Human purified interleukin-1 inhibits DNA synthesis and cell growth of osteoblastic cell line (MC3T3-E1), but enhances alkaline phosphatase activity in the cells

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We examined the effects of human purified interleukin-1 (IL-1) on DNA synthesis, cell growth, and alkaline phosphatase activity in the osteoblastic cell line MC3T3-E1 under both preconfluent and confluent culture conditions. Addition of IL-1 to the cells markedly inhibited their DNA synthesis and growth over the range 1–10 U/ml. Such significant inhibitory effects were observed in cells cultivated in 1 or 5% fetal calf serum (FCS)-containing alpha modification Eagle's medium (alpha-MEM), but not in alpha-MEM containing 10% FCS. In contrast, alkaline phosphatase activity was enhanced significantly by IL-1 in the cell line cultivated in 1% FCS-containing alpha-MEM. These results demonstrate that human purified IL-1 is effective in inducing the differentiation of osteoblastic cell MC3T3-E1.

Interleukin 1 *Osteoblast* *DNA synthesis* *Cell growth* *Alkaline phosphatase* *Differentiation*

1. INTRODUCTION

Some recent studies have suggested that macrophage-derived interleukin-1 (IL-1), a peptide hormone, may be involved in the regulatory mechanisms at play in the bone remodeling system [1–5]. Gowen et al. [1] have shown that a human monocyte-derived IL-1-like factor stimulates bone resorption. It has been reported that IL-1 inhibits the production of the bone specific protein osteocalcin in human osteoblast-like cells [3]. Also, Krakauer et al. [4] have shown that human IL-1 mediates cartilage matrix degradation. Interestingly, a very recent study [6] has demonstrated that human recombinant IL-1 β is the major protein with osteoclast-activating factor activity produced by lectin-stimulated human peripheral blood mononuclear cells.

Although many studies have shown effects of several hormones on cell growth, collagen synthesis, and alkaline phosphatase activity of osteoblast-like cells, it is also very important to understand the effect of IL-1 on the biological activities in osteoblastic cells for elucidation of its regulatory mechanisms in the bone remodeling process.

We have now investigated the effect of IL-1 or IL-1-like factor on the bone remodeling process. Because bone remodeling is regulated by interactions among bone cells, macrophages, lymphocytes, and their products, it is very important to use a clonal bone cell population and homogeneous IL-1 for analysis of the functional role of IL-1 in the bone remodeling process. Kodama et al. [7] have recently established a clonal osteoblastic cell line (MC3T3-E1) from newborn mouse calvaria, and this cell line exhibits various osteoblastic functions [8]. Here, we examined the effect of human purified IL-1 on some parameters of differentiation, such as DNA synthesis, cell growth, and alkaline phosphatase activity in this clonal osteoblastic cell line.

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The results show that human purified IL-1 is effective in inducing the differentiation of clonal osteoblastic cells.

2. MATERIALS AND METHODS

2.1. *Osteoblastic cell line*

The MC3T3-E1 cell line, derived from C57BL/6 newborn mouse calvaria, was kindly provided by Dr Kodama (Tohoku Dental University, Fukushima). The cells were grown in 10% fetal calf serum (FCS, Irvine Scientific, Santa Ana, USA)-containing the alpha modification Eagle's medium (alpha-MEM, Flow Labs, McLean, USA) supplemented with antibiotics. The cells were maintained at 37°C in 5% CO_2 in air. Cells were passaged twice a week by treatment with 0.02% pronase and 0.02% EDTA in Mg^{2+}- and Ca^{2+}-free phosphate-buffered saline.

2.2. *Assay for DNA synthesis and cell growth*

MC3T3-E1 cells were seeded into Falcon 96-well flat-bottomed type microculture plates (5×10^3 cells/well) and incubated overnight in 200 μl of 10% FCS-containing alpha-MEM. In some experiments, the cells were incubated until the monolayer had reached confluence. These cells were washed twice with alpha-MEM, and then 100 μl of various doses of human ultrapure IL-1 (Genzyme, Boston, USA) was added to each well, along with 100 μl of 1% FCS-containing alpha-MEM, unless stated otherwise. After 48 h, the cells were pulsed for 16 h with 0.2 μCi [^{14}C]thymidine (spec. act. 50.5 mCi/ml, New England Nuclear, Boston, USA) and then washed twice with phosphate-buffered saline. The washed cells were treated with 0.2% SDS, and the radioactivities in the lysate were measured by liquid scintillation counting. The number of [^{14}C]thymidine counts in the SDS lysate was similar to that found in the trichloroacetic acid-insoluble material.

To determine the effects of IL-1 on the growth of MC3T3-E1 cell, the cells in alpha-MEM containing 10% FCS were seeded into wells of Falcon 24-well flat-bottomed type microculture plates (8×10^3 cells/well) and incubated overnight. The culture medium was then removed and replaced with alpha-MEM containing 1% FCS, and various concentrations of IL-1. At chosen intervals during the following culture period, cells were treated with 0.02% pronase, and the dispersed cells were counted in a hemocytometer. The experiments were carried out with triplicate cultures and the results expressed as mean cell number ± SD.

2.3. *Assay for alkaline phosphatase activity*

MC3T3-E1 cells were seeded in Falcon 96-well flat-bottomed type microculture plates (5×10^3 cells/well), and cultured in 10% FCS-containing alpha-MEM until the cell monolayer had reached confluence. The cultured cells were washed twice with alpha-MEM and then cultured for various times in alpha-MEM with or without serum and various concentrations of IL-1. The IL-1-treated cells were then washed twice with phosphate-buffered saline, followed by the addition of 200 μl of 0.2% Nonidate P-40 containing 1 mM $MgCl_2$ to the cells. Thereafter, the cells were sonicated for 5 s with a Branson model 200 ultrasonifier. The lysate was used for the assay of alkaline phosphatase activity. Alkaline phosphatase activity was measured as described by Lowry et al. [9] with *p*-nitrophenyl phosphate as substrate. Protein content was measured by the dye-binding method of Bradford [10].

2.4. *Assay for IL-1 activity*

IL-1 activity was measured as described [11]; briefly, C3H/HeJ mouse thymocytes (1.5×10^6 cells/well) were cultured for 72 h at 37°C in 5% FCS-containing RPMI 1640 (Nissui Pharmaceutical, Tokyo) supplemented with 2-mercaptoethanol and phytohemagglutinin-p (Difco, Detroit) and test sample. Cell proliferation was measured by pulsing with 0.5 μCi [^{3}H]thymidine (New England Nuclear, spec. act. 6.7 Ci/mM) for the last 24 h of the culture period. The results were expressed as the arithmetic mean of cpm ± SD as determined from triplicate cultures.

3. RESULTS

Figs 1 and 2 show that addition of purified IL-1 to preconfluent monolayers of MC3T3-E1 cells markedly inhibited DNA synthesis and cell growth. DNA synthesis decreased to 42% of the control value when the cells were treated with 1 U/ml of IL-1. Also, cell growth was significantly inhibited as early as 24 h after initiation of treatment with 1 or 5 U/ml of IL-1. However, signifi-

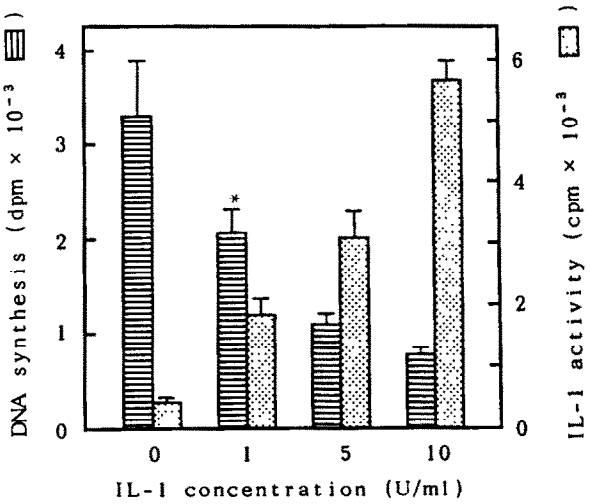

Fig.1. Effect of purified IL-1 on DNA synthesis in MC3T3-E1 cells under preconfluent cultures. MC3T3-E1 cells were plated in 96-well flat-bottomed type culture plates (5×10^3 cells/well) in 1% FCS-containing alpha-MEM with or without IL-1 at various concentrations. After 48 h, the cells were pulsed for 16 h with 0.2 μCi [^{14}C]thymidine. The results are expressed as mean ± SD from triplicate cultures. * Significant difference from control at $p < 0.01$.

cant inhibitory effect at 1 U/ml was not observed at 72 h after its addition. Fig.3 shows that alkaline phosphatase activity of the cells cultured with 1 U/ml of IL-1 was enhanced in a time-dependent manner.

The inhibitory effect of IL-1 on DNA synthesis of the cells in media supplemented with different serum concentrations was also examined (fig.4). No significant inhibitory activity with IL-1 at 5 U/ml was detected in the cells cultivated in alpha-MEM containing 10% FCS. However, IL-1 remarkably inhibited DNA synthesis of the cells cultivated in alpha-MEM containing 1% FCS. These results indicate that some factor(s) contained in the serum affects the action of IL-1 on DNA synthesis and cell growth in this cell line.

These results led us to suppose that IL-1 may be actively involved in the differentiation of MC3T3-E1 cells under preconfluent culture conditions. Therefore, we further examined IL-1 for a possible effect on the differentiation of these cells under confluent culture conditions by assaying for their DNA synthesis and alkaline phosphatase activity. The cells were seeded at a density of 5×10^3 cells/well into Falcon 96-well flat-bottomed type microculture plates. After cell monolayers had reached confluence, the cells were incubated for 4 days in serum-free alpha-MEM and various doses of IL-1. As shown in fig.5, significant enhancement of alkaline phosphatase activity was observed in the cells cultivated in the presence of 5 U/ml of IL-1. However, the cellular protein content per

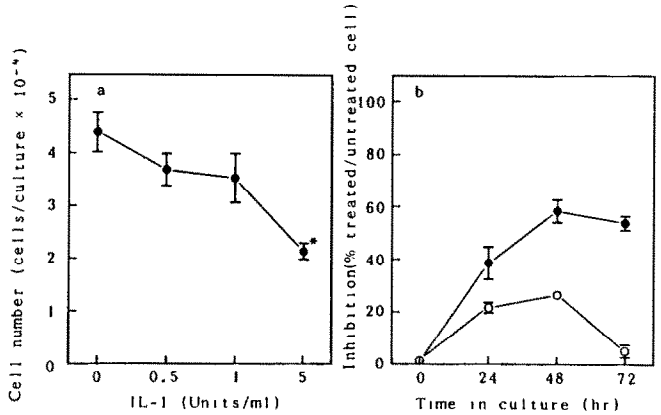

Fig.2. Effect of purified IL-1 on growth of MC3T3-E1 cells. The cells were plated in 24-well flat-bottomed type culture plates (8×10^3 cells/well) in 1% FCS-containing alpha-MEM with or without IL-1 at various concentrations. (a) After 72 h the medium was removed and the cells treated with pronase and then counted. Each point represents the mean ± SD of triplicate cultures. * Significant difference from control at $p < 0.01$. (b) Cells were cultured with 1 U/ml (○) or 5 U/ml (●) of IL-1. At various intervals, some of the cells were treated with pronase and then counted. The results are expressed as % inhibition by IL-1 (treated/untreated cells). Each point represents the mean ± SD of triplicate cultures.

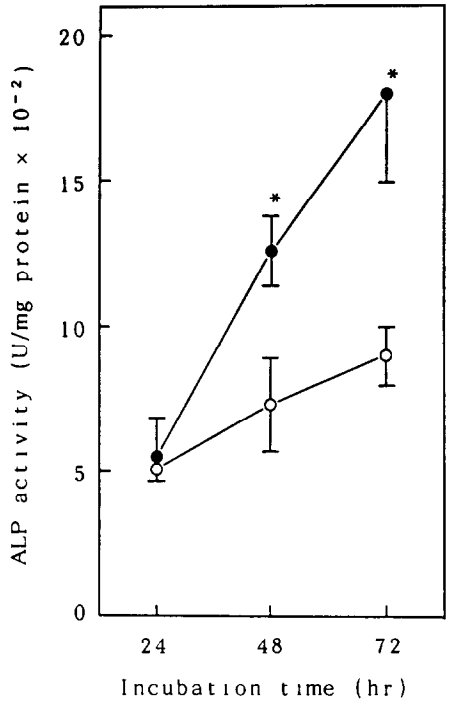

Fig.3. Effect of purified IL-1 on alkaline phosphatase activity of MC3T3-E1 cells. MC3T3-E1 cells were plated in 96-well flat-bottomed type culture plates (5×10^3 cells/well) in 1% FCS containing alpha-MEM with (●) or without (○) IL-1 at 1 U/ml. After each culture period, the cells were assayed for alkaline phosphatase activity. The results are expressed as the mean ± SD of triplicate cultures. * Significant difference from control at $p < 0.01$.

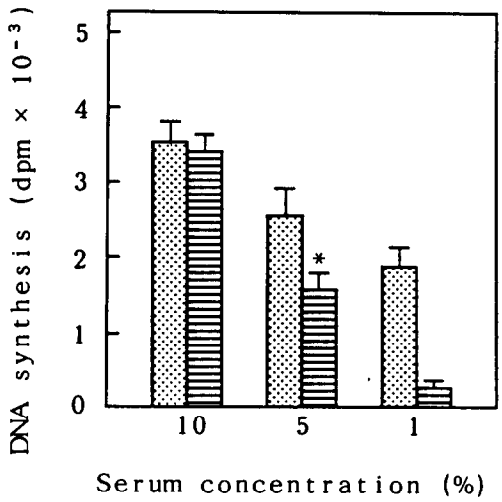

Fig.4. Effect of serum on DNA synthesis of MC3T3-E1 cells treated with IL-1. Cells were plated in 96-well flat-bottomed type culture plates (5×10^3 cells/well) in alpha-MEM containing various concentrations of FCS with ( ) or without ( ) 5 U/ml of IL-1. After 48 h, cells were pulsed for 16 h with 0.2 μCi [^{14}C]thymidine, and the radioactivity measured. Each point represents the mean ± SD of triplicate cultures. * Significant difference from control at $p < 0.01$.

culture was not affected under the same conditions. On the other hand, remarkable inhibition of DNA synthesis was found in cells treated with IL-1 at the same concentrations. The inhibition of DNA synthesis occurred in a dose-dependent fashion over the concentration range 1–10 U/ml.

These findings show that the IL-1 is an effective mediator for inducing clonal osteoblastic cell differentiation in vitro.

4. DISCUSSION

This paper reports that purified IL-1 is effective in inducing the differentiation of clonal osteoblastic cell lines (MC3T3-E1), which were derived from newborn mouse calvaria. The effects of this cytokine on some parameters of differentiation, such as DNA synthesis, cell growth, and alkaline phosphatase activity, were examined in this osteoblastic cell line. IL-1 inhibited DNA synthesis and cell growth of the cells, but enhanced their alkaline phosphatase activity. Sudo et al. [8] have demonstrated that MC3T3-E1 cells exhibit properties of osteoprogenitor cells in their active growing stage and have the capacity to differentiate into osteoblasts and osteocytes in vitro. Our present findings show that purified IL-1 can act as an agent for differentiation of these cells in vitro. Very recently, Gowen et al. [12] have shown that human monocyte products with IL-1 activity stimulate proliferation of human bone cells. Since they have no evidence that the stimulatory factor is IL-1, nor do they know which cell types respond to the stimulatory effect, identification of the monocyte products and their target cells has yet to be made. The answers are very important for an understanding of the regulatory role of monocyte- and macrophage-derived factors in the bone remodeling process.

IL-1 has been recently recognized as a polypep-

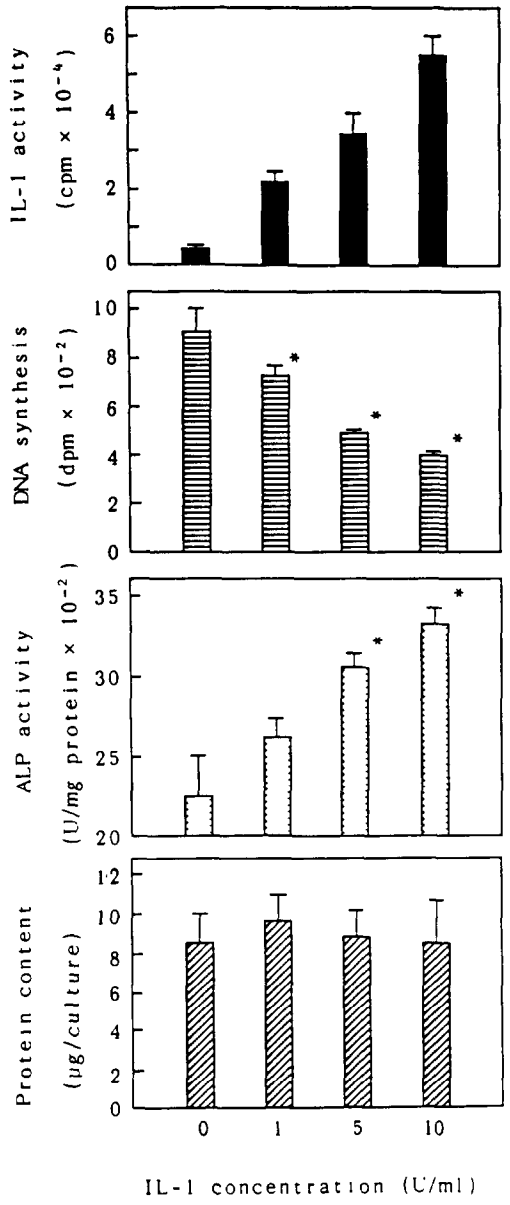

Fig.5. Effect of IL-1 on DNA synthesis and alkaline phosphatase activity of MC3T3-E1 cells under confluent culture conditions. Cells were cultured in 96-well flat-bottomed type culture plates (5×10^3 cells/well) with 10% FCS-containing alpha-MEM. After the cell monolayer had reached confluence, cells were cultured in serum free alpha-MEM with or without IL-1. For assay of DNA synthesis, after 3 days the cells were pulsed for 16 h with 0.2 μCi [^{14}C]thymidine and then the radioactivity in the cells counted. On the other hand, for alkaline phosphatase activity, the cells were cultured for 4 days and then assayed for enzyme activity. Results are expressed as the mean ± SD of triplicate cultures. (a) IL-1 activity, (b) DNA synthesis ([^{14}C]thymidine uptake dpm/culture), (c) alkaline phosphatase activity (U/mg protein), (d) protein concentration (μg/culture). * Significant difference from control at $p < 0.01$.

tide hormone produced by activated macrophages. Dower et al. [13] have shown that IL-1 receptor(s) distributed on T and B lymphocytes, fibroblasts, and epithelial cells, and have suggested that the apparently diverse biological activities of IL-1 are presumably mediated by its specific cell surface receptor(s). Although we have not yet determined whether the IL-1 receptor(s) is present on osteoblastic MC3T3-E1 cells, it is a very attractive proposal that some of the effects of IL-1 on DNA synthesis, cell growth, and/or alkaline phosphatase activity in MC3T3-E1 may result from interaction between IL-1 and its receptor on the cells.

Recent studies [1,14] have shown that both human and pig IL-1-like factors stimulate bone resorption. Interestingly, Dewhirst et al. [6] have indicated that human recombinant IL-1β is the major protein with osteoclast-activating factor activity produced by lectin-stimulated peripheral blood mononuclear cells. Therefore, it is clear from these findings that IL-1 plays a regulatory role in the bone remodelling process.

The role played by IL-1 in the regulation of bone remodeling is unclear. However, further studies employing recombinant IL-1 and these MC3T3-E1 clonal osteoblasts should lead to a better understanding of the regulatory mechanisms underlying the bone remodeling process.

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