Correlation between Human Cell Growth Response to Interleukin 1 and Receptor Binding

E. V. Gaffney, G. Koch, S-C. Tsai, T. Loucks, and S. E. Lingenfelter

Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, Pennsylvania 16802 [E. V. G., S-C. T., T. L., S. E. L.], and Cistron Biotechnology, Pine Brook, New Jersey 07058 [G. K.]

ABSTRACT

Human recombinant interleukin 1α (IL-1α) and IL-1β inhibited the replication of the mammary tumor cell line, MDA-MB-415; stimulated division in the colon carcinoma, SW-48; and had no effect on the growth of the milk mammary line, HBL-100. Inhibition of growth was reflected in a significant decrease in DNA synthesis accompanying a transient increase in RNA synthesis. Specific binding of 125I-labeled recombinant IL-1β by MDA-MB-415 and SW-48 reached a maximum by 2 h of incubation, and an equivalent amount was bound by each cell type. Binding was inhibited in a dose-dependent manner by unlabeled IL-1α or IL-1β. Scatchard plot analysis revealed that MDA-MB-415 cells expressed approximately 700 binding sites with an apparent dissociation constant of 8.8 x 10⁻¹⁰ M. Reversibility of growth inhibition was independent of dose or time of incubation, but DNA synthesis did not return to control values. Flow cytometric analysis of DNA content showed that growth inhibition was cell cycle phase nonspecific with a slight reduction in the proportion of cells in S phase. The major conclusion from these studies was that inhibition or stimulation of malignant cell growth by IL-1 was related to the presence of receptor sites.

INTRODUCTION

Originally described for its enhancing effect on thymocyte proliferation (1), IL-1 is now recognized as a key mediator amplifying many immunological and inflammatory reactions (2, 3). Furthermore, IL-1 has been shown to influence tumor cell growth by promoting monocyte-mediated tumoricidal activity (4) and inducing cytotoxic T-cell responses (5). Our laboratory reported that human IL-1 exerted direct antiproliferative effects on cell lines established from malignant breast tissues (6). Growth inhibition was not mediated by IL-1-induced prostaglandin production. Highly purified IL-1 was also reported by others to be a cytoidal or cytokastic factor for certain tumor lines (7, 8). Further investigation revealed that the growth-inhibitory activity was common to both native and recombinant human IL-1 from a variety of sources (9, 10). Most recently, we observed that IL-1 possessed both growth-stimulating and -inhibiting properties with tumor cells depending on the target cell type (11).

Human IL-1 comprises a family of at least two different polypeptides termed IL-1α and IL-1β. The α form isofocuses a pI 5, and the β form has an isoelectric point of 7. Both IL-1α forms show only 26% homology in their amino acid sequences and 45% homology in their nucleotide sequences (12); however, there are five regions of amino acid sequence homology in the primary translation products between the two molecules (12, 13). Although biochemically and antigenically distinct, IL-1α and IL-1β appear to have indistinguishable biological activities. We previously showed that both forms of recombinant IL-1 had comparable growth-regulatory activities and similar target cell specificities (10). The susceptibility of cells to both IL-1 forms was different from that of y-interferon (11).

The current investigation begins to elucidate the mechanisms by which IL-1 regulates cell proliferation. The target cell lines used in this study represent those that are inhibited, stimulated, or show no response in the presence of IL-1. In this paper we examine the effect of rIL-1 on in vitro growth, macromolecular synthesis, and cell cycle distribution. In addition, we show that MDA-MB-415 cells, a sensitive responder to IL-1, have receptors for 125I-IL-1.

MATERIALS AND METHODS

Cell Culture. Assays were performed with cell lines whose growth is inhibited, stimulated, or unaffected with exposure to human recombinant IL-1. rIL-1 stocks were provided by Cistron Biotechnology (Pine Brook, NJ). The original concentration of rIL-1α was 3.9 mg per ml and expressed 1.6 x 10⁷ half-maximal units of biological activity per mg in a standard comitogenic thymocyte assay (6, 10). rIL-1β was provided at a concentration of 7.5 mg per ml containing 1.3 x 10⁷ units per mg. Both proteins appear to be homogeneous when electrophoresed under reducing conditions. The malignant mammary line, MDA-MB-415, was maintained in a medium consisting of 4 parts L-15 and 1 part DMEM. SW-48, derived from a colon carcinoma, was cultured in DMEM. HBL-100, established from a primary culture of human milk cells, was grown in McCoy's Medium Sa. Media were supplemented with 10% FBS (GIBCO, Grand Island, NY) and 50 µg of gentamycin/ml (Schering Corp., Kenilworth, NJ). Cell lines were obtained from the American Type Culture Collection (Rockville, MD) and were maintained free of Mycoplasma.

DNA, RNA, and Protein Synthesis. MDA-MB-415 or SW-48 cells were seeded at 10⁴ in 200 µl of medium to 96-well microtiter plates. One day later cultures received rIL-1. At daily intervals, sets of control and treated cultures were pulsed with 1 µCi/well of [³H]Thd (6.7 Ci/mmol), 0.5 µCi/well of [³H]Urd (43.1 Ci/mmol), or 1 µCi/well of [³H]leucine (58.4 Ci/mmol) (New England Nuclear) for 6 h. Cultures were trypsinized and harvested onto filter paper with a Titertek cell harvester (Flow Industries, Rockville, MD). Unpulsed cultures in parallel wells were trypsinized, and cell numbers were counted using a Coulter Counter ZM. The values were converted to cpm per cell number, and results were expressed as the percentage of control incorporation.

Cell Cycle Analysis. MDA-MB-415 cells were plated at 10⁵ cells per 60-mm dish. Twenty-four h later cultures were fluid changed with fresh medium alone or medium containing rIL-1. Sets of control and treated cultures were washed, resuspended in phosphate-buffered saline to 10⁶ cells per ml, and incubated with ribonuclease (40 µg/ml) (Worthington Biochemical Corp., Freehold, NJ) for 30 min at room temperature. The cells were then stained with propidium iodide (18 µg/ml), filtered through nylon mesh, and analyzed on a Coulter EPICS V fluorescent activated cell sorter (Coulter Electronics, Inc., Hialeah, FL) at 488-nm laser wavelength. The data from samples with 20,000 cells were collected, and DNA analyses of resultant histograms were performed on the M.D.A.D.S. computer according to the method of Bagwell et al. (14).
Radioiodination of Interleukin 1. IL-1β was diluted with 0.1 m sodium borate buffer to a concentration of 20 µg/ml. Fifty µl were added to 1 mCi of 125I-Bolton Hunter reagent (2200 Ci/mmol) (New England Nuclear) which had been dried to nitrogen gas. Incubation was continued for 45 min on ice with intermittent shaking. Fifty µl of 0.2 M glycine in buffer were added, and radiolabeled rIL-1β was chromatographed to remove free isotope on a 1 x 20-cm Sephadex G-25 column equilibrated with phosphate-buffered saline containing 10% glycerol and 0.1% bovine serum albumin. Both radioactivity by γ-counting and biological activity measured by thymocyte mitogenic assay were determined for each fraction. There was no loss of biological activity in any of the four labeling experiments. The specific activity of 125I-IL-1β was 2.6 to 3.6 x 10^8 cpm/µg of 125I-IL-1β alone, or with a 100-fold excess of unlabeled rIL-1. Cultures were incubated at 4°C on a rocker platform. Monolayer cultures were washed 5 times with binding buffer and solubilized in 2 N NaOH. Cell-associated radioactivity was determined by counting 0.5 ml of extract. Counts were expressed per 10^6 cells.

RESULTS

Effect of IL-1 on Cell Growth. The effects of human rIL-1α and rIL-1β on cell growth and macromolecular synthesis were followed by measuring incorporation of the appropriate radio-labeled precursors. MDA-MB-415 cells and SW-48 cells incubated in 96-well plates with medium containing rIL-1β were sampled following 6-h incubation each day in [3H]dThd, [3H]Urd, or [3H]leucine. The number of cells in corresponding control and treated cultures was counted, and the amounts of incorporation were corrected for cell number.

Fig. 1 shows that DNA synthesis in MDA-MB-415 cells was decreased 80 to 90% on Day 1 and remained inhibited during the period of observation. Although RNA synthesis decreased slightly by Day 1, an increase to 60% above control was observed by Day 3 of IL-1 exposure. [3H]Urd incorporation gradually decreased to approximately 20% above control levels by Day 6 (data not shown). Protein synthesis, measured by [3H]leucine incorporation, increased about 20% above controls 2 days after IL-1 addition and then rapidly declined to control levels. SW-48 cells showed slight increases in DNA, RNA, and protein synthesis during the first 3 days of the experiment. [3H]dThd incorporation returned to the control level by Day 4. Identical changes in the synthesis of DNA, RNA, and protein/cell were observed with exposure to the same concentration of rIL-1α.

Neither rIL-1α nor rIL-1β had any effect on replication or synthesis by HBL-100 cells.

Detection of IL-1 Receptors. The relationship between incubation time and receptor binding was studied using 125I-IL-1β. Confluent cultures of MDA-MB-415 cells, SW-48, or HBL-100 cells were incubated at 4°C in 0.8 ml of binding buffer containing 0.1 µCi of 125I-IL-1β in the presence or absence of 100-fold excess of unlabeled rIL-1β. After washing with cold binding buffer and hydrolysis in 2 N NaOH, the radioactivity of cell-associated 125I-IL-1β was measured. Fig. 2 illustrates the data from one of four experiments with similar binding kinetics. Specific binding was observed in cultures of MDA-MB-415 and SW-48 cells. Binding reached a maximum by 2 h of incubation, and an equivalent amount of binding occurred with both cell types. In contrast, no significant level of binding was observed in cultures of HBL-100 cells.

Next, the specificity of the IL-1 binding was investigated by the competitive binding of 125I-IL-1β in the presence of unlabeled rIL-1α or rIL-1β during a 3-h incubation period. Data were obtained with the confluent cultures of MDA-MB-415 cells. Fig. 3 illustrates that the binding of 125I-IL-1β was inhibited by unlabeled rIL-1α and rIL-1β in a dose-dependent manner. These results suggest that a receptor on MDA-MB-415 cells was specific for IL-1 and that the receptor recognized both the α and β forms.

Fig. 4 shows the dose-response curve of the specific binding of 125I-IL-1β following 3-h incubation with confluent cultures of MDA-MB-415 cells. The IL-1 concentration required to reach half saturation of binding sites was approximately 8.8 x 10^-10 M. Scatchard plot analysis showed that MDA-MB-415 cells bound approximately 700 molecules of 125I-IL-1β per cell under these conditions.

Reversibility. Two types of experiments were used to dem-
Fig. 3. Competitive inhibition of the binding of 125I-IL-1β by unlabeled rIL-1α or rIL-1β. MDA-MB-415 cells cultured to confluency in 35-mm dishes were incubated at 4°C for 3 h in 0.8 ml of binding buffer containing 3.6 x 10^3 cpm per μg of 125I-IL-1β at a final concentration of 4.9 nM in the presence of various concentrations of unlabeled rIL-1α (C) or rIL-1β (○). After washing, cell-bound radioactivities were counted. Results are expressed as average counts for duplicate samples.

Fig. 4. Specific binding curve of 125I-IL-1β to MDA-MB-415 cells and Scatchard plot analysis. Confluent cultures in 35-mm dishes were incubated with various concentrations of 125I-IL-1β at 4°C for 3 h, and specific binding (A) was determined in the presence (○) or absence (O) of a 100-fold excess of unlabeled rIL-1β. Monolayer cultures were washed 5 times with cold binding buffer and incubated in 2 N NaOH, and radioactivity was counted by a gamma counter. The results are expressed as the mean for duplicate samples. Data are representative of three experiments.

Fig. 5. Recovery of MDA-MB-415 cell growth following incubation in rIL-1β. Microtiter wells were seeded at 10^4 cells and maintained for 16 h. IL-1β was added at concentrations of 0.05 (○), 0.25 (●), 0.5 (△), or 1.0 (□) ng/well. Three days later cells were washed, fluid was changed to fresh medium, and samples were taken 1, 2, or 3 days later. [3H]dThd at 0.5 μCi/well was added 4 h before samples were collected. Results are the average percentage of control calculated from triplicate cultures; bars, SD.

Fig. 6. Time-dependent reversibility of growth arrest. Microtiter wells were seeded with 10^4 MDA-MB-415 cells in 200 μl of medium and incubated for 24 h. IL-1 was added at 1 ng/well. Sets of treated and untreated wells were washed 3 times, and fluid was changed to fresh medium after 1, 2, or 3 days of exposure to IL-1 (○). Cultures were also maintained without fluid renewal throughout the experiment (△). Triplicate control and treated cultures from each set were incubated each day for 4 h in 0.5 μCi of [3H]dThd. Data are expressed as the average percentage of control incorporation for each set; bars, SD.

Demonstrate that the inhibition of MDA-MB-415 cell growth by IL-1 was reversible. The first examined dose-dependent reversibility by incubating microtiter well cultures in rIL-1β at concentrations of 0.05 ng to 1.0 ng (Fig. 5). After 3 days, inhibited cultures were washed, and fluid was changed with fresh medium. Triplicate control and IL-1-treated cultures were then incubated in [3H]dThd for 4 h each day and harvested. The data showed that DNA synthesis inhibition was reversible and that reversibility was independent of the IL-1 concentration to 1 ng. However, recovery from growth arrest was not immediate. Cells exposed to 0.05 ng of IL-1 returned to control values 2 days after removal of the monokine. In contrast, cells exposed to 1 ng of IL-1 had achieved only 75% of the control level by Day 3.

Time-dependent reversibility studies were initiated by incubating 24-h-old microtiter well cultures of MDA-MB-415 cells with 1 ng per well of rIL-1β. Each day for 3 days, sets of control and treated wells were washed with fresh medium and refed. Cultures from each set were incubated for 4 h in [3H]dThd on subsequent days. Reversibility was expressed as a percentage of control incorporation (Fig. 6). The data show that, following exposure to IL-1, DNA synthesis resumed independently of the length of exposure within 1 day. However, synthesis did not return to control levels during the length of this experiment. Identical results were obtained when cells were incubated in the presence of 1 ng of rIL-1α (data not shown).
Cell Cycle Analysis. Flow cytometric analysis of DNA content was performed to determine if the cytostatic effect of IL-1 involved an arrest of target cells at a particular stage of the cell cycle. Sets of control and treated cultures were harvested and analyzed for 5 days. Fig. 7 shows the data from compartment analysis of the population distribution in each cell cycle phase following exposure to rIL-1β. Although 1 ng/ml of IL-1 inhibited cell growth by more than 50%, the fraction of cells in the different phases of the cell cycle was not markedly changed. A small reduction in the fraction of treated cells in S phase was observed with an accompanying increase in the proportion of cells in G1 phase. These differences were not apparent in cultures sampled during Days 4 and 5 after the addition of IL-1. The results suggested that IL-1 treatment increased the duration of phases of the cell cycle and transiently reduced the populati...
with the expression of an unusually high number of binding sites. Their results also suggested that a small number of high affinity receptors was responsible for the induction of biological activity. Scatchard analysis of equilibrium binding revealed two classes of IL-1 receptors on a number of T-cell populations. The low number of receptors and specific activity of the 125I-labeled human IL-1 receptor populations on a number of T-cell populations. However, the differences between concentrations required to achieve either binding or growth inhibition tended to argue that biological activity requires less than maximal receptor occupancy.

The biological activity of lymphokines similar to IL-1 also depends on binding to specific receptors. It was initially reported that human and mouse cells sensitive to the cytotoxic action of TNF have high affinity cell surface receptors, whereas cells made resistant to TNF fail to bind appreciable amounts of 125I-TNF (27, 28). In contrast, Tsujimoto et al. (29) showed that some cell lines with large numbers of TNF receptors were highly resistant to TNF cytotoxicity. Thus, receptor concentration may not be directly related to biological activity. This was confirmed in a survey with human tumor lines where the number of TNF binding sites ranged from 5 to 20,000 (30). This study showed no correlation between the receptor density and susceptibility to the cytotoxic action of TNF. It has yet to be established whether the degree of growth response in the presence of IL-1 can be correlated with the level of IL-1 receptor, since the current work was limited to one inhibited cell type. However, the data do establish a qualitative correlation between receptor presence and growth response.

The molecular mechanism of signal transduction by IL-1 is currently under study. Martin et al. (31) recently showed that IL-1 purified from the conditioned medium of the murine macrophage cell line P388D1 induced the phosphorylation of a Mr 41,000 membrane protein of the human leukemic line, K562. Receptor binding and membrane protein phosphorylation are a general mechanism through which mammalian cell functions are controlled by exogenous factors. The accumulation of data on second messenger induction by IL-1 coupled with information on phosphorylation pathways in specific cell types may eventually explain the pleiotropic effects of IL-1 in both immune and nonimmune cells. An examination of the activities of common protein kinases may also provide the basis for understanding how IL-1 inhibits, stimulates, or has no effect on the growth of certain malignant cell lines.

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