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DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS IS ACCOMPANIED BY AN INCREASE IN INSULIN RECEPTORS

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SUMMARY: Changes in insulin receptors accompanying cell differentiation in human promyelocytic leukemia cells (HL-60) were studied. Cell differentiation was induced by 1α,25-dihydroxyvitamin D_3, vitamin A, dimethyl sulfoxide, or phorbol esters. 1α,25-dihydroxyvitamin D_3 increased the ability of HL-60 cells to bind insulin in a dose-dependent manner. The increase in insulin binding was due to an increase in the number of insulin receptors. Vitamin A, dimethyl sulfoxide and phorbol esters were also effective in increasing insulin receptors. Thus, the differentiation of HL-60 cells was accompanied by an increase in insulin receptors.

Introduction

The human promyelocytic leukemia cell line (HL-60) can be induced to differentiate to macrophages or granulocyte-like cells by a number of reagents including phorbol esters (1), retinoic acid (2), dimethyl sulfoxide (3), and 1α,25-dihydroxyvitamin D_3 (4), and serves a useful model of *in vitro* cell differentiation. The changes of cell membranes accompanying cell differentiation have been demonstrated in a number of cells. Recently, Gahmberg *et al.* (5) reported that the cell surface glycoproteins of HL-60 cells alter in association with differentiation induced by dimethyl sulfoxide. They showed the loss of major glycoprotein (Molecular Weight 160,000) and the appearance of the MW 130,000 species, which correlated with the appearance of phagocytic and chemotactic

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activity. Recently, we have found receptors on HL-60 cells with a high affinity and specificity for insulin. Insulin receptors have now been characterized as glycoproteins in various tissues. We inferred that insulin receptors might be altered during the differentiation of HL-60 cells. It has been reported that differentiation of 3T3-L1 preadipocytes into mature adipocytes is accompanied by a significant increase in insulin receptors (6). By contrast, differentiation induced by dimethyl sulfoxide was associated with a decrease in insulin receptors in Friend leukemia cells (7). In the present study, we show that the insulin receptor of HL-60 cells increases in association with differentiation to granulocyte-like cells or macrophages.

Materials and Methods

Hormones and Chemicals: 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], 1α-hydroxyvitamin D_3, and 24,25-dihydroxyvitamin D_3 were supplied by Chugai Pharmaceuticals (Tokyo). Monocomponent porcine insulin was from Ely Lilly (Indianapolis, IN), and [^{125}I]-insulin was prepared by the chloramin T method modified as reported (8) to a specific activity of 100 mCi/mg. Retinoic acid and 12-*O*-tetradecanoylphorbol-13-acetate[TPA] were purchased from Sigma Chemicals (St Louis,MO).

Cells and Cell Culture: HL-60 cells were provided by Dr.S.Sato, the National Cancer Research Institute, Tokyo. Cells were cultured at 37°C in RPMI-1640 medium (Gibco., Grand Island,NY) supplemented with 10 % fetal calf serum [FCS] in a humidified atmosphere of 5 % CO_2 in air. All experiments were performed using cells in the late log phase of growth.

Determination of insulin binding: Cells were allowed to grow to a density of 1.5 x 10^6/ml in 10 ml of medium in 75 cm^2 culture flasks (Falcon). The cells were cultured for 48 or 72 h in the presence or absence of vitamin D_3 or other reagents. The cells were washed once with the medium, and suspended in Hepes binding buffer (9). An aliquot of the suspension (10^6cells per tube) was incubated with [^{125}I]-insulin (100,000 cpm) for 18 h at 4°C in the presence or absence of 1 μg/ml of unlabelled insulin in a total volume of 1 ml of Hepes binding buffer containing 0.5 % bovine serum albumin (BSA) and 1 mg/ml Bacitracin. Then the tubes were centrifuged and the pellet was counted to determine the specific binding of ^{125}I-insulin.
Morphological examination: Cytospin slide preparation were stained with Wright-Giemsa, and differential counts were performed under a light microscope at a minimum of 200 cells for each preparation. Cell viability was monitored by Trypan blue exclusion.

Results and Discussion

Confirming a recent report by Miyaura *et al.*(4), 1α,25(OH)$_2$D$_3$ caused a significant morphological alteration of HL-60 cells. Most of the untreated cells were promyelocytes with a large nucleus and basophilic cytoplasma. Culture with 1α,25(OH)$_2$D$_3$ resulted in the appearance of more mature cells with prominent granules in less basophilic cytoplasma. The nuclear/cytoplasmic ratio decreased significantly. The appearance of mature types of cells (myelocyte- or metamyelocyte-like cells) was dependent upon the concentration of 1α,25(OH)$_2$D$_3$ as shown in Fig.1 (left panel). It can be seen that the ability of HL-60 cells to bind [^{125}I]-insulin increased by culture with 1α,25(OH)$_2$D$_3$ in a dose-dependent manner (Fig.1 right panel). The dose-response curve for inducing cell differentiation was similar to that of enhancement in insulin binding. The effect of 1α,25(OH)$_2$D$_3$ was detected at a concentration as low as 10^{-10}M, and binding was increased by 3-fold at 10^{-8}M.

Analogs of vitamin D$_3$ were also tested for their ability to increase insulin binding (Fig.2). 1α-hydroxyvitamin D$_3$ [1α(OH)D$_3$] was approximately one-tenth as potent as 1α,25(OH)$_2$D$_3$, and 24,25-

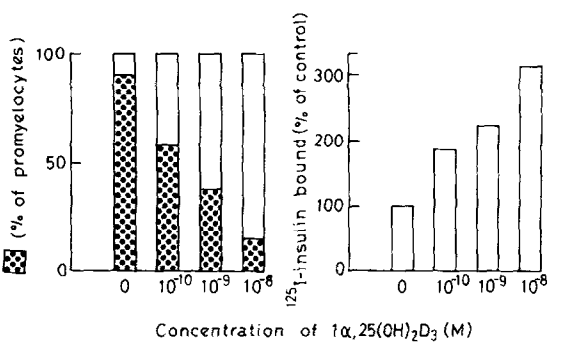

Fig.1. Effect of 1α,25(OH)$_2$D$_3$ on differentiation and the ability of HL-60 cells to bind [^{125}I]-insulin. HL-60 cells were cultured in the absence or presence of 1α,25(OH)$_2$D$_3$ at concentrations indicated. 48 hours later, the cells were examined for morphological alteration (left panel) and [^{125}I]-insulin binding (right panel). Specific binding of [^{125}I]-insulin was expressed as percentage of binding in untreated cells (the mean of triplicate determinations).

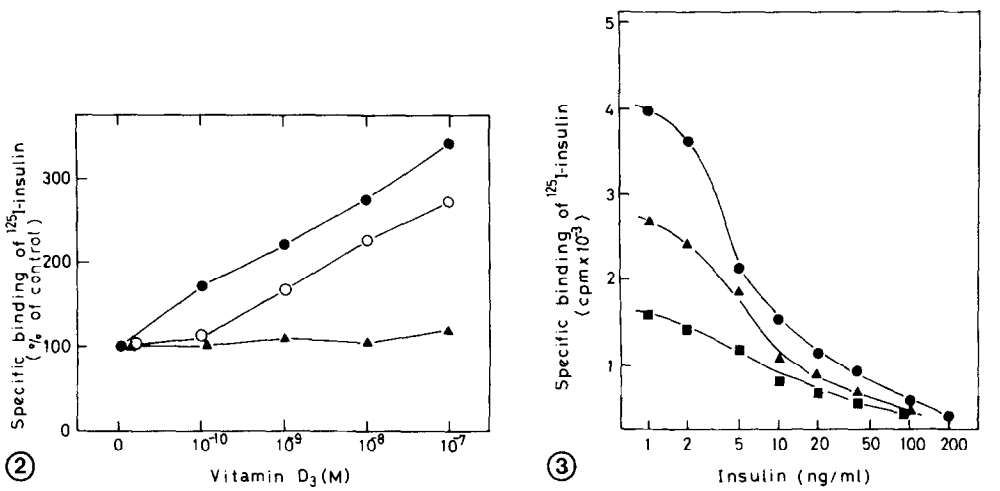

Fig.2. Effect of vitamin D_3 derivatives on insulin binding. HL-60 cells were incubated for 48 h in the absence or presence of various concentrations of 1α,25(OH)$_2$D$_3$ [●], 1α(OH)D$_3$ [○] or 24,25-(OH)$_2$D$_3$ [▲], and the specific binding of [^{125}I]-insulin was determined. Each point represents the percentage of binding in untreated cells (the mean of triplicate determinations).

Fig.3. Insulin binding of HL-60 cells treated with 1α,25(OH)$_2$D$_3$. HL-60 cells were cultured for 48 h in the absence [■] or presence of 10^{-9} [▲] or 10^{-8} M [●] of 1α,25(OH)$_2$D$_3$. Then the specific binding of [^{125}I]-insulin to 10^6 cells was determined in the presence of various concentrations of unlabelled insulin. The number of insulin receptors on the untreated cells was approximately 2000 per cell.

dihydroxyvitamin D_3 [24,25(OH)$_2$D$_3$] was without effect. The potencies of these derivatives in increasing insulin binding were parallel to their ability to induce differentiation (data not shown).

The nature of the increase in insulin binding by 1α,25(OH)$_2$D$_3$ was studied in more detail. The increase in [^{125}I]-insulin binding was demonstrated in the presence of various concentrations of unlabelled insulin (Fig.3). Scatchard analysis of the data revealed that the increase of [^{125}I]-insulin binding was primarily due to an increase in the number of insulin receptors, but was not due to a change of affinity. As shown in Fig.4, an increase of insulin binding was dose-dependently prevented when varying concentrations of cycloheximide was included in the culture medium. Cell differentiation was inhibited in a similar manner (data not shown). These observations indicate that protein synthesis is required for the increase of insulin receptors.

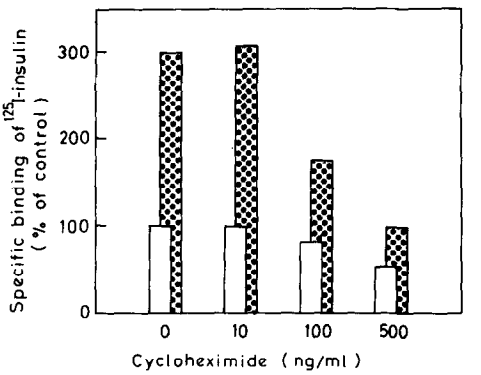

Fig.4. Effect of cycloheximide on vitamin D_3-induced increase of insulin binding. HL-60 cells were cultured with [dotted column], or without [open column] 10^{-8}M 1α,25(OH)$_2$D$_3$ for 48 h in the presence or absence of cycloheximide. Then specific binding of [^{125}I]-insulin was determined. Data are expressed as percentage of binding in control cells (the mean of triplicate determinations).

We were next interested in determining whether insulin receptors would increase by treating cells with other compounds known to induce differentiation of HL-60 cells. Table 1 summarizes the results. Retinoic acid induced HL-60 cells to differentiate to myelocyte- or metamyelocyte-like cells (data not shown) as reported by others (2), and it increased the number of insulin receptors in a dose-dependent manner. Dimethyl sulfoxide showed a similar effect. TPA, a potent cocarcinogen has been reported to induce HL-60 cells to differentiate to macrophages (1). Interestingly, TPA was also able to increase insulin binding. By contrast, an inactive form of phorbol esters PDA (4β-phorbol 12β,13α-diacetate) had no effect on either cell differentiation or insulin receptors.

These observations strongly suggest that differentiation of HL-60 cells into either granulocytes or macrophages is accompanied by an increase in the number of insulin receptors. The functional significance of the increase of insulin receptors is not clear at present. The differentiation of 3T3-L1 preadipocytes is accompanied by a 20-fold increase in the number of insulin receptor (10), and the differentiated cells (adipocytes) acquire an increased sensitivity to insulin (11,12). Our present findings may support

Table 1. Effect of retinoic acid, dimethyl sulfoxide or TPA on insulin binding of HL-60 cells.

Reagents added	Concentrations	[^{125}I]-insulin binding (% of control)
None		100
Retinoic acid	10^{-8} M	103
	10^{-7} M	124
	10^{-6} M	150
	10^{-5} M	205
Dimethyl sulfoxide	0.1 %	103
	1.0 %	130
TPA	10^{-7} M	353
PDA	10^{-7} M	98

HL-60 cells were cultured with compounds indicated for 72 h and the specific binding of [^{125}I]-insulin was determined. The values are percentage of the binding in untreated control cells (the mean of triplicate determinations).

the hypothesis that differentiated HL-60 cells develop sensitivity to the yet unrecognized metabolic effect(s) of insulin on the cells.

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