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VARIABLES THAT REGULATE PRODUCTION OF INSULIN-LIKE PEPTIDE(S) IN HUMAN LEUKEMIA CELL LINE (HL-60)

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Summary: A human myeloid leukemia cell line (HL-60) produces a peptide or peptides with insulin-like activity which is distinct from insulin or insulin-like growth factors (somatomedins). Factors regulating the production of this peptide (HL-ILP) were explored in the present study. The production of HL-ILP was maximal in the early log phase of cell growth and declined with increasing cell density. Differentiation of HL-60 cells to macrophages, induced by dihydroxyvitamin D_3 or phorbol esters, was also associated with a decrease in HL-ILP production. Glucose consumption by the cells in the early log phase was closely related with HL-ILP production, and HL-ILP was found to stimulate glucose consumption by HL-60 cells. Production of HL-ILP was dependent on glucose concentrations in the culture medium and glucose concentrations higher than 1mg/dl suppressed the release of HL-ILP. These observations are not inconsistent with a hypothesis that HL-ILP is involved in the glucose metabolism of the HL-60 cells that produce this peptide.

Recently an "autocrine growth" hypothesis has been proposed by Todaro, DeLarco and others (1,2). They have suggested that the growth of tumor cells may be initiated and/or promoted by growth factors produced by the cells themselves. Although it is difficult to demonstrate this "autocrine growth system", well-recognized growth factors have been identified in the conditioned media of a variety of normal and malignant cells. The production of somatomedin(s) by human fibroblasts (3) and osteosarcoma (4), platelet-derived growth factor by sarcoma or glioma cells (5) and transforming growth factor by neuroblastoma cells (6) have been reported. In addition, a number of novel

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growth factors have been identified in the culture medium conditioned by neoplastic cells (7-9). We recently reported a peptide with potent insulin-like activity in the conditioned medium of a human leukemia cell line (HL-60) which is distinct from insulin (10). This peptide (Human leukemic cell derived insulin-like peptide, HL-ILP) stimulated ^{14}C-glucose uptake and lipogenesis in rat adipocytes. When subjected to gel-chromatography with Sephadex G-100, HL-ILP eluted in fractions corresponding to 10-12 K daltons. Although the functional significance of HL-ILP in HL-60 cells is at present unknown, in this report we have described the alteration in secretion of HL-ILP during the proliferation or differentiation of HL-60 cells and the relationship between HL-ILP production and glucose metabolism in HL-60 cells has also been discussed.

Materials and Methods

Hormones and chemicals: 1α,25 dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] was supplied by Chugai Pharmaceuticals (Tokyo), 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was purchased from Sigma Chemicals (St Louis, MO) and [D-3-^3H]Glucose (SA, 11.5 Ci/mmol) was a product of New England Nuclear (Boston, MA).

Cells and cell culture: HL-60 cells were cultured at 37°C in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with fetal calf serum (Boehringer Mannheim, W. Germany, lot 674603) in an atmosphere of 5% CO_2 in air. Unless otherwise noted, the cells were plated at a density of 10^5/ml in 75 cm^2 culture flasks. Under these conditions, the doubling time of HL-60 cells was 24 h. To induce HL-60 cells to differentiate to macrophages, 1,25$(OH)_2D_3$ or TPA was added to the culture medium. In some experiments the concentrations of glucose in the culture medium were adjusted to desired levels using RPMI-1640 select amine kits (Gibco). Glucose concentrations in the medium were determined by the Fuji DRI-CHEM analyzer system (11).

Partial purification of HL-ILP: HL-ILP was partially purified from the conditioned medium prior to the assay for bioactivity. The culture medium was centrifuged at 3000 rpm for 30 min to clarity, and the supernatant was concentrated by ultrafiltration with Amicon UM-05 membrane (nominal exclusion 5000 daltons). The concentrate was applied to CM-Sepharose column (Pharmacia, Uppsala) of 3.5 x 16 cm. HL-LIP was adsorbed to the column and was eluted with a linear gradient of sodium chloride of 0.05 M to 0.3 M in 50 mM MES-NaOH, pH 5.8. (The detailed procedure will be described elsewhere in a manuscript under preparation). This procedure was employed to remove small molecular weight substances such as glucose, amino acids and additives which may affect the bioassay for insulin-like activity.

Assay for insulin-like activity: Insulin-like activity of HL-ILP was determined according to the method of Moody et al (12). The activity of HL-ILP in the culture medium was expressed as unit/ml/10^5 cells,

where 1 mU was defined as the potency which cause 100% increment of [^{3}H]-glucose incorporation above basal in the bioassay.

Differentiation of HL-60 cells: The differentiation of HL-60 cells induced by 1,25 $(OH)_2$ D_3 or TPA was judged by cell morphology and by an increase of ^{125}I-insulin binding (13).

Results and Discussion

The production of HL-ILP was dependent on cell density, as shown in Figure 1. When the cells were plated at a density of 10 x 10^5 cells/ml, HL-ILP in the conditioned medium increased for 2 days and then declined with increasing cell density. This is similar to the observation reported by Clemmons and Shaw (14) that the production of somatomedin-C by human fibroblasts decreases at a high cell density. The mechanism by which high cell density reduces the production of HL-ILP is unknown; it may be due to a decrease in protein synthesis in high

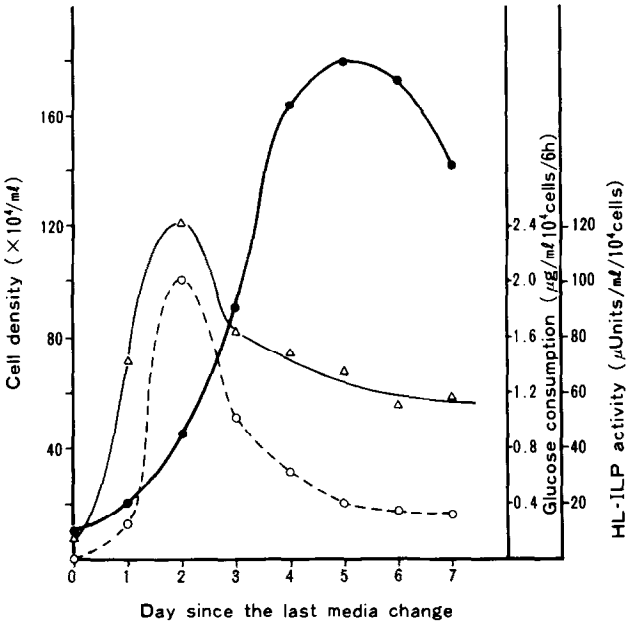

Fig. 1 Production of HL-ILP and glucose consumption by HL-60 cells.

HL-60 cells from stock cultures were plated at a density of 10^5 cells/ml and allowed to grow. Cells were counted by trypan blue exclusion. HL-ILP in the medium was determined as described in the text. Glucose consumption rate was calculated by the rate of reduction of glucose in the medium. This figure shows the cell density (●——●), glucose consumption (o---o), and HL-ILP activity (△——△). The values are the mean of 5 experiments.

density cultures. We examined the relationship between HL-ILP and glucose utilization by the cells, since the peptide has a potent insulin-like activity in rat adipocytes. Figure 1 also shows that the change in the rate of glucose consumption of HL-60 cells is very similar to that of HL-ILP production, both being maximal at day 2. This result suggests that HL-ILP production and glucose metabolism are closely related events, and accordingly, we studied whether glucose consumption by HL-60 cells is stimulated by HL-ILP itself. The results are presented in Table 1. It is clear that HL-ILP stimulates glucose consumption in a dose-related manner. Although fetal calf serum (FCS) also stimulated glucose consumption significantly, the effect of insulin was marginal.
The production of HL-ILP was, on the other hand, greatly affected by glucose concentrations in the culture medium (Fig.2). HL-ILP production was maximal in the absence of glucose and was reduced by more than 50% when glucose was added to achieve concentrations higher than 2 mg/ml, indicating that glucose concentration in the medium is one of the factors regulating HL-ILP production.

Table 1. Stimulation of glucose consumption by HL-ILP

Additives	Concentration	Glucose consumption rate ($\mu g/ml/10^4$ cells/2 h)
None		0.04 ± 0.01
FCS	10 %	0.80 ± 0.32
Insulin	50 ng/ml	0.08 ± 0.02
HL-ILP	5 μU/ml	0.06 ± 0.01
	50 μU/ml	0.21 ± 0.03
	500 μU/ml	0.62 ± 0.10

HL-60 cells were washed once with serum-free RPMI-1640 containing 1.92 mg/ml glucose, and resuspended in the same medium. A one-ml aliquot of the suspension adjusted to 5 x 10^5 cells/ml was added to flasks containing test samples of indicated concentrations. The mixture was then incubated at 37°C for 2 h. Glucose consumption rate was determined by the reduction of glucose levels in the culture medium. Data are the mean ± SEM of five determinations.

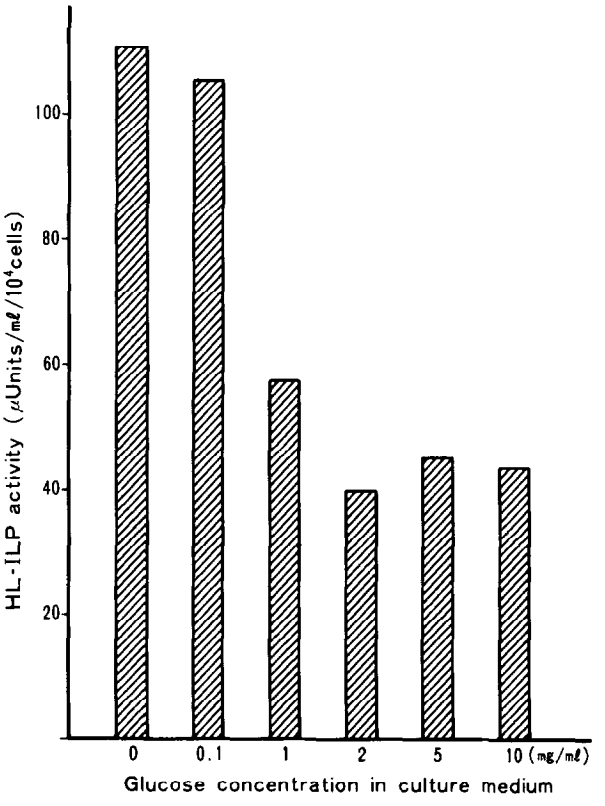

Fig. 2 Effect of glucose on the release of HL-ILP from HL-60 cells.

HL-60 cells (2×10^5 cells/ml) were cultured for 2 days in RPMI-1640 medium supplemented with indicated concentrations of glucose as described in Materials and Methods.

Finally we examined the effect of cell differentiation on HL-ILP production. As previously reported by others (15), $1,25(OH)_2D_3$, as well as TPA (16) - a potent tumor promoter- suppressed growth of HL-60 cells and induced the cells to differentiate to macrophage-like cells, as judged by morphology and by an increase of insulin binding activity (13). As shown in Table 2, the cells treated with either agent produced significantly less HL-ILP compared to the non-treated cells. The effect of $1,25(OH)_2D_3$ and TPA was dose-dependent and inversely related to the percentage of the differentiated cells (data not shown). Thus, it appears that the maturation of HL-60 cells is associated with a decrease in HL-ILP production, whether differentiation is achieved either by $1,25(OH)_2D_3$ or TPA.

Table 2. Suppression of HL-ILP production by differentiation of HL-60 cells induced by $1,25(OH)_2D_3$ and TPA

Additives	Concentration (M)	HL-ILP in medium (μ units/ml/10^4 cells)
None		85 ± 17
$1,25(OH)_2D_3$	10^{-10}	72 ± 11
	10^{-9}	45 ± 8
	10^{-8}	17 ± 7
TPA	10^{-10}	41 ± 8
	10^{-9}	14 ± 5
	10^{-8}	11 ± 3

HL-60 cells were plated at a density of 4 x 10^5 cells/ml and incubated for 2 days in the presence of indicated concentrations of $1,25(OH)_2D_3$ or TPA. HL-ILP in the medium was measured as described in the text. Values are the mean ± SEM of five determinations.

The present study showed that HL-ILP production was affected by a number of factors. The HL-ILP production was maximal in the early log phase of cell growth and was suppressed with increasing cell density or cell differentiation. Together with our unpublished observations that HL-ILP stimulates DNA synthesis of HL-60 cells, the present observations suggest that HL-ILP possibly plays a role in the growth of HL-60 cells. In addition, we have shown that the glucose consumption by HL-60 cells is closely related to the production of HL-ILP. The biological significance of this phenomenon at present remains to be determined. It may be that HL-ILP itself is involved in the regulation of glucose metabolism in HL-60 cells. The stimulation of glucose conusumption by HL-ILP would seem to support this hypothesis. However, further studies are required to establish that HL-ILP acts as an autoregulator in glucose metabolism as well as in cell growth.

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