Decrease in IGF-I Binding Sites on Human Promyelocytic Leukemia Cell Line (HL-60) with Differentiation

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Abstract

Specific insulin-like growth factor I (IGF-I) receptors on human promyelocytic leukemia cell line (HL-60) were identified and characterized. [125 I]IGF-I specifically bound to the cells, and [125 I]IGF-I binding to the cells was displaced by unlabeled IGF-I in a dose dependent manner. [125 I]IGF-I binding to the cells were displaced by multiplication stimulating activity (MSA) and porcine insulin, with potencies that were 10 and 100 times less than that of IGF-I, respectively. By an affinity labeling technique, IGF type I receptors were found to be present on the HL-60 cells.

After the cells were differentiated to the macrophage-like cells by 12-o-tetra-decanoyl-phorbol-13-acetate (TPA) and 1, 25-dihydroxy-vitamin D₃ (1, 25(OH)₂D₃), [125 I]IGF-I binding to the cells decreased significantly. By Scatchard analysis, it was found to be due to a decrease in the number of IGF-I receptors.

Thus, the differentiation of HL-60 cells to the macrophage-like cells was accompanied by a decrease in IGF-I receptors.

The HL-60 cells can be selectively induced to mature to granulocytes or macrophage-like cells by culture with a number of reagents; i.e. the differentiation to granulocyte is induced by retinoic acid (Reiss *et al.*, 1985), butyric acid (Fischkoff and Con-

don, 1985) and dimethyl sulfoxide (DMSO) (Collins *et al.*, 1978), and the differentiation to macrophage is induced by phorbol esters (Rovera, 1979) and vitamin D₃ (Reitsma *et al.*, 1983).

The number of transferrin receptors (Yeh *et al.*, 1982) and β -adrenergic receptors (Hoshino *et al.*, 1986) on HL-60 cells increased with differentiation. Yamanouchi *et al.* (1982) reported that differentiation of HL-60 cells into granulocytes or macrophage-like cells was accompanied by an increase in the number of insulin receptors. Specific receptors for insulin and IGF-I were

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located in a variety of cells and both receptors coexist in most cells (Nissley and Rechler, 1984).

In the present study, we investigated IGF-I receptors on the HL-60 cells and the change in IGF-I receptor sites with the differentiation to macrophage-like cells.

Materials and Methods

Hormones and reagents

IGF-I was kindly provided by Fujisawa Pharmaceutical Co. (Osaka). The IGF-I preparation was synthesized by recombinant DNA technology as described (Niwa *et al.*, 1986), and the amino acid sequence of the preparation is the same as that of natural IGF-I. Crystalline porcine insulin and MSA were gifts from Eli Lilly Co. (Indianapolis, IN) and from Dr. M. Kasuga, the University of Tokyo (originally obtained from Dr. M. P. Czech (Worcester, MA)), respectively. Na¹²⁵I was purchased from New England Nuclear (Boston, MA). Both [¹²⁵I]IGF-I and [¹²⁵I]insulin were prepared by the chloramine-T method modified as reported (Zapf *et al.*, 1975; Roth, 1975) with a specific activity of 180 $\mu\text{Ci}/\mu\text{g}$ and 100 $\mu\text{Ci}/\mu\text{g}$, respectively. TPA was purchased from Sigma Chemicals (St Louis, MO), and 1,25(OH)₂D₃ was kindly provided by Chugai Pharmaceuticals (Tokyo). Disuccinimidyl suberate (DSS) was purchased from Pierce Chemical Co. (Rockford, IL).

Cells and cell differentiation

Cultured HL-60 cells were provided by Dr. T. Yamanouchi of the University of Tokyo. The cells were grown at 37°C in RPMI-1640 medium (GIBCO, Grand Island, NY), containing 10% fetal calf serum (FCS) and under 5% CO₂ atmosphere. TPA dissolved in acetone or 1,25(OH)₂D₃ dissolved in ethanol was added to the cell suspension at a final concentration of 10 ng/ml or 2.0 $\times 10^{-9}$ M, respectively. Control cultures contained equivalent amounts of the solvents. Cytospan slide preparations of 0.1 ml aliquots of suspension were prepared using a Shandon-Elliot Cytospin centrifuge (model SCA-0031, Cheshire, England), and the cells were stained with May-Grünwald-Giemsa.

Binding studies for IGF-I and insulin

The HL-60 cells were allowed to grow to a concentration of 5 $\times 10^5$ cells/ml in culture flasks and centrifuged at 400 $\times g$ for 5 min. The cells were resuspended in RPMI-1640 medium containing 10% FCS, and then the cells were cultured for 24 h or 48 h in the presence or absence of TPA or 1,25(OH)₂D₃, respectively. At the end of the incubation, the cells were washed once with assay buffer (50 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES) lymphocyte buffer with 1% bovine serum albumin (BSA)), and suspended in the same buffer. The cells (1 $\times 10^7$ cells/ml) were incubated with [¹²⁵I]-IGF-I in the presence or absence of IGF-I in a total volume of 0.5 ml. The cell viability examined by the trypan blue exclusion method was greater than 95% in the assay buffer for 48 h. At the end of the incubation, replicate 200 μl aliquots were layered onto ice-cold buffer and centrifuged for 8000 $\times g$ in a Beckman B Microfuge (Beckman, Palo Alto, CA). After aspiration of the supernatant, the cell-associated radioactivity was counted. Nonspecific binding was defined as cell-associated radioactivity in the presence of an excess of unlabeled IGF-I (1 $\mu\text{g}/\text{ml}$). Specific binding is the difference between total binding and nonspecific binding. The binding study for porcine insulin was carried out similarly to that for IGF-I.

Cross-linking of [¹²⁵I]IGF-I to HL-60 cells

The HL-60 cells, 5 $\times 10^6$ cells/ml, were incubated in 50 mM HEPES lymphocyte buffer with 0.1% BSA at 4°C overnight with [¹²⁵I]IGF-I in the presence or absence of unlabeled IGF-I (1 $\mu\text{g}/\text{ml}$). The cells were washed in ice-cold buffer without albumin and resuspended in the same buffer. DSS, dissolved in DMSO, was added at a final concentration of 1 mM. After 30 min, the reaction was stopped by 10 mM Tris-(hydroxymethyl) aminomethane and 1 mM EDTA. The cells were solubilized in 50 mM HEPES buffer containing 1% Triton X-100, 0.15 M NaCl and 2 mM phenylmethylsulfonyl fluoride for one hour at 4°C and centrifuged at 100,000 $\times g$ for 45 min. The supernatant was precipitated with ice-cold 10% trichloroacetic acid and the pellet was boiled for 5 min in 2% sodium dodecyl sulfate (SDS), 10% (v/v) glycerol, 0.5 M 2-mercaptoethanol, 0.01% bromophenol blue and 10 mM sodium phosphate buffer, pH 7.0. Samples were analyzed on 0.1% SDS/7.5% polyacrylamide gel electrophoresis according to

Laemmli (1970). Autoradiography was carried out using Kodak X-Omat film with an intensifying screen.

Results

Binding of [¹²⁵I]IGF-I to the HL-60 cells

[¹²⁵I]IGF-I specifically bound to the HL-60 cells, and the binding increased as a function of the cell concentration. The binding of [¹²⁵I]IGF-I to the cells was time and temperature dependent (Fig. 1). At

37°C, the maximal binding which occurred at 30 min was greater than that at 15°C and 4°C, but declined rapidly and the condition was too unstable for the binding study. At 15°C, there was a slower association of [¹²⁵I]IGF-I with the cells, and the maximal binding occurring at 2 h. At 4°C, the binding reached a plateau by 18 h, and the maximal binding was the same as that at 15°C. From these data, the binding studies were performed at 15°C for 2 h. At a cell concentration of 1×10^7 cells/ml, the specific binding of [¹²⁵I]IGF-I averaged 8%.

Fig. 1. Effect of time and temperature on specific [¹²⁵I]IGF-I binding to the HL-60 cells. The cells (5×10^6 cells/ml) were incubated at 37°C, 15°C and 4°C for varying times.

Fig. 2. Competition for specific [¹²⁵I]IGF-I binding to the HL-60 cells (8×10^6 cells/ml) by IGF-I, MSA and porcine insulin.

The nonspecific binding was less than 2%.

Specificity of IGF-I receptor

[¹²⁵I]IGF-I binding to the HL-60 cells was displaced by unlabeled IGF-I in a dose dependent manner (Fig. 2). A 50% inhibition of [¹²⁵I]IGF-I binding was obtained at a concentration of 2 ng/ml IGF-I. The binding of [¹²⁵I]IGF-I to the cells was displaced by MSA and porcine insulin, with potencies 10 and 100 times less than that of IGF-I, respectively (Fig. 2). In addition, [¹²⁵I]insulin specifically bound to the cells, and IGF-I inhibited insulin binding with a potency at least 100 times lower than that of insulin (data not shown). When the HL-60 cells were cross-linked to [¹²⁵I]IGF-I with DSS, solubilized and electrophoresed in the presence of reductant, the major band migrated at Mr=130,000. An excess of unlabeled IGF-I blocked the formation of this band (Fig. 3).

Changes in morphology of HL-60 cells induced by reagents

The morphological changes induced by TPA in the HL-60 cells are illustrated in Fig. 4. When untreated, HL-60 cells were predominantly typical promyelocytes with large round nuclei, basophilic cytoplasm with azurophilic granules, and the nuclear/cytoplasmic (N/C) ratio was relatively high. The addition of TPA at a final concentration of 10 ng/ml to the culture for 24 h induced striking morphological differentiation of monocyte or macrophage-like cells in the majority of the cells. The morphological changes in the cells were dramatic; the azurophilic granules disappeared and the N/C ratio became small. Furthermore, the cell changed similarly to that of TPA and the most striking change was observed at 48 h incubation with 1,25(OH)₂D₃.

Changes in IGF-I receptors with cell differentiation

Fig. 5 shows [¹²⁵I]IGF-I binding to the

Fig. 3. Cross-linking of [¹²⁵I]IGF-I to the HL-60 cells. Left lane: unlabeled IGF-I (-), Right lane: unlabeled IGF-I (+).

HL-60 cells after incubation with or without TPA. After treatment with TPA for 24 h, the specific binding of [¹²⁵I]IGF-I to the HL-60 cells was decreased to $67.4 \pm 16.5\%$ (mean $\pm$ SEM, n=3) of that of untreated cells. The Scatchard analysis of the binding revealed a curvilinear pattern and the cause of the decrease in [¹²⁵I]IGF-I binding to the treated cells was primarily a decrease in the number of IGF-I binding sites but not to a change in affinity. Assuming the existence of two classes of binding sites, the number of IGF-I receptors for high affinity sites decreased from 3,500/cell to 2,300/cell. The affinity constant (K_a) for the TPA

Fig. 4. Morphological changes induced in HL-60 cells. Cytospan preparation cells cultured with or without TPA. Left side: untreated cells ($\times 400$). Right side: treated with TPA, 10 ng/ml, for 24 h ($\times 400$).

Fig. 5. IGF-I binding to the HL-60 cells (9.1×10^6 cells/ml) treated with TPA. HL-60 cells were cultured with (○) or without (●) 10 ng/ml TPA for 24 h at 37°C. At the end of the incubation, the cells were washed as described in the Materials and Methods and then [125 I]-IGF-I binding to the cells was determined. Inset: Scatchard plot for the binding of [125 I]IGF-I to the cells. Results of a single representative experiment are shown.

Fig. 6. IGF-I binding to the HL-60 cells (8.1×10^6 cells/ml) treated with $1,25(\text{OH})_2\text{D}_3$. The HL-60 cells were incubated with ($\circ$) or without ($\bullet$) 2×10^{-9} M $1,25(\text{OH})_2\text{D}_3$ for 48 h. Then the binding studies were carried out similarly to those described in Fig. 5. Results of a single representative experiment are shown.

treated cells was similar to that for untreated cells ($K_a = 3.36 \times 10^9 \text{ M}^{-1}$ vs. $K_a = 3.51 \times 10^9 \text{ M}^{-1}$).

When the HL-60 cells were incubated with $1,25(\text{OH})_2\text{D}_3$ at a final concentration 2.0×10^{-9} M for 48 h, the specific binding of the $[^{125}\text{I}]\text{IGF-I}$ to the cells was $64.1 \pm 12.1\%$ (mean $\pm$ SEM, $n=3$) of that of before treatment (Fig. 6). The number of binding sites decreased similarly to that of TPA.

Discussion

In the present study, specific IGF-I receptors on the HL-60 cells were identified and characterized. $[^{125}\text{I}]\text{IGF-I}$ specifically bound to the HL-60 cells and the binding characteristics are similar to those demonstrated in the other cells. Compared to IGF-I, the relative potencies of MSA and porcine insulin in displacing the $[^{125}\text{I}]\text{IGF-I}$ binding were 10 and 1%, respectively. In addition, IGF-I displaced $[^{125}\text{I}]\text{insulin}$ binding to HL-60 cells with a potency that was 100 times lower than that of insulin. These results show that HL-60 cells have specific receptors for IGF-I as well as for insulin. The IGF-I binding subunit was $\text{Mr}=130,000$ by an affinity labeling technique. These

data indicate that an IGF type I receptor is present on the HL-60 cells.

It has been reported that some reagents such as $1,25(\text{OH})_2\text{D}_3$, TPA, retinoic acid and DMSO increased insulin binding sites in a dose dependent manner and the increase was due to an increase in the number of insulin binding sites along with the differentiation to granulocytes or macrophage-like cells (Yamanouchi *et al.*, 1982). In contrast to the insulin receptors on HL-60 cells, we observed that the $[^{125}\text{I}]\text{IGF-I}$ binding to the cells differentiated to macrophage-like cells by TPA or $1,25(\text{OH})_2\text{D}_3$ declined to 67 or 64% of the control, respectively. The Scatchard analysis showed that diminution of the specific binding with the differentiation was due to the decrease in the number of IGF-I binding sites, but not to the change in affinity. The decrease in $[^{125}\text{I}]\text{IGF-I}$ binding to the HL-60 cells treated with TPA or $1,25(\text{OH})_2\text{D}_3$ might be due to the presence of these reagents themselves. However, $[^{125}\text{I}]\text{IGF-I}$ binding to the cells treated with TPA or $1,25(\text{OH})_2\text{D}_3$ for 1 hour did not differ from the binding to untreated cells (data not shown). Therefore, the decrease in the binding to the treated cells may be due to the cell differentiation.

We observed that IGF-I and insulin

binding sites of the erythroleukemia cell line, K-562, decreased with differentiation to mature erythrocytes (Hizuka *et al.*, 1987), suggesting that both IGF-I and insulin receptors might be regulated by the same mechanism. On HL-60 cells, however, IGF-I and insulin receptors seem to be regulated by different mechanisms with cell differentiation. Shimizu and Roth (1985) reported that the TAI preadipocytes had low numbers of insulin receptors and poor sensitivity to insulin, whereas the TAI adipocytes had high numbers of receptors and good sensitivity to insulin. In contrast to insulin receptors, the preadipocytes had high numbers of receptors for IGF-I. They suggested that IGFs plays an important part in cell differentiation, especially immature stages. Our results agreed with their suggestion.

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