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INTERLEUKIN-1 DERIVED FROM HUMAN MONOCYTIC LEUKEMIA CELL LINE JOSK-I ACTS AS AN AUTOCRINE GROWTH FACTOR

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SUMMARY: Interleukin-1 (IL-1) enhances the growth of human monocytic leukemia cell line JOSK-I cells, which were recently established in our laboratory and which were demonstrated to produce a high level of IL-1 constitutively, in liquid as well as semisolid culture systems. Concomitantly, IL-1 stimulated the prostaglandin E2 synthesis and nitroblue tetrazolium dye-reducing capacity of JOSK-I cells. This indicates that IL-1 may act as autocrine growth factor for monocytes, and also suggests the possibility that this autocrine stimulation may play an important role in the pathophysiology of monocytic leukemia in vivo.

Interleukin-1 (IL-1) is a glycosylated polypeptide mediator mainly produced by monocytes or B-lymphocytes, which plays many roles in host defense mechanisms (1,2), and has a wide variety of effects on various cells which are involved in immune and inflammatory systems (3). It is well known that IL-1 acts as a growth factor for thymocytes (4) or fibroblasts (5), and some authors reported that IL-1 also stimulated monocytes, i.e., it enhanced their prostaglandin (PG) synthesis (6), chemotaxis (7) or cytotoxic activity (8) in an autostimulatory manner.

JOSK-I is a monocytic leukemia cell line established from peripheral blood of a patient with acute myelomonocytic leukemia. The cells possess many monocytic features and constitutively produce a high level of IL-1 when

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Abbreviations used: IL-1, interleukin-1; PG, prostaglandin; CSF, colony stimulating factor; FCS, fetal calf serum; NBT, nitroblue tetrazolium dye; LPS, lipopolysaccharide ; hrIL-1, human recombinant interleukin-1β.

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they are appropriately incubated at the saturation density, as previously reported (9). JOSK-I-derived IL-1 was found to have molecular weights of 14K and 30K, and exhibited two isoelectric points of pI 6.9 and pI 5.9 on chromatofocusing on a DEAE-5PW column (10). Neither interleukin-2 nor colony stimulating factor (CSF) activity was detected in the conditioned medium of JOSK-I cells.

Here we describe that IL-1 enhances the growth of JOSK-I cells as autocrine growth factor.

MATERIALS AND METHODS

JOSK-I Cell Line and Liquid Culture: JOSK-I is a human monocytic leukemia cell line, which possesses many monocytic features and produces a high level of IL-1 constitutively (9). The cells were seeded at an initial concentration of $5x10^4$ cells/ml in RPMI 1640 medium (Flow Laboratories, McLean, VA) supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum (FCS)(Commonwealth Serum Laboratories, Melbourne, Australia), in Falcon 3047 or 3915 tissue culture plates (Becton Dickinson Labware, Oxnard, Calif.), and then grown in the absence or presence of varying amounts of IL-1 at 37°C under a humidified atmosphere of 5% carbon dioxide. Cell growth was monitored by determining the cell concentration after given culture periods, and viability was checked by means of the Erythrosine B dye-exclusion test. As a control, the growth of human promyelocytic leukemia cell line HL-60 cells was examined in exactly the same way.

Semisolid Culture: The effect of IL-1 on JOSK-I cells in a semisolid culture system was examined according to the method of Minden et al. with some modifications (11). Briefly described, given numbers of cells were plated in a mixture consisting of 0.9% methylcellulose (Methocel A-4M, standard; Dow Chemical Co.) in RPMI 1640 medium containing 20% heat-inactivated FCS in the absence or presence of varying amounts of IL-1. One ml aliquots of the cell mixture were plated in 10x30 mm plastic Petri dishes (No.1008; Falcon), and then incubated for 10 days at 37°C under a humidified atmosphere of 5% carbon dioxide. The number of colonies consisting of more than 40 cells was determined under an inverted microscope.

IL-1 Preparation: Partial purification of JOSK-I-derived IL-1 was performed by high-performance liquid chromatography on HPHT hydroxylapatite and TSK gel G3000SW columns. After the gel filtration step, the specific activity was $1.1x10^4$ U/mg protein of IL-1 (10). One unit of activity was defined according to Mizel et al (12). We performed the experiment using partial-purified JOSK-I-derived IL-1 and human recombinant IL-1β with a specific activity of $2x10^7$ U/mg of protein, which was kindly provided by Otsuka Pharmaceutical Co. (Tokushima, Japan).

Assay for NBT-Reducing Capacity: For measurement of one of the monocyte functions, quantitative assaying of nitroblue tetrazolium dye (NBT)-reduction was performed according to the method of Schopf, R.E. et al. with some modifications (13). Briefly, the assay mixture consisted of 50 μl of the NBT working solution (270mM NaCl, 10.4mM Na_2HPO_4 and 3.16mM KH_2PO_4) and 50 μl of IL-1 solution in a 96-well microplate. A non-opsonized zymosan solution (final, 330 μg/ml; Sigma Chemical Co., St. Louis) and RPMI 1640 medium were used as positive and negative controls, respectively. Fifty μl of a

JOSK-I cell suspension adjusted to $2x10^6$ cells/ml was added to the assay mixture at the beginning of the incubation, and then incubated for 60 min at 37°C. The reaction was stopped by adding 20 μl of a 1M HCl solution, and then the cells were spun down at 600xg for 15 min and the supernatant was carefully decanted. The reduced NBT was extracted with 100 μl of dimethyl sulfoxide (Wako Chemical Co., Tokyo, Japan) after the cell pellets had been dried overnight at 37°C. The absorbance at 550 nm was measured with a spectrophotometer.

Assay for PGE_2 Production: PGE_2 concentrations were determined by the means of a radioimmunoassay using a radioimmunoassay kit (NEK-020; New England Nuclear, Boston, MA). The antibody used shows the following cross-reactivities; PGE_2:100%, PGE_1:3.7% and PGA_1, PGB_2 and PGD_2:0.001%. Samples were prepared according to the method of Khansari, N. et al (14). Briefly, JOSK-I cells were plated at $1x10^6$ cells/well in 24-well culture plates (No. 25820; Corning, NY) in 1 ml of RPMI 1640 medium with 1% heat-inactivated FCS in the absence or presence of varying amounts of IL-1 or lipopolysaccharide (LPS)(final, 20 μg/ml; Escherichia coli, 055:B5; Sigma). Conditioned media were prepared after 24 hr-incubation and then their PGE_2 contents were determined.

Evaluation of IL-1 Effect on DNA Synthesis: Effect of IL-1 on [^{3}H]thymidine incorporation by JOSK-I cells was determined according to the method of Lusis and Koeffler, originally described for the rapid microassay for CSF activity using KG-1 cells (15). The cells were seeded at $2x10^5$ cells per well in 200 μl of RPMI-1640 medium without FCS in 96-well tissue culture plate (Falcon 3915) in the absence or presence of varying amounts of IL-1, and then incubated for 20 hr. The cells were labeled for 2 hr with [^{3}H]thymidine (10 μCi/ml; Amersham Japan, Tokyo, Japan) and the incorporated radioactivity was determined after collecting on glass-fiber filters with an automatic cell harvester.

RESULTS

As shown in Fig.1a, the growth of JOSK-I cells was enhanced in the presence of JOSK-I-derived IL-1, although the growth of HL-60 cells was not affected by the addition of IL-1 at all. This JOSK-I growth enhancement was maximal at 50 U/ml of IL-1, and a lower (10 U/ml) or higher (100 U/ml) IL-1 concentration was less stimulatory. Because the IL-1 produced by JOSK-I cells was predominantly IL-1 β (10), we performed the above experiment using human recombinant IL-1 β (hrIL-1). As shown in Fig.1b, the growth of JOSK-I cells was also significantly enhanced by hrIL-1, the optimal dose being 50 U/ml. To confirm the enhancing effect of IL-1 on the JOSK-I cell growth, we examined whether or not IL-1 could stimulate [^{3}H]thymidine incorporation by JOSK-I cells. As shown in Table 1, [^{3}H]thymidine uptake was increased by approximately 3-fold in the presence of the optimal dose of IL-1, whereas FCS could stimulate the uptake only by nearly 50% over the control in the serum-free condition.

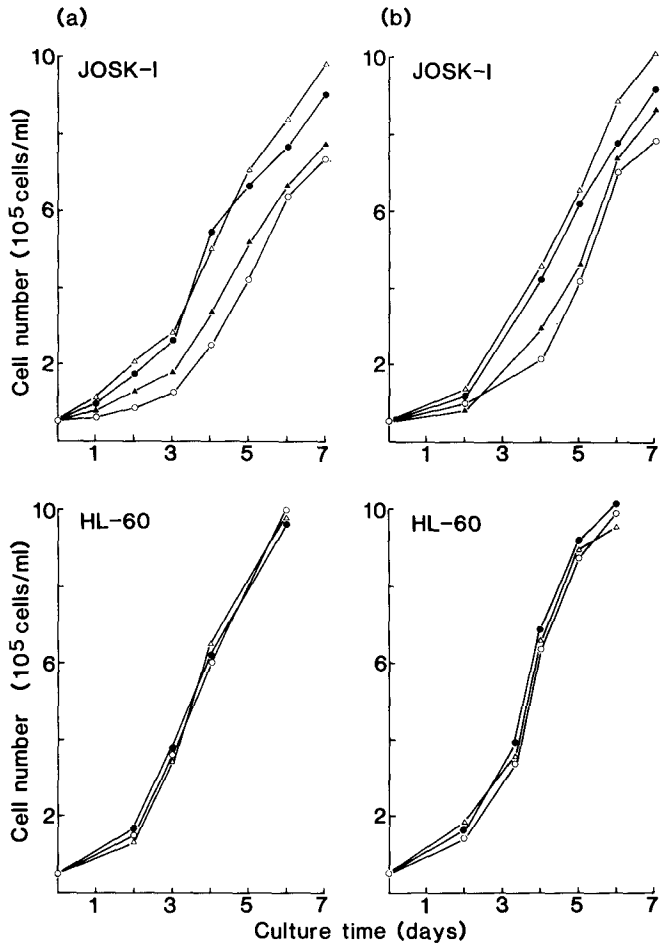

Fig. 1. Interleukin-1 response of JOSK-I cells in a liquid culture system. The cells were seeded as described in MATERIALS AND METHODS, and grown in the absence (-○-) or presence of 10 U/ml (-●-), 50 U/ml (-△-) and 100 U/ml (-▲-) of (a) JOSK-I-derived IL-1 or (b) human recombinant IL-1β. The dose of IL-1 added was adjusted by means of the conventional thymocyte co-mitogenic assay (25). Cell growth was monitored by determining the cell concentration after given culture periods. Values represent the means of triplicate determinations in two separate experiments. Standard errors of means were always less than 10%.

Next, we examined the effect of IL-1 on JOSK-I cells in a semisolid culture system. As shown in Fig.2a, the plating efficiency of 10-day cultures ranged from 0.5% with $1x10^2$ cells to 8.0% with $1x10^3$ cells, when no stimulants were added. IL-1 stimulated the colony formation of JOSK-I cells in a dose dependent manner, i.e., the plating efficiency ranged from 4.5% with $1x10^2$ cells to 26.7% with $1x10^3$ cells in the presence of 10 U/ml of IL-1, and from 11.5% with $1x10^2$ cells to 49.9% with $1x10^3$ cells in the presence

Table 1. Effect of IL-1 on DNA synthesis by JOSK-I cells

Additions	[^{3}H]thymidine incorporation	Stimulation index
None	6098.2 ± 85.4	1.0
10% FCS	9041.7 ± 611.0	1.48
IL-1 5 U/ml	10005.5 ± 374.0	1.64
10 U/ml	15541.2 ± 1803.9	2.54
50 U/ml	18228.6 ± 844.6	2.99
100 U/ml	14584.5 ± 6311.7	2.40
250 U/ml	9936.4 ± 165.2	1.62

The cells were seeded as described in MATERIALS AND METHODS, and incubated for 20 hr. Then the cells were labeled for 2 hr with [^{3}H]thymidine and the incorporated radioactivity was determined. The values represent the means ± SD of triplicate samples.

of 50 U/ml of IL-1. The hrIL-1 also enhanced the colony formation, but the extent of enhancement was less than that with JOSK-I-derived IL-1 (Fig.2b). However, the colony formation of HL-60 cells was not affected at all by the addition of IL-1 (data not shown).

We further examined whether or not IL-1 could enhance the PGE_2 production and NBT reducing activity of JOSK-I cells. As shown in Fig.3a, when stimu-

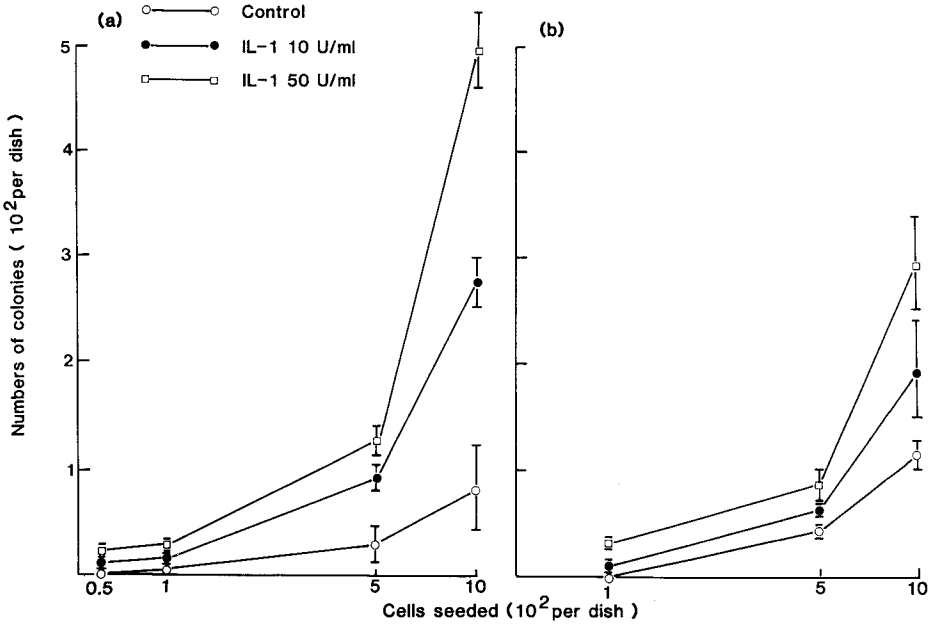

Fig. 2. Colony formation of JOSK-I cells in the absence (-○-) or presence of 10 U/ml (-●-) and 50 U/ml (-□-) of (a) JOSK-I-derived IL-1 or (b) human recombinant IL-1β. After the cell mixtures were incubated for 10 days, the number of colonies consisting of more than 40 cells was determined under an inverted microscope.

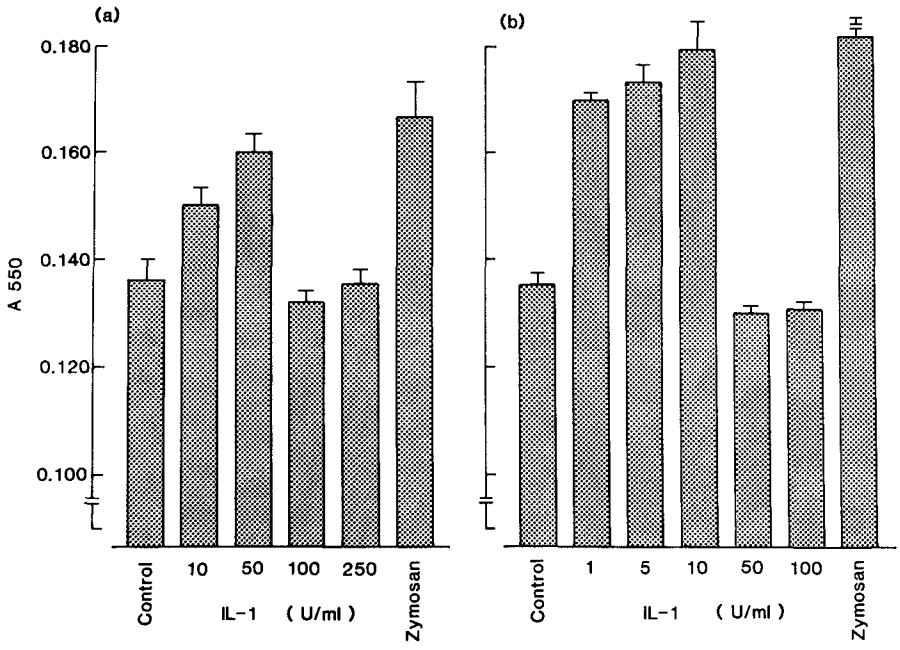

Fig. 3. NBT-reducing activity of JOSK-I cells was determined in the presence of varying amounts of (a) JOSK-I-derived IL-1 or (b) human recombinant IL-1β. A non-opsonized zymosan solution and RPMI 1640 medium were used as positive and negative controls, respectively.

lated with 50 U/ml of JOSK-I-derived IL-1, the cells exhibited an about 20% increase in NBT reduction as compared with in the case of non-stimulated control cells. Human recombinant IL-1 also enhanced the NBT reduction, but it should be noted that the optimal concentration was lower than that in the case of JOSK-I-derived IL-1, and the extent of enhancement was as high as that with a well-known monocyte stimulator, zymosan (Fig.3b). No morphological changes indicating cellular differentiation were seen after the IL-1 treatment. PGE_2 production was markedly increased in the presence of 50 U/ml of IL-1, although the baseline production of PGE_2 by JOSK-I cells was relatively low and LPS failed to stimulate PGE_2 production (Fig.4).

DISCUSSION

Since the first description by Todaro et al. in 1978 (16), the autocrine growth concept for cancer cells has involved one of the mechanisms which explain the autonomous proliferation of transformed cells (17). Four poly-

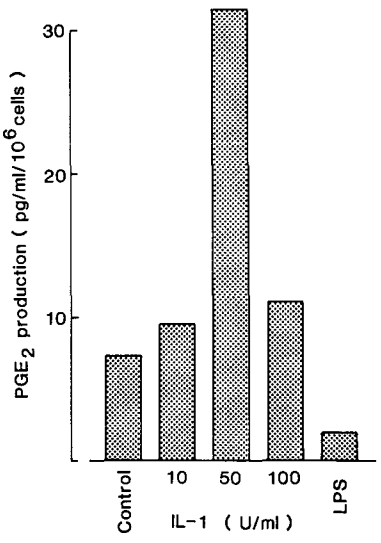

Fig. 4. PGE_2 production by JOSK-I cells. The cells were stimulated with IL-1 or LPS and PGE_2 contents in conditioned media were determined by the means of a radioimmunoassay. The results are expressed in picograms of PGE_2 produced per ml of the conditioned medium from $1x10^6$ cells.

peptides, the α and β transforming growth factors (18,19), platelet-derived growth factor (20) and bombesin (21), have so far been reported as autocrine growth factors. Here we have demonstrated that another polypeptide, IL-1, acts as an autocrine growth factor for a human monocytic leukemia cell line that produces the molecule constitutively. The results reported here indicate that the constitutive production of IL-1 might be closely related to the growth of JOSK-I cells. It should be noted that there was a slight difference in stimulatory ability between JOSK-I-derived IL-1 and hrIL-1. Some hypotheses have been presented to explain this difference, as follows; JOSK-I produces a molecule which has the same biological activity and antigenicity as the known IL-1s, but which is different in its affinity to JOSK-I cells, or some co-existing humoral factors modulate the action of IL-1. It is possible that IL-1 can promote autonomous proliferation of monocytic leukemic cells in vivo, since fresh leukemic cells from patients with monocytic leukemia can also produce IL-1, as we recently reported (22). Recently, some authors reported that IL-1 exhibited an antitumor activity toward some cancer cells (23,24), but our results suggest that care should be taken when IL-1 is used for cancer treatment in some instances.

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