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Lymphocyte-activating and Growth-inhibitory Activities for Several Sources of Native and Recombinant Interleukin 1[1]

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ABSTRACT

Several native and recombinant forms of human interleukin-1 (IL-1) and recombinant murine IL-1 were assayed for their ability to inhibit the growth of cell lines established from malignant and nonmalignant human sources. The amount of growth-inhibitory activity was compared to the units of half-maximal [^{3}H]thymidine incorporation in mouse thymocyte cultures exposed to IL-1. Three malignant human mammary cell lines (MCF-7, T47D, and MDA-MB-415) were growth inhibited in the presence of both native and the α and β forms of recombinant human IL-1. MDA-MB-415 was most sensitive. Although most sources of IL-1 showed good correlation between units of activity and percentage of growth inhibition, native IL-1 from Genzyme Corporation induced a cytotoxic effect. Murine IL-1 was less growth inhibitory than the human forms of the monokine. Human embryonic lung (HEL), adult fibroblast (CRL 1445), and transformed milk (HBL-100) lines were not growth inhibited when tested against any IL-1 source. A lung carcinoma (CALU-1) and a colon carcinoma (SW-48) were not inhibited by either the α or β forms of human recombinant IL-1.

INTRODUCTION

Interleukin 1 (1) was initially defined as a factor which enhances the mitogenic response of cultured thymocytes to phytohemagglutinin or concanavalin A. Several other major biological functions ascribed to IL-1[3] include induction of IL-2 and the expression of IL-2 receptors by activated T-lymphocytes (2), endogenous pyrogen activity (3), induction or enhancement of the cyclooxygenase and lipooxygenase pathways of arachidonic acid metabolism (4), induction of B-cell proliferation (5), and the synthesis of proteases in synovial fibroblasts and chondrocytes (6). In addition, IL-1 has been reported to function as a negative regulator of cell behavior by increasing natural killer cell activity (7), antibody-dependent cytotoxicity (8), and macrophage tumoricidal killing (9). More recently, this laboratory reported that an IL-1 activity purified to homogeneity from the serum-free culture supernatants of a mezerein-treated acute monocytic leukemia cell line, THP-1, and a commercial product purified from peripheral blood monocytes were capable of directly inhibiting the replication of cell lines established from malignant breast tissues. IL-1 derived from THP-1 cells had an isoelectric point of 7 and an apparent molecular weight of 17,000 (10).

This partial list of the diverse and, at times, antagonistic activities has raised the possibility that a family of IL-1 molecules exists with slightly differing biochemical features. Several sources of native (11–13) and recombinant (14–16) IL-1 have now been characterized. There appear to be at least two forms of human IL-1, both derived from parent M_r 31,000 molecules but with only 26% homology in amino acid sequence. The α form has an isoelectric point of 5, similar to that derived from murine macrophages, while the β form isofocuses at pI 7 (16). Based on isoelectric point, the M_r 17,000 THP-1 cell-derived inhibitory factor is a β-type IL-1.

The purpose of the current investigation was to determine if growth-inhibitory activity was a function unique to IL-1 from THP-1 cells or if this represented a new activity common to IL-1 purified by several different laboratories. Both native and recombinant human and murine IL-1 from a variety of sources were tested for inhibitory activity against a limited number of malignant and nonmalignant cell lines. The data show that all of the human IL-1 sources examined express a growth-inhibitory function.

MATERIALS AND METHODS

Thymocyte Assay. Cell suspensions prepared from 5- to 8-wk-old LPS-unresponsive C3H/HeJ mice were inoculated at 1.5×10^6 cells per well into 96-well trays with RPMI-1640 medium supplemented with 10% fetal bovine serum, 2.5×10^{-5} M 2-mercaptoethanol, and phytohemagglutinin (1 μg/ml). The final volume of each well was 200 μl following the addition of test samples. Cultures were pulsed with 0.5 μCi of [^{3}H]thymidine (2 mCi/mM) per well for the final 6 h of 72-h incubation.

Sources of IL-1. The purification of IL-1 from the conditioned culture fluids of THP-1 cells was previously described (10). Briefly, crude serum-free medium was concentrated 100-fold and isoelectrofocused. IL-1 recovered at pH 7 was further purified by molecular-sieving chromatography through AcA-54 and ion-exchange chromatography through DEAE-cellulose. Fractions eluted in 30 to 70 mM NaCl were concentrated and electrophoresed under nondenaturing conditions. Gel slices containing IL-1 activity were shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to contain a single component with an apparent molecular weight of 17,000. Recombinant murine IL-1 was a gift of Dr. P. Lomedico (Hoffmann La Roche, Nutley, NJ). Dr. George Koch of Cistron Technology (Pine Brook, NJ) provided human recombinant β IL-1 for assay. Other human IL-1 materials were purchased from: Collaborative Research (Lexington, MA), native IL-1; Genzyme Corporation (Boston, MA), recombinant IL-1, α and β forms (developed by Immunex Corporation, Seattle, WA), and native IL-1; and Cistron Technology, native IL-1 from peripheral blood lymphocytes.

All IL-1 samples were reassayed. For the purpose of this study a unit of IL-1 was defined as that amount which resulted in half-maximal incorporation of [^{3}H]thymidine in the comitogenesis mouse thymocyte assay. The number of units of THP-1 material was calculated from the reciprocal of the dilution of a sample which resulted in half-maximal incorporation.

Growth Assays. The effect of IL-1 on cell replication was monitored with several human cell lines. Lines established from malignant tissues included: MCF-7, MDA-MB-415, and T47D, all pleural effusions from breast; CALU-1, a lung carcinoma; and SW-48, a colon carcinoma. Other lines included: HBL-100, established from breast milk; and HEL, an embryonic lung fibroblast obtained from the American Type Culture Collection (Rockville, MD). MCF-7, MDA-MB-415, and SW-48 cells were maintained in Eagle's minimal essential medium. T47D cells were grown in RPMI-1640 medium. Lines HBL-100, CALU-1, and ME-180 were cultured in McCoy's medium 5A, and HEL was grown in a 1:1 mixture of Dulbecco's minimal essential medium:Ham's F-12. Media were supplemented with 10% fetal bovine serum (Gibco Laboratories, Grand Island, NY) and gentamycin (50 μg/ml) (Schering-Plough Corporation, Kennilworth, NJ).

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[3] The abbreviations used are: IL-1, interleukin 1; IL-2, interleukin 2; LPS, lipopolysaccharide; rIL-1, recombinant interleukin 1.

Table 1 *Properties of interleukin-1 sources*

Type	Species	Source	Class	Inducer	Carrier	Specific activity (units/mg)
Native	Human	Collaborative Research	β	LPS	Human SA[a]	8.7×10^5
		Genzyme Corp.	β	LPS	FBS	8×10^9
		Cistron Technology	β	Staphylococcal proteoglycan	BSA	2×10^7
		THP-1	β	Mezerein		6×10^5
Recombinant	Human	Genzyme Corp.	α		BSA	10^8
		Genzyme Corp.	β		BSA	10^8
		Cistron Technology	β		BSA, DDT	2×10^6
	Murine	Hoffmann-LaRoche			Guanidine	6.7×10^7

[a] SA, serum albumin; FBS, fetal bovine serum; BSA, bovine serum albumin; DDT, dithiothreitol.

Growth assays were conducted as described (17). Cells were inoculated to 16-mm wells at 0.5 to 1.0×10^4, and the starting cell numbers were determined after 24 h. Remaining cultures were fluid changed with medium containing various concentrations of IL-1. The percentage of growth inhibition was calculated 7 days later as

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

Cell numbers were corrected by subtracting the 24-h values. The average numbers of cells present in control cultures at 7 days were: MCF-7, 1.8×10^5; MDA-MB-415, 0.8×10^5; T47D, 1.9×10^5; CALU-1, 1.1×10^5; SW-48, 4.9×10^5; HBL-100, 2.3×10^5; CRL-1445, 0.3×10^5; and HEL, 0.6×10^5.

RESULTS

Comparison of IL-1 Activity. The three commercial sources of native human IL-1 were produced by peripheral blood leukocytes exposed to either LPS or *Staphylococcus* proteoglycan and were supplied in a phosphate-buffered saline containing serum protein. The amount of residual endotoxin as determined by chromogenic LAL assay was below detectable levels at the highest concentrations of native or recombinant IL-1 sources used in this study. Table 1 outlines the properties of these IL-1 preparations and their reported specific activities.

IL-1 was assayed to standardize that amount which resulted in half-maximal [^{3}H]thymidine incorporation by thymocyte cultures. Fig. 1 illustrates typical titration curves established within the same assay for all sources of both native and recombinant IL-1 used in this study. Each lot of IL-1 was assayed twice with similar results. Data are expressed as the incorporation of [^{3}H]thymidine *versus* the number of IL-1 units reported by the supplier for each sample. A portion of the titration curve for each source showed a linear relationship between units of IL-1 and [^{3}H]thymidine incorporation. Two of the four lots of native IL-1 from Genzyme Corporation inhibited thymocyte proliferation when tested above 8 units per ml.

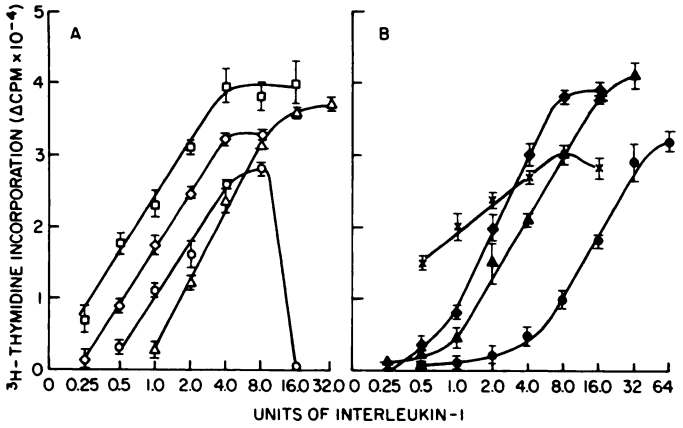

Fig. 1. Titration of interleukin 1 activity assayed in cultured thymocytes from C3H/HeJ mice. Data compare stated units of activity for each source with [^{3}H]-thymidine incorporation. All samples were compared in the same assay. *A* shows four sources of native IL-1 purified from human monocytes: Genzyme Corporation (○); Cistron Technology (△); Collaborative Research (□); THP-1 cells (◇). *B* shows three types of recombinant human IL-1: Genzyme α (◆); Genzyme β (●); Cistron (▲); and recombinant murine IL-1, Hoffmann-LaRoche (×). Each *point* represents the average of triplicate thymocyte cultures; *bars*, SD.

Differences between the reported number of IL-1 units and the amount required to induce half-maximal activity are evident. These differences could arise from variations among laboratories in thymocyte assay procedures, the lack of homogeneity within samples, or differences among suppliers in the definition of a unit of IL-1 activity. Approximately 10% of the IL-1 activity purified from peripheral blood cells was reported in literature from suppliers to be due to species with isoelectric points below 7. Previous studies reported multiple forms of IL-1 with induction schedules which included LPS (18). In addition, Cistron Technology defines a unit of native IL-1 as that which doubles the proliferative response of thymocytes to phytohemagglutinin (1 μg/ml). Other suppliers calculate IL-1 units according to half-maximal response.

Growth Inhibition by IL-1 Sources. Based on these data we conducted growth inhibition studies with quantities of each source of IL-1 standardized, so that 1 unit was defined as that amount which induced a half-maximal thymocyte response.

Fig. 2 compares growth of three cell lines derived from malignant mammary tissue in the presence of four sources of both native and recombinant IL-1. All sources of IL-1 were simultaneously tested for growth-inhibitory activity against target cell lines in 1-ml cultures. The data are plotted as the percentage of growth inhibition *versus* the number of half-maximal units established through thymocyte assays in our laboratory.

The results show some quantitative differences in effects among native IL-1 samples purified from peripheral blood cells or the THP-1 cell line. Based on the number of cells recovered at the end of each assay, it appeared that native Genzyme IL-1 was cytotoxic when tested at concentrations above four half-maximal units. An inhibition of [^{3}H]thymidine incorporation was also noted in the thymocyte assay (Fig. 1). Cytotoxicity in this study was defined as the recovery of fewer cells at the end of the assay than were inoculated to culture dishes. Cells treated with Genzyme native IL-1 had a rounded appearance, and a significant number of detached cells could not be recovered from culture supernatants. A sample of IL-1 carrier solution provided by Genzyme Corporation did not inhibit cell growth. Native IL-1 from Collaborative Research appeared to inhibit less growth at higher concentrations than the other sources.

Recombinant forms of human IL-1 showed closer agreement in the amount of growth inhibition induced per half-maximal unit than did native forms. Murine rIL-1 was less inhibitory

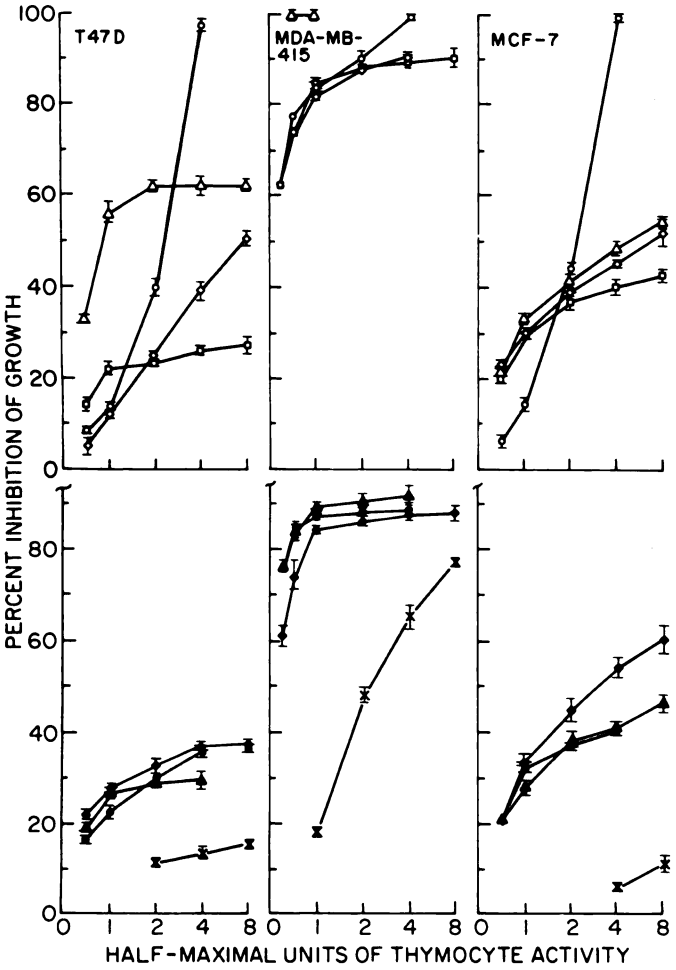

Fig. 2. Percentage of inhibition of growth in three mammary cell lines, T47D, MDA-MB-415, and MCF-7, incubated in the presence of increasing half-maximal units of IL-1. Native IL-1 sources (*top*): Genzyme Corporation (○); Cistron Technology (△); Collaborative Research (□); THP-1 cells (◇). Recombinant IL-1 sources (*bottom*): Genzyme α (◆); Genzyme β (●); Cistron (▲); and Hoffmann-LaRoche murine IL-1 (×). Each *point* represents the average of a triplicate culture; *bars*, SD.

when compared to other sources. For example, when T47D and MCF-7 cells were tested, murine rIL-1 induced only 12% growth inhibition at eight half-maximal units. The addition of an equivalent concentration of murine IL-1 carrier solution (10^{-7} M guanidine) did not inhibit cell growth, and the addition of guanidine to Cistron rIL-1 did not diminish growth-inhibitory activity against MCF-7 cells.

There was a large variation among these three cell lines in their response to IL-1. The MDA-MB-415 cell line was the most susceptible to the effects of all IL-1 types, displaying greater than 80% growth inhibition in the presence of one unit of any native or recombinant human source. Inhibited cultures were observed to contain enlarged flat cells with numerous cytoplasmic vacuoles.

Other transformed lines including CALU-1, SW-48, and HBL-100 were not growth inhibited when maintained for 7 days in the presence of 2 to 16 units of either the α or β forms of Genzyme IL-1 per ml. The nonmalignant fibroblast line, HEL, showed no response to any of the human native or recombinant IL-1 sources.

DISCUSSION

Enhanced tumoricidal activity by peripheral blood monocytes following exposure to lymphokines, γ-interferon, or lipopolysaccharide is well documented (19–21). However, the mechanism by which activated monocytes are involved in tumor cell destruction is not clearly understood. We recently demonstrated that cells of an acute monocytic leukemia line, THP-1, expressed mature monocyte features following exposure to the tumor-promoting agent, mezerein (17). Activated THP-1 cells were capable of producing IL-1, growth inhibitors, growth promoters, and a colony-stimulating factor. An effort to characterize the factor responsible for growth inhibition led to the discovery that a portion of this activity was attributable to interleukin 1 (10). Onoyaki *et al.* first suggested that IL-1 prolonged the cytotoxic state of monocytes by acting as an autostimulating factor (22). Subsequent findings showed that IL-1 was a direct cytocidal factor when assayed against the human melanoma cell line, A375 (23). In contrast, we were previously unable to show growth inhibition of A375 cells by IL-1 purified from THP-1 cells. However, growth inhibition was observed with several other lines derived from malignant tissue (10). The contrasting results with A375 cells probably reflect the existence of cell populations which differ from a parental phenotype. Two laboratories (10, 23), therefore, have related negative growth regulation to the IL-1 family of molecules. The current study establishes the ubiquitous nature of this IL-1 function by examining several sources of both recombinant and native IL-1 and leads to the conclusion that all human forms of the molecule inhibit the growth of some human cell lines.

When growth-inhibitory activity was related to half-maximal incorporation of [^{3}H]thymidine in the thymocyte assay, a very good correlation was observed among sources of both the α and β forms of recombinant material. In contrast, less growth inhibition was associated with recombinant murine IL-1 when assayed at concentrations identical to the human forms. Differences were observed among native sources of IL-1 when comparing thymocyte activation and growth inhibition. Most notable was the cytotoxic effect induced by native Genzyme IL-1 when tested above four half-maximal units. We have used four different lots of Genzyme material for this and previous studies (10), and two of those induced cytotoxicity.

The cloning and expression of complementary DNAs for both murine and human forms of IL-1 have been accomplished. March *et al.* (16) reported the isolation of two complementary DNAs responsible for the expression of the α and β forms of human IL-1 from lipopolysaccharide-stimulated macrophages. Auron *et al.* (15) reported the sequence of a complimentary DNA with striking similarities to the gene encoding the β form of IL-1. Lomedico *et al.* (14) isolated the complementary DNA responsible for murine IL-1 from the P388D$_1$ line. All studies concluded that IL-1 is synthesized as larger precursors which are processed to smaller forms with an apparent molecular weight of 17,000. Sequence analysis of the translation products revealed a greater homology between the α form of human IL-1 and murine IL-1 than between the α and β human forms (16). In spite of these similarities, our data show greater and equivalent inhibition of cell growth with the two human forms than with murine rIL-1. Variations in amino acid sequences may be responsible for species differences in cell line susceptibility or differences in the expression of the numerous functions attributable to IL-1.

The data also show differences in sensitivity among lines to IL-1 growth inhibition. Morphological changes were readily apparent in the growth-inhibited lines with exposure to all of the IL-1 sources. Increased cell spreading and vacuolization were seen in cultures of MDA-MB-415 cells within 48 h of

exposure to IL-1, and the MDA-MB-415 line was the most sensitive of the three mammary cell lines to the growth-inhibitory effects of IL-1. Previous data showed no inhibition or stimulation of fibroblast cell growth by IL-1 purified from THP-1 cells (10). The current work extends this preliminary finding for fibroblast lines by testing both native and rIL-1 forms against HEL cells.

Onozaki *et al.* (22) suggested that IL-1-promoted monocyte cytotoxicity was mediated by prostaglandins and cyclic AMP. However, the direct cytocidal effects they observed were not blocked by indomethacin, an inhibitor of the cyclooxygenase pathway of arachidonic acid metabolism (23). We previously showed that THP-1-derived IL-1 did not enhance prostaglandin synthesis in MCF-7 cells (10). Although these results imply that inhibition occurs independently of prostaglandins, they do not eliminate the possible involvement of metabolites of the lipooxygenase pathway. In addition, it is known that prostaglandin induction in response to IL-1 varies among cell types.

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