levels of both $PGE_2$ (Table II) and collagenase (data not shown).

To verify that a true collagenase is synthesized by these cells, media were passed over an affinity column (Moore & Spilburg, 1986), and the purified enzyme was then incubated with type I collagen. Visualization of the cleavage products on SDS–polyacrylamide gels showed only a $^3/_4$–$^1/_4$ cleavage, indicating that a true collagenase has been synthesized and secreted. This confirmation is especially important since the collagenase assay used here is not specific for the enzyme. Thus, virtually any protease can cleave the globular, terminal portions of the collagen α-chains producing soluble $^{14}C$-fragments. The appearance of these fragments can be erroneously interpreted as collagenase activity (Seifter & Harper, 1970).

*LAF Activity.* Samples of the 18 000-dalton fraction were assayed in the conventional LAF assay by following the uptake of [$^3H$]thymidine by mouse thymocytes. As shown in Table III, this fraction markedly stimulates thymocyte proliferation. Supernatants of $P388D_1$ cells, a rich source of IL-1, were also tested for activity as a positive control. While there were more units of activity in the SK hepatoma sample, this was found in a purified fraction so that it may not be concluded at this time that this tumor cell is a superior source of the cytokine

Table III: Effect of Tumor-Derived IL-1 on Thymocyte Proliferation

| addition | [$^3H$]thymidine (cpm) | IL-1 (units/mL)[a] |
|---|---|---|
| none | 230 ± 55 | 0 |
| Con A (0.5 μg/mL) | 7021 ± 414 | 0 |
| $P388D_1$ cell supernatant + Con A | 54418 ± 2242 | 16 |
| SK hepatoma cell (18 000-dalton fraction) + Con A | 27985 ± 1940 | 256 |

[a] One unit of activity is defined as the reciprocal of the dilution which gives half-maximum response.

Table IV: Effect of Parathormone and Tumor-Derived IL-1 on Bone Resorption

| sample | mM $Ca^{2+}$ | μg of hydroxyPro/calvarium | resorption of lacunae (score) |
|---|---|---|---|
| control | 1.21 | 14.90 | 0.00 |
| parathormone (5 units/mL) | 2.62 | 11.92 | 3.75 |
| SK hepatoma cell (18 000-dalton fraction), 10 μg/mL | 2.55 | 8.64 | 3.88 |

Table V: Comparison of IL-1 Activity[a] as a Function of Molecular Weight for SK Hepatoma Cells and SK Hepatoma Conditioned Media

| | mol wt | |
|---|---|---|
| SK hepatoma sample | 30 000 | 18 000 |
| cells | 19300 | 5600 |
| conditioned media | 1790 | 6820 |

[a] Nanograms of $PGE_2$ per milliliter per 16 h.

than the $P388D_1$ cell line. Studies are in progress to quantitate and to compare IL-1 production by these two cell lines.

*Bone Resorption.* The effect of this fraction on bone resorption of mouse calvaria was measured by three different methods, each based on a physiological consequence of bone resorption, and the results were compared to those obtained by using parathormone. As shown in Table IV, in the presence of the 18 000-dalton fraction, $Ca^{2+}$ accumulates in the media, and the hydroxyproline content of the bone decreases. In addition, by simple visual observation, the calvaria become more porous as the resorptive process progresses. Finally, as shown in Table IV, the resorptive properties of this tumor-derived material were similar to those produced by 5 units/mL parathormone.

*Pyrogenicity.* When material is injected into rabbits, within 30 min there is a rapid temperature rise. After 1-h postinjection, the mean temperature increase was 0.65 °C. Since the sample was verified to be endotoxin free (<0.12 ng/mL), this pyrogenicity is consistent with the presence of IL-1 in the 18 000-dalton fraction.

*Physical–Chemical Characterization.* To determine some physical–chemical properties of the cytokine, lyophilized protein from either SK hepatoma cells or media (see Materials and Methods) was dissolved in 1.0 M NaCl and 50 mM sodium phosphate, pH 6.0, and applied to a Bio-Gel P-30 column (2.6 × 95 cm) at 16 mL/h. As shown in Figure 1, two peaks of activity were found, but their relative abundance depended on the starting material. As summarized in Table V, for the cell extracts, 80% of the activity was found at 30 000 daltons and 20% was found at 18 000 daltons. On the other hand, for tumor-conditioned media, 80% of the activity was eluted at 18 000 daltons while only 20% appeared at 30 000 daltons.

Gel chromatography of the cell extract dissolved in dissociating agents, such as 6 M urea, resulted in substantially the same elution profile, indicating that the two peaks were not

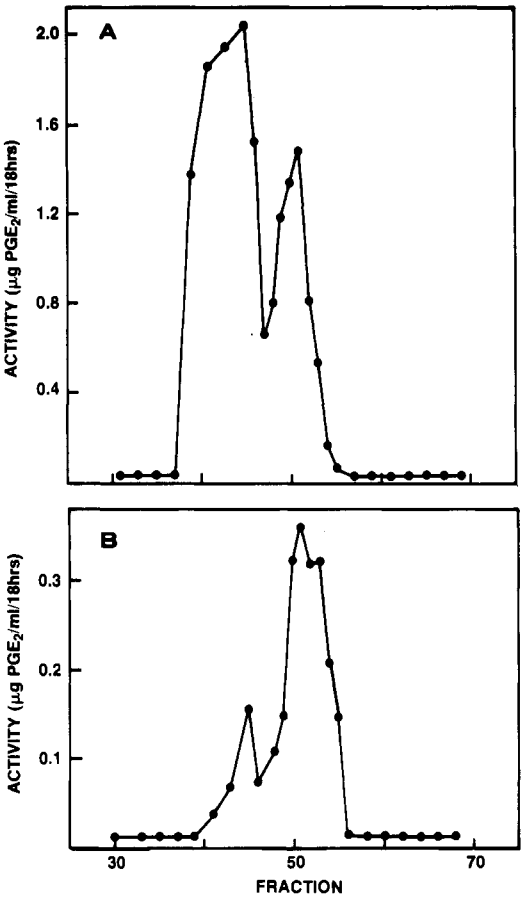


FIGURE 1: P-30 gel filtration of SK hepatoma cell extract (A) and SK hepatoma conditioned media (B). Fractions of 3.5 mL were collected.

related by protein/protein interaction (data not shown). However, as shown in Figure 2, if the starting cell extract was first treated with trypsin, quenched with soybean trypsin inhibitor, and then chromatographed, only one peak of activity was found at a molecular weight of 18 000.

Active fractions from the P-30 column were pooled and dialyzed against 25 mM Bis-Tris (iminodiacetic acid), pH 7.1, and chromatofocusing was then performed. Specifically, the sample was applied to a Mono P column equilibrated with 25 mM Bis-Tris, pH 7.1, and then eluted with Polybuffer 74 (diluted 1:10 with water and adjusted to pH 4.0 with iminodiacetic acid) at a flow rate of 0.75 mL/min. One-milliliter fractions were collected and measured for pH, $A_{280}$, $PGE_2$ activity, and collagenase activity. As shown in Figure 3, active fractions were found at pH 5.1, 4.7, and 4.1 while a fourth very acidic species remained bound to the column at pH 4.0 and could only be removed with high salt. The same elution profile was found regardless of the molecular weight of the starting material or its source.

## Discussion

It was recently reported that SK hepatoma cells can produce IL-1 (Doyle et al., 1985), and the results presented here confirm this finding by showing that all the activities commonly associated with this cytokine are found in an 18 000-dalton fraction of this tumor cell extract. Thus, as shown in Tables I–IV, this fraction can stimulate collagenase and prostaglandin production in human synovial cells (Mizel et al., 1981); it is pyrogenic (Rosenwasser et al., 1979); it induces bone resorption in mouse calvaria (Gowen et al., 1983); finally, it stimulates the proliferation of murine thymocytes, the classic biological assay for IL-1 (Gery et al., 1972).

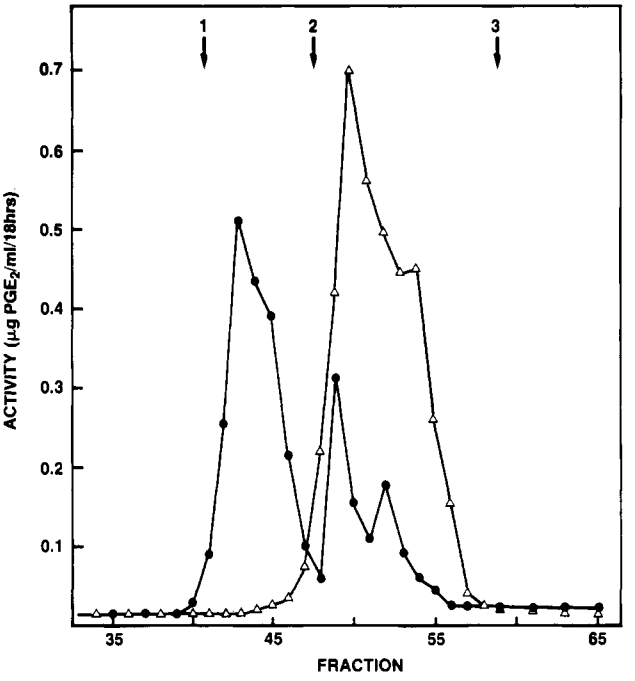


FIGURE 2: P-30 gel filtration of the 30 000-dalton fraction from Figure 1A before and after treatment with trypsin. Fractions 39–45 from Figure 1A were pooled, dialyzed overnight at 4 °C against 4 L of 0.01 M sodium phosphate and 0.15 M sodium chloride, pH 7.5, and then lyophilized. Identical aliquots were dissolved in 3.5 mL of 0.1 M Tris-HCl and 0.01 M calcium chloride, pH 7.5. One aliquot was treated with 2.1 mg of trypsin for 30 min at 23 °C followed by the addition of 2.2 mg of soybean trypsin inhibitor. This sample was applied to the P-30 gel filtration column (see Results), and 3.50-mL fractions were collected and assayed for activity (△). The control sample (●) was treated the same way without the addition of trypsin or soybean trypsin inhibitor. Molecular weight standards are as follows: (1) ovalbumin, 43 000; (2) chymotrypsinogen, 25 000; (3) ribonuclease, 13 700.

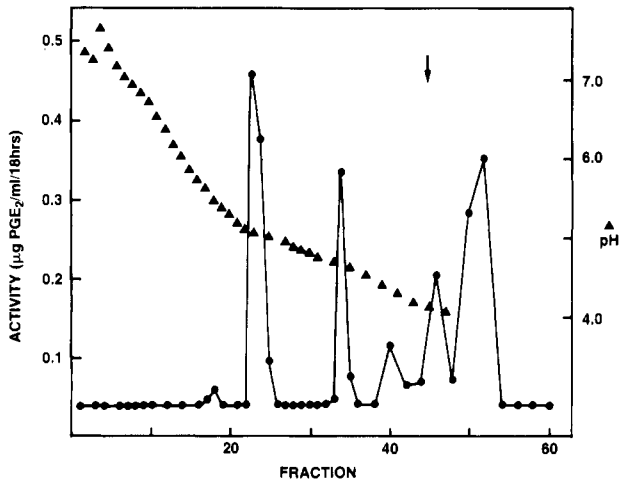


FIGURE 3: Chromatofocusing of P-30 gel filtration fractions on a Mono P column. Active fractions were pooled, dialyzed against 25 mM Bis-Tris/iminodiacetic acid, pH 7.1, and applied to a Mono P HR 5/20 column followed by at least 2 column volumes of buffer, and the fractions were collected. No activity was detected in the fractions. The column was eluted with 45 mL of Polybuffer 74 solution (see Results) followed by a 5-mL linear gradient from 0 to 1.0 M sodium chloride and finally with a 10-mL wash of 1.0 M sodium chloride. The arrow indicates the beginning of the sodium chloride elution.

Moscatelli and co-workers (Moscatelli et al., 1985) have shown that sonicates from SK hepatoma cells contain a factor which stimulates human umbilical vein endothelial cells to produce collagenase. From this observation, they have proposed that this factor plays a critical role in angiogenesis by producing the proteolytic activity necessary for new vessel

growth. The results presented here suggest that one component of their "angiogenesis factor" may be IL-1. When SK hepatoma cell extracts are added either to human synovial cells or to human skin cells, the level of collagenase activity increases dramatically. Indeed, for the synovial cells, using the conventional [$^{14}$C]collagen assay, no activity is observed until the normal medium is replaced with SK hepatoma conditioned medium. Confluent skin cells produce small quantities of enzyme in normal medium (Stricklin et al., 1977), but when the tumor-conditioned medium is added, collagenase levels increase over 1000-fold. Chua and co-workers (Chua et al., 1985) found collagenase induction when various growth-promoting factors were added to skin cells, but there was only a 3-fold enhancement, not nearly as striking as that described here. The capacity of a tumor to generate this kind and level of proteolytic activity has long been recognized as a key element of tumor invasiveness (Liotta et al., 1983). In the past, it was assumed that the proteolytic activity was produced by the tumor itself. However, as shown here, certain tumors may produce IL-1 which can interact with a variety of the host's cells to induce these normal cells to produce the proteolytic activity necessary for the tumor's survival.

The data presented here bear importantly on the biochemical basis for neoplastic hypercalcemia, one of the most common metabolic complications of cancer in general (Mundy et al., 1984) and hepatomas in particular (Margolis & Homey, 1972). For those patients without bone metastases, elevated serum calcium levels are caused by the secretion of a tumor-derived factor which stimulates bone resorption. To date, the identification and characterization of this humoral mediator have not been possible. In this work, the fact that bone resorption activity coelutes with all the other IL-1 activities strongly suggests that this cytokine is the causal agent. While tumor cells can produce a variety of hormones and activators, none of the properties of known, human bone resorbing agents corresponds to the physical–chemical properties of the activity contained in the 18 000 molecular weight fraction described here. Thus, epidermal growth factor (Tashjian & Levine, 1978) and parathormone are much smaller proteins (6000 and 9000 daltons, respectively), platelet-derived growth factor (Antoniades & Williams, 1983) is very cationic (p*I* 9.8–10.2), and transforming growth factors (Roberts et al., 1983) are stable in acetonitrile and 0.1% trifluoroacetic acid, conditions which irreversibly destroy the activity described here.

Recently, an IL-1-like molecule from normal human monocytes was also shown to possess both bone resorption activity and LAF activity (Gowen et al., 1983). There were at least two active species, one with p*I* 6–6.7 and another with p*I* 4.5–5.2, both with molecular weight 12 000–18 000. The properties of the more acidic species closely resemble those described here, providing additional evidence that some forms of neoplastic hypercalcemia are probably derived from this cytokine.

While any of the biological activities described above can be used for monitoring a purification protocol or for studying cytokine/cell interactions, the prostaglandin assay and the collagenase assay are the most desirable because they are rapid and highly reproducible. However, before using either one on a routine basis, it was necessary to verify that the assay product was being actively synthesized as a result of the stimulus of only IL-1 and was not being simply released as a cytotoxic effect. This was demonstrated in three ways. First, cycloheximide and actinomycin D both inhibit the production of $PGE_2$ and collagenase, indicating that both protein and RNA syntheses are required for their appearance. Second, the cells continuously secrete either collagenase or $PGE_2$ over long time periods as long as SK hepatoma conditioned media are presented. There is no evidence that production diminishes with time, as would be expected if preexisting molecules were simply being released from the cells. Third, addition of an IL-1 antibody inhibits the appearance of $PGE_2$ or collagenase in the media, indicating that this specific cytokine is responsible for the observed activity. Therefore, monitoring the appearance of either $PGE_2$ or collagenase is an appropriate way to study the activity of this mediator.

March and co-workers (March et al., 1985) have isolated two proteins with IL-1 activity (IL-1$\alpha$ and IL-1$\beta$) as the translation products of two distinct macrophage cDNAs. Both cytokines are first synthesized as a high molecular weight species (30 000), but only IL-1$\alpha$ has activity in this form. It was postulated that proteolytic degradation of either high molecular weight species results in a fully active, 18 000 molecular weight protein. This cytokine is then like many other hormones, such as nerve growth factor (Berger & Shooter, 1977) and epidermal growth factor (Frey et al., 1979), which are first synthesized as high molecular weight precursors and then cleaved to a smaller form by proteolytic enzymes. From the published sequence, it was proposed that the high molecular weight species of IL-1$\alpha$ is cleaved by a trypsin-like protease since the N-terminal serine of the low molecular weight form is attached to a basic arginine residue in the precursor molecule. In contrast, the N-terminal alanine of the low molecular weight $\beta$-form is attached to an acidic aspartic acid residue in the precursor molecule so that a different kind of protease is required to generate this species. Finally, both forms have distinctly different isoelectric points; the p*I* value for IL-1$\alpha$ is 4.6–5.2 while the p*I* value for IL-1$\beta$ is 6.8.

By comparing the data here with the work above, it is most likely that the active species secreted by SK hepatoma tumor cells is IL-1$\alpha$. When the activity from tumor-conditioned media is applied to a gel filtration column, greater than 80% of this activity appears at 18 000 daltons, and less than 20% of the activity is found at 30 000 daltons (Figure 1B). Moreover, chromatofocusing of the active, low molecular weight material results in a heterogeneous mixture of very acidic species from p*I* less than 4.0 to p*I* 5.1. Activity has not been detected at p*I* 6.8, the characteristic isoelectric point of the $\beta$-form (Figure 3).

Unanue and Kiely (1977) found that when murine macrophages are induced to produce "mitogenic protein", the newly synthesized cytokine remains largely in the cell. Since only one of the two interleukin species was found in this work, it was possible that one form was actively secreted and the other was stored within the SK hepatoma cell. Therefore, the molecular weight and isoelectric point of the active species found in the cells were compared to those found for the species secreted into the media. Unlike the activity in the tumor-conditioned media, for the cell extracts, greater than 80% of the activity was found at 30 000 daltons (Figure 1A). Since only the $\alpha$-form has activity in the precursor form, this indicates that there is no active IL-1$\beta$ stored within the cell. This was verified further by chromatofocusing both this 30 000- and 18 000-dalton material from the cells. Once again, activity was found only between p*I* 4.0 and 5.1.

These data also indicate that once IL-1 is synthesized by the cell further processing is required before it is secreted into the media. According to the formalism developed by Unanue for mitogenic protein, there is a "control protein" for the $\alpha$-form which converts an active, stored precursor to another active form which can then be secreted by the SK hepatoma

cell. It is interesting that when the active 30000-dalton species described above is incubated with trypsin, all the activity is converted to another active species of 18000 daltons (Figure 2). This observation is consistent with the presence of a trypsin-cleavable bond, such as that which exists in high molecular weight IL-1$\alpha$ at $Arg^{112}$–$Ser^{113}$. In this regard, it should be noted that SK hepatoma extracts have been shown to stimulate a variety of cells to produce plasminogen activator (Gross et al., 1983), which interacts with plasminogen to produce the general protease plasmin. Both of these hydrolytic enzymes cleave at Arg–X or Lys–X bonds, and, therefore, either could possibly convert the high molecular weight $\alpha$-species to its low molecular weight form. Work is currently in progress to determine if this kind of proteolytic activity is generated either by SK hepatoma cells themselves or, alternatively, by the interaction of SK hepatoma conditioned media with the synovial cells or skin cells used in this study.

While these observations strongly point to the presence of IL-1$\alpha$ as the active cytokine, these data cannot exclude the possibility that IL-1$\beta$ in the high molecular weight form is present. Since this form is inactive, it cannot be detected with any of the in vitro assays used here. It is interesting that others have detected IL-1$\beta$ in lipopolysaccharide-stimulated SK hepatoma cells (Doyle et al., 1985), using a 21-base oligonucleotide probe complementary to the IL-1$\beta$ sequence between amino acids 48 and 55. Taken together, these observations indicate that the control protein which converts the inactive, high molecular weight $\beta$-form to the active low molecular weight form may not be synthesized by this tumor cell. Hence, this form of the cytokine can be detected with structural probes but not with the activity assays used here.

In conclusion, the results presented here are similar to those reported for the IL-1 found in murine macrophages (Giri et al., 1985). Using anti-IL-1 antibodies, this cytokine was found to be synthesized as a 33000-dalton precursor which was then rapidly converted to the native 17000–19000-dalton species found in the culture supernatant. This active form has a similar p*I* range (4.9–5.2) as that found in the tumor cell described here (4.0–5.1), and it also has a similar charge microheterogeneity (Mizel & Mizel, 1981). Since the p*I* of the major human species is 6.8, in the past it has been assumed that the murine cytokine is quite different than that found in humans. However, as shown here, this difference may result from different processing after synthesis in the cell. Thus, following the notation used for the human system, there may be a $\beta$-form synthesized by murine cells which has not been detected because conditions which induce its conversion to an active, low molecular weight form have not been found as yet. More detailed studies on the biochemical steps which favor the activation of one form over the other should resolve this question.

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