Inhibition of Cell Proliferation by Interleukin-1 Derived from Monocytic Leukemia Cells

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ABSTRACT

Growth inhibitors and interleukin-1 (IL-1) are two biological response modifiers produced by mezerein-treated THP-1 cells maintained in serum-free medium. The activities comigrated with isoelectrofocusing in a pH range of 6.7 to 7.3. Subsequent molecular sieving on an AcA-54 column revealed that a portion of the growth-inhibitory activity for the marnmary cell line MCF-7 remained associated with IL-1. IL-1-containing fractions were further analyzed by chromatography with DEAE-Sephacel, phenyl-Sepharose, and concanavalin A-Sepharose. In each instance, IL-1 coloeluted with growth-inhibitory activity. IL-1 and growth-inhibitory activities partially purified by sequential isoelectrofocusing, AcA-54 chromatography, and DEAE-Sephacel were located in a single region following preparative polyacrylamide gel electrophoresis. Elution, concentration, and analytical polyacrylamide gel electrophoresis of this region resulted in a single band with an apparent molecular weight of 17,000. Stability studies revealed similarities between the IL-1 activity and growth-inhibitory activity in their sensitivity to a variety of physical and chemical treatments. A commercial source of human IL-1 also inhibited the growth of MCF-7 cells. DEAE-purified IL-1 derived from THP-1 cells inhibited the growth of 7 of 11 cell types tested, and all inhibited cell lines were established from malignant sources. Prostaglandin synthesis by MCF-7 cells in response to IL-1 was not responsible for growth inhibition.

INTRODUCTION

Earlier studies reported that media conditioned by primary macrophage cultures inhibited growth in cell populations from malignant tissue sources. Cytotoxins (1–3), interferon (4), and tumor necrosis factor (5) are a few examples of the macrophage-derived negative growth regulators that have been identified. The limited quantities of these components available from primary macrophage culture prompted preliminary studies in this laboratory to detect the production of similar growth factors by established monocytic leukemia cell lines.

The THP-1 line of human cells was established from a 1-yr-old male with acute monocytic leukemia. This line expresses certain leukemia lines (7–9). We subsequently demonstrated that mezerein-treated THP-1 cells become adherent to plastic culture dishes, lose division potential, acquire Fc receptors, display phagocytic activity, and express increased nonspecific esterase staining (10). Medium conditioned by adherent THP-1 cells was found to contain both growth-inhibitory and growth-stimulatory activities for lines from a variety of tissue sources (11).

Conditioned medium was mitogenic for diploid human fibroblasts only in the presence of whole serum or plasma-derived serum supplemented with platelet-derived growth factor. Thus, in contrast to previous results with macrophage products (12, 13), the THP-1 growth-stimulating activity did not fulfill the role of a competence factor for fibroblasts (14).

Preparative isoelectrofocusing revealed that THP-1 cell-conditioned medium also contained a colony-stimulating factor active with bone marrow cells and IL-1 assayed with mouse thymocyte cultures (10). IL-1 activity was detected only after partial purification from crude medium. Mizel and Anderson (15) had previously reported that THP-1 synthesized low levels of IL-1.

The current study stemmed from our initial attempts to elucidate the nature of the inhibitory activities in THP-1 medium (11). During the course of this investigation, it became apparent that a portion of the growth-inhibitory activity could not be biochemically separated from IL-1. The experiments described herein recount our attempts at separation of these activities and lead to the conclusion that human IL-1 produced by THP-1 cells directly inhibits the growth of certain cell lines.

MATERIALS AND METHODS

Preparation of Conditioned Medium. THP-1 cells were seeded at a concentration of 1.5 x 10⁶ per ml in 100 ml of RPMI-1640 medium supplemented with 1% FBS and 2 mM l-glutamine into 530-cm² tissue culture plates. Mezerein (CCR, Inc., Eden Prairie, MN) was added to a final concentration of 10⁻⁷ M, and cells were incubated for 24 h. Adherent cells were washed 3 times with serum-free RPMI-1640 medium and maintained for an additional 36 h in RPMI-1640 medium supplemented with insulin (5 µg/ml) and transferrin (5 ng/ml). Conditioned medium was concentrated 100-fold by ultrafiltration through a YM-10 membrane in an Amicon-stirred cell.

Isoelectrofocusing. Preparative flat-bed electrophoresis of concentrated THP-1 medium was conducted in a granulated gel using an LKB 2117 Multiphor system (LKB-Produkter AB, Bromma, Sweden). A 4% Ultralow (LKB) slurry was prepared containing a 3% mixture of Ampholine amphoteries (LKB) with a pH range of 3.5 to 10.0. A 5-ml sample of 100-fold concentrated medium was dialyzed against 1% glycine and added to the gel. Electrophoresis was performed at a constant power of 8 W for 16 h at 7°C. The gel was fractionated with a grid, and each fraction was eluted twice with 2 ml of 0.15 M NaCl containing 0.1% PEG 6000. Eluted components were dialyzed against PBS. Interleukin-1 and growth-inhibitory activities were observed in fractions between pH 6.7 and 7.3. These fractions were pooled, concentrated by ultrafiltration, and used as the starting material for subsequent purification procedures.

3 The abbreviations used are: IL-1, interleukin-1 (lymphocyte activating factor); FBS, fetal bovine serum; PBS, phosphate-buffered saline; PBSa, calcium/magnesium-free PBS; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; PHThdh, phytate thymidine; PEG, polyethylene glycol; PGB, prostaglandin B₂ (other prostaglandins defined similarly); TNF, tumor necrosis factor.
INHIBITION OF CELL PROLIFERATION BY IL-1

Molecular Sizing Chromatography. Separation of isoformulated fractions according to molecular size was accomplished by molecular sieve chromatography with an AcA-54 column (1 x 120 cm). The sample volume was 0.5 ml and was eluted with PBS containing 0.1% PEG at a rate of 15 ml/h. Absorption was monitored at 280 nm.

Molecular weight markers included: ovalbumin, M, 45,000; chymotrypsin, M, 25,000; and RNase, M, 13,500.

Ion Exchange Chromatography. AcA-54 fractions with IL-1 activity were pooled, concentrated to 5 ml, dialyzed against 20 mM Tris-HCl (pH 8.5), and applied to a 1 x 10-cm column of DEAE-Sephalose (Pharmacia Fine Chemicals) equilibrated with the same buffer. The column was washed with 10 bed volumes of starting buffer and eluted in a 200-ml gradient of 0 to 0.4 M NaCl at the flow rate of 15 ml/h. Four-mI fractions were collected.

Chromatography on Phenyl-Sepharose. IL-1-containing fractions from the AcA-54 chromatographic separation were dialyzed against a buffer of 0.8 mM ammonium sulfate and 10 mM sodium phosphate (pH 6.8) and centrifuged at 10,000 x g for 10 min. The supernatant was applied to a 1.2 x 6-cm column of phenyl-Sepharose CL-48 (Pharmacia Fine Chemicals) equilibrated with the same buffer. The column was washed with 5 bed volumes of starting buffer and then eluted with a 140-ml linear gradient of 0 to 50% ethylene glycol. Fractions of 2.5 ml were collected and exhaustively dialyzed with PBS before assay.

Concanavalin A-Sepharose Chromatography. A 5-ml syringe column of concanavalin A-Sepharose (Pharmacia Fine Chemicals) was equilibrated in a buffer of 20 mM Tris-HCl and 0.5 mM NaCl (pH 7.4). Pooled fractions from the AcA-54 step were concentrated and adsorbed onto the column in the equilibrium buffer. Unbound materials were removed with 10 bed volumes of starting buffer. Elution was continued with an 80-ml linear gradient of 0 to 0.5 mM αmethyl-o-mannoside in equilibrium buffer. Five-mI fractions were dialyzed against PBS for assay.

Electrophoresis. Preparative and analytical electrophoresis was performed according to the alkaline gel systems of Ornstein and Davis (16) and Weber and Osborn (17). Preparative PAGE was accomplished using a 10- x 14-cm slab consisting of a 3% stacking and an 8% separating gel. Pooled IL-1-containing fractions following sequential isoelectric focusing, molecular-sieve chromatography on AcA-54, and ion-exchange chromatography with DEAE of conditioned THP-1 medium were dialyzed against 50 mM Tris-HCl containing 0.1% PEG (pH 6.8) and concentrated by ultrafiltration through a YM10 membrane. After electrophoresis at a constant current of 15 mA, the gel was sliced and eluted in 10 mM Tris:acetate (pH 8.6) with 0.1% PEG using an ISCO sample concentrator. A corresponding lane was stained with silver nitrate (18). The gel fraction containing IL-1 activity was prepared by analytical PAGE. Components were resolved using reduction in the presence of 2-mercaptoethanol on a 12% polyacrylamide gel containing SDS. Electrophoresis was conducted at a constant current of 15 mA, and the gel was stained with silver nitrate.

Growth Assays. Growth-inhibitory activity during biochemical isolation steps was assayed with the human mammary carcinoma line, MCF-7. Several additional lines were used to test for cell type specificity. These included: malignant mammary cell lines (BT-474, MDA-MB-134 IV, MDA-MB-231, MDA-MB-415, T47D, and ZR-75); a cervical carcinoma (HeLa); a malignant melanoma (A375Ag5); a diploid embryonic lung fibroblast (MRC-5); and a primary fibroblast (FDC-P1). A375Ag5, HeLa, and HEL cells were grown in a 1:1 mixture of Dulbecco-Vogt-modified Eagle’s minimal essential medium:Ham’s F-12 medium. BT-474, T47D, and ZR-75 were assayed with RPMI-1640 medium. Media were supplemented with 10% FBS (Flow Laboratories, Inc., Rockville, MD) and gentamicin (50 μg/ml) (Schering-Plough Corp., Kenilworth, NJ).

Growth assays were conducted by inoculating 5 x 10⁴ cells in 16-mm wells with 1 ml of medium. Cell number in triplicate wells was determined at 24 h, and culture fluids were changed with fresh medium containing various concentrations of test samples. After 7 days, cells were detached by incubation in a 0.5% trypsin:versene solution (2 μg/ml), and the number of cells was recorded with a Model B Coulter Counter. The percentage of inhibition of growth was calculated according to the formula:

\[
\% \text{ of growth inhibition} = 100 \times \frac{1 - \text{corrected cell no. in test sample}}{\text{corrected cell no. in control}}
\]

Cell numbers were corrected by subtracting the number of cells present before test samples were added. The final number of MCF-7 cells present in control cultures ranged from 1.2 to 2.0 x 10⁵.

Lymphocyte Activating Factor. A single-cell suspension of thymocytes prepared from 5- to 6-week-old lopinosa sarcomide-unresponsive C3H/HeJ mice was inoculated at 1.5 x 10⁶ cells per well into 96-well flat-bottomed trays with RPMI-1640 containing 10% FBS. 2.5 x 10⁻⁶ M 2-mercaptoethanol, and phytohemagglutinin (1 μg/ml). Various concentrations of test samples were added, and the final volume of each well was 200 μl. Cultures were pulsed with 0.5 μCi of [³H]dThd (2 mCi/mM) per well for the final 6 h of the 72-h incubation. Samples positive for IL-1 induced an increase in incorporation over that obtained with phytohemagglutinin alone. The biological unit of activity for both the THP-1 product and the commercial human IL-1 (Genzyme Corp., Boston, MA) was defined by the reciprocal of that dilution supporting 50% of the maximal [³H]dThd incorporation. Three lots of Genzyme IL-1 were retired for these studies.

Stability Assays. Biochemical stability tests were performed using 5-fold concentrates of the DEAE-Sephalose-purified materials (approximately 60 μg of protein per ml). Samples were incubated with 1% phenylglyoxal (Sigma Chemical Co., St. Louis, MO) in 200 mM imidazole (pH 8.0) at 25 °C. After 2- or 4-h incubation, the treated samples were separated from unreacted phenylglyoxal by gel filtration on a column of Bio-Rad P-10. Control samples were passed over the P-10 column immediately after the addition of phenylglyoxal (19).

Samples were dialyzed against PBS with HCl or NaOH to a pH between 2 and 10 in increments of 2 pH units and incubated for 24 h. Samples were then dialyzed against PBS at pH 7.4. The effects of trypsin, chymotrypsin, and protease (Sigma Chemical Co.) were examined by incubating IL-1-containing samples at 37°C for 2 h in enzyme (100 μg/ml). The direct effect of enzymes on MCF-7 cell or thymocyte proliferation was also studied. In addition, the heat stability of the IL-1 and growth-inhibitory activities was followed by incubation of samples at 37°C, 56°C, or 72°C for 1 h. Samples were chilled on ice immediately after incubation.

SDS was added at a final concentration of 0.1% to samples and incubated at 37°C for 1 h. A control sample and the SDS-treated materials were passed through a column of Ag501-X8-mixed bed resin (Bio-Rad Laboratories, Rockville Centre, NY), eluted with water, and concentrated to the original volume.

Prostaglandin Synthesis. MCF-7 cells were incubated at 5 x 10⁴ in 4 ml of medium to 60-mm tissue culture plastic dishes. After 24 h, 2 μCi of [¹⁴C]arachidonic acid were added in the presence or absence of 4 units of IL-1 purified from THP-1 cell supernatants. Cultures were maintained for 5 additional days. Media were removed, and cells were washed with PBS and dissolved in 0.1% SDS. Aqueous solutions were acidified with 1 ml of n citric acid and extracted 3 times with 10 ml of chloroform. Chloroform layers were pooled and frozen overnight. Chloroform was evaporated under a stream of nitrogen, and the sample was applied to a plastic backed preactivated thin-layer chromatography plate. The plate was developed in ethyl acetate:trimethyl pentane:acetic acid: H₂O (110:50:20:100). Unlabeled prostaglandins were added to the plate, UV light-absorbing materials were removed from the plate, and the amount of radioactivity was quantitated in an LKB scintillation counter.

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RESULTS

Association of IL-1 and Growth Inhibitors. The starting material for the studies described herein was derived from the IL-1-containing fractions collected following isoelectrofocusing of concentrated THP-1 cell-conditioned medium. Both IL-1 and the growth-inhibitory activity for MCF-7 cells comigrated with maximum activity at a pH of 7.

Fractions between pH 6.7 and 7.3 were pooled and concentrated for subsequent purification. Further separation was initially attempted on a molecular weight basis.

Fig. 1 illustrates the results following chromatography through an AcA-54 column of the fractions containing IL-1 activity following preparative isoelectrofocusing. This chromatographic step gave the first indication that the inhibitory activity recovered at pH 6.7 to 7.3 may be resolved into two components, one with a molecular weight of approximately 45,000 and a second associated with the peak of IL-1 activity. IL-1 activity was eluted with a molecular weight between 13,500 and 25,000.

The associated inhibitory and IL-1 activities following molecular sieving chromatography on AcA-54 were further purified by ion-exchange chromatography. Fractions collected between 57.5 and 65 ml from the AcA-54 column shown in Fig. 1 were pooled, concentrated to 5 ml, loaded onto a DEAE-Sephacel column equilibrated in Tris-HCl buffer (pH 8.5), and eluted with a gradient of NaCl. Data are expressed as the average percentage of growth inhibition of MCF-7 cell growth (O) or [3H]dThd incorporation for IL-1 (•).

Fig. 2. Ion-exchange chromatography on DEAE-Sephacel of IL-1 activity. Fractions collected between elution volumes 57.5 and 65 ml from the AcA-54 column separation shown in Fig. 1 were pooled and concentrated to 5 ml. Bound material was eluted at a flow rate of 8 ml/h in a gradient of NaCl. DEAE-Sephacel was equilibrated in 20 mm Tris-HCl buffer (pH 8.5). Data are expressed as the average percentage of growth inhibition of MCF-7 cell growth (O) or [3H]dThd incorporation for IL-1 (•).

Fig. 3. Hydrophobic chromatography on phenyl-Sepharose. IL-1 activity was first separated by isoelectrofocusing followed by AcA-54 chromatography. Active fractions were pooled and concentrated. Column was equilibrated in 10 mm sodium phosphate containing 0.8 M (NH4)2SO4 (pH 6.8). Bound materials were eluted in a linear gradient of ethylene glycol (0 to 50%). Growth inhibition was assayed with MCF-7 cells (O), and IL-1 activity was followed by [3H]dThd incorporation (•).

All detectable IL-1 and growth-inhibitory activities were recovered as unbound material. This result agreed with previous reports characterizing IL-1 from both the P388D1 murine cell line (20) and human peripheral blood monocytes (21) as lacking accessible mannosyl or glycosyl residues.

Electrophoresis. IL-1 activity separated by sequential isoelectrofocusing, AcA-54 gel filtration, and DEAE-Sephacel ion-exchange chromatography was further purified on 8% Tris-glycine discontinuous polyacrylamide gels. As shown in Fig. 4, both IL-1 and growth-inhibitory activities were recovered in the same region of the gel.

SDS-PAGE was also performed with pooled IL-1 eluted from Gel Slices 17 and 18 of the nondenaturing gel shown in Fig. 4. Repeated analyses revealed a single visible band after staining with silver nitrate. This component had an apparent molecular weight of 17,000 estimated by comparison with molecular weight standards (Fig. 5). Elution, concentration, and testing of this material resulted in no detectable IL-1 activity and a minimal level.
INHIBITION OF CELL PROLIFERATION BY IL-1

Fig. 4. Preparative PAGE of IL-1 activity from the ion-exchange chromatographic separation shown in Fig. 3. Material was resolved under nondenaturing conditions in an 8% acrylamide slab gel. The gel was sliced, eluted, and concentrated in 10 mM Tris-acetate (pH 8.6). Growth inhibition was assayed with MCF-7 cells (O), while IL-1 activity was measured in thymocyte cultures (●).

Fig. 5. SDS-PAGE analysis of IL-1 eluted from preparative PAGE (Fig. 4, Slice Nos. 17 and 18); Lane 1, molecular weight standards; Lane 2, 200 units IL-1; Lane 3, 80 units IL-1. The gel reveals a single component with an apparent molecular weight of 17,000.

Table 1
Comparison of the specific activities of IL-1 and associated growth inhibitor during purification

<table>
<thead>
<tr>
<th>Purification</th>
<th>Interleukin-1 (units/mg)</th>
<th>Growth inhibitor (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcA-54</td>
<td>7.4 x 10^3</td>
<td>7.9 x 10^3</td>
</tr>
<tr>
<td>DEAE-Sephacel</td>
<td>1.9 x 10^4</td>
<td>1.5 x 10^4</td>
</tr>
<tr>
<td>PAGE, prep.</td>
<td>6.6 x 10^4</td>
<td>5.8 x 10^4</td>
</tr>
</tbody>
</table>

* A unit of IL-1 is defined as the reciprocal value of the dilution resulting in half-maximum response in the thymocyte proliferation assay.

† A unit of growth-inhibitory activity is defined as the amount which induces a 50% reduction in the number of MCF-7 cells at the end of the 7-day growth assay as outlined in "Materials and Methods."

Table 2
Stability of IL-1 and growth-inhibitory activities partially purified from THP-1 cell-conditioned medium

<table>
<thead>
<tr>
<th>Treatment (assay)</th>
<th>Exposure time (h)</th>
<th>Temperature (°C)</th>
<th>Growth inhibition IL-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyglyoxal (1%)</td>
<td>2</td>
<td>37</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>Trypsin (100 µg/ml)</td>
<td>2</td>
<td>37</td>
<td>81</td>
</tr>
<tr>
<td>Chymotrypsin (100 µg/ml)</td>
<td>2</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Pronase (100 µg/ml)</td>
<td>2</td>
<td>37</td>
<td>61</td>
</tr>
<tr>
<td>Heat</td>
<td>1</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>72</td>
<td>87</td>
</tr>
<tr>
<td>pH 2</td>
<td>24</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>24</td>
<td>41</td>
</tr>
<tr>
<td>SDS (0.1%)</td>
<td>1</td>
<td>37</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>90</td>
</tr>
</tbody>
</table>
Incubation with 0.1% SDS at 37°C for 1 h induced 90% loss of the IL-1 activity with a 65% loss in the growth-inhibitory activity.

Comparison of IL-1 Sources. Inhibitory activity for MCF-7 cells was assayed in a commercial source of IL-1 (Genzyme Corp., Boston, MA) (22) and compared to the activity in partially purified THP-1 preparations. IL-1 from THP-1-conditioned medium was purified by stepwise isoelectrofocusing, molecular sieve, and ion-exchange chromatography. The data in Table 3 show that both sources of IL-1 inhibited the growth of MCF-7 cells and that this response was dose dependent.

It was important to preliminarily survey the response of a variety of human cell types to IL-1, since MCF-7 cells had been used exclusively as a target cell line during biochemical purifications. Growth assays were performed in the presence of 2 units of IL-1 per ml of culture medium from THP-1 cells or Genzyme Corp. No effect on the growth of the transformed milk cell (HBL-100), the diploid human embryonic lung fibroblast (HEL), the cervical carcinoma (HeLa), or the melanoma (A375Ag5) lines was observed (Table 3). In contrast, several cell lines established from malignant mammary tissue including BT-474, MDA-MB-134 IV, MDA-MB-231, MDA-MB-415, T47D, and ZR-75 showed a growth-inhibitory response in the presence of IL-1-containing material.

Prostaglandins. Korn et al. (23) reported that an adherent mononuclear cell-derived factor inhibited fibroblast growth by stimulating prostaglandin production. Dayer et al. (24) demonstrated that a mononuclear cell factor, later shown to be IL-1 (25), inhibited synovial cell proliferation and thymidine incorporation in a dose-dependent manner. Prostaglandin-dependent suppression could be reversed in part by the addition of indomethacin, a potent inhibitor of the cyclooxygenase pathway (26). Schmidt et al. (30) demonstrated that IL-1 can stimulate the proliferation of fibroblasts, while Estes et al. (31) reported that IL-1 and macrophage-derived growth factor are different entities. Recent studies have attributed a multiplicity of biological activities to IL-1 on a wide spectrum of target cells (21, 32, 33).

Macrophages are responsible for the synthesis of several biological response modifiers that affect the behavior of a wide variety of cell types. Among these modifiers is a thymocyte-activating factor which significantly influences immune response. The primary function ascribed to this macrophage product, now defined as interleukin-1 (27), is the induction of interleukin-2 production by T cell subsets of thymus tissue (28, 29). Partially purified IL-1 was shown by Mizel et al. (25) to induce prostaglandin and collagenase synthesis by synovial cells. Schmidt et al. (30) demonstrated that IL-1 can stimulate the proliferation of fibroblasts, while Estes et al. (31) reported that IL-1 and macrophage-derived growth factor are different entities. Recent studies have attributed a multiplicity of biological activities to IL-1 on a wide spectrum of target cells (21, 32, 33).

DISCUSSION

In our continuing effort to discover sources of natural inhibitors of malignant cell proliferation, we observed a cytostatic activity in serum-free medium conditioned by a mezerein-treated acute monocytic human leukemia cell line, THP-1 (11). Mezerein is a plant diterpene ester which appears to induce a series of steps whereby THP-1 cells acquire characteristics typical of fully differentiated macrophages. In addition to inhibitory activity, THP-1 cells produce a colony-stimulating factor, stimulators of fibroblast proliferation, and a thymocyte-activating factor (10). Isofocured fractions of conditioned medium containing thymocyte-proliferative activity did not stimulate the proliferation of murine IL-1-dependent cytotoxic T-cells (data not shown).

Attempts to dissociate IL-1 and growth-inhibitory activities on the basis of net charge by isoelectrofocusing were not successful. Although some separation was effected by molecular sieve chromatography, a second peak of inhibition was observed (Table 4). A list of lines assayed with two sources of human IL-1 is shown in Table 3.

Comparison of growth-inhibitory activities assayed with two sources of human IL-1

<table>
<thead>
<tr>
<th>Units of IL-1</th>
<th>% of inhibition by</th>
<th>% of inhibition by</th>
<th>% of inhibition by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>THP-1-derived</td>
<td>commercial</td>
<td>commercial</td>
</tr>
<tr>
<td>2</td>
<td>63 ± 5</td>
<td>55 ± 5</td>
<td>55 ± 5</td>
</tr>
<tr>
<td>0.5</td>
<td>46 ± 6</td>
<td>42 ± 4</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>0.25</td>
<td>37 ± 5</td>
<td>30 ± 5</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>0.125</td>
<td>29 ± 4</td>
<td>10 ± 3</td>
<td>10 ± 3</td>
</tr>
</tbody>
</table>

*Mean ± SD.

Response of human cell lines to partially purified IL-1 from THP-1-conditioned medium and pure IL-1 (2 units) from a commercial source

<table>
<thead>
<tr>
<th>Line designation</th>
<th>Characteristics*</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-415</td>
<td>PE, AC</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>BT-474</td>
<td>P, IDC</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>T47D</td>
<td>PE, IDC</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>ZR-75</td>
<td>A, IDC</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>MDA-MB-134 IV</td>
<td>PE, IDC</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>PE, IDC</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>A375Ag5</td>
<td>Melanoma</td>
<td>0</td>
</tr>
<tr>
<td>HeLa S3</td>
<td>Cervical carcinoma</td>
<td>0</td>
</tr>
<tr>
<td>HBL-100</td>
<td>Milk</td>
<td>0</td>
</tr>
<tr>
<td>HEL</td>
<td>Embryonic lung fibroblast</td>
<td>0</td>
</tr>
</tbody>
</table>

*Characteristics of mammary cell lines are presented as tissue source and histopathology. P, primary tumor; PE, pleural effusion; A, ascitic fluid; IDC, infiltrating ductal carcinoma; AC, adenocarcinoma.

DISCUSSION

Macrophages are responsible for the synthesis of several biological response modifiers that affect the behavior of a wide variety of cell types. Among these modifiers is a thymocyte-activating factor which significantly influences immune response. The primary function ascribed to this macrophage product, now defined as interleukin-1 (27), is the induction of interleukin-2 synthesis leading to the stimulation of proliferation by T-cell subsets of thymus tissue (28, 29). Partially purified IL-1 was shown by Mizel et al. (25) to induce prostaglandin and collagenase synthesis by synovial cells. Schmidt et al. (30) demonstrated that IL-1 can stimulate the proliferation of fibroblasts, while Estes et al. (31) reported that IL-1 and macrophage-derived growth factor are different entities. Recent studies have attributed a multiplicity of biological activities to IL-1 on a wide spectrum of target cells (21, 32, 33).

Macrophages are also responsible for a variety of growth-inhibitory substances including TNF. Characterization and purification of TNF were initially accomplished by Green et al. (34) and Ruff et al. (35). The work of Månell et al. (20) subsequently identified the macrophage as the source of TNF.
 Cultures were incubated with DEAE-purified IL-1 fractions in the defense. The work presented here indicates that an apparently was proposed (32, 33) that IL-1, like the interferons, could be a exerted multiple biological effects. Interferon inhibited tumor cell observed in immunological and nonimmunological reactions. Par in supernatants of U937, THP-1, and HL60 lines. Mizel and cell lines. In addition, a nondialyzable IL-1 inhibitor was detected thymocyte-stimulating activity and the levels of growth inhibition. [14C]arachidonic acid, the precursor for synthesis of prosta glandin. To exclude this possibility that growth inhibition was suppresion by SDS treatment. These may reflect in the IL-1 family differ in their structures but share some common homogeneous molecule was responsible for both the IL-1 and a portion of the growth-inhibitory activities produced by THP-1 cells. Although both activities shared a number of physicochemical features, some quantitative differences in sensitivities were observed with trypsin and SDS treatment. These may reflect differences in the assay systems for IL-1 and growth inhibition. These observations may support the conclusion that molecules in the IL-1 family differ in their structures but share some common regions, and that microheterogeneity enables IL-1 to exert its variety of effects by different mechanisms.

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