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Inhibition by islet-activating protein, pertussis toxin, of retinoic acid-induced differentiation of human leukemic (HL-60) cells

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Human promyelocytic leukemic (HL-60) cells were induced to differentiate into neutrophil- or macrophage-like cells by incubation of the cells with retinoic acid, dibutyryl cyclic AMP (Bt_2cAMP) or phorbol 12-myristate 13-acetate (PMA). Differentiation was determined by an increase in the percentage of morphologically mature cells. The retinoic acid-induced differentiation of HL-60 cells was, but the Bt_2cAMP- or PMA-induced one was not, inhibited by prior exposure of the cells to islet-activating protein (IAP), pertussis toxin. The IAP-induced inhibition was correlated with the toxin-catalyzed ADP-ribosylation of a membrane GTP-binding protein with a molecular mass of 40 kDa. Thus, the IAP-substrate GTP-binding protein appears to be involved in the retinoic acid-induced differentiation of HL-60 cells.

ADP-ribosylation; GTP-binding protein; Islet-activating protein; Retinoic acid; (HL-60 cell)

1. INTRODUCTION

The HL-60 cell line, derived from a patient with acute promyelocytic leukemia [1], proliferates continuously in suspension culture and consists predominantly of promyelocytes. The cells can be induced to terminally differentiate into morphologically mature neutrophils or macrophages by incubation with wide variety of compounds, including retinoic acid [2], phorbol 12-myristate 13-acetate (PMA) [3], and dibutyryl cyclic AMP (Bt_2cAMP) [4]. The differentiated cells can be characterized by morphological, enzymatic and antigenic markers. Thus, this cell line provides a unique system for studying human myeloid differentiation.

Islet-activating protein (IAP), persussis toxin, is known to ADP-ribosylate and inactivate GTP-binding proteins (G-proteins) and, hence, to be useful for the identification of processes mediated by IAP-sensitive G-protein(s) [5]. Recent reports have shown that IAP-sensitive G-proteins are also involved in the signal-transduction systems of some growth factors which induce cell proliferation. For example, Fujinaga et al. [6] reported that an IAP-sensitive G-protein played a role in the G_0-G_1 transition of rat hepatocytes. The involvement of G-proteins in growth factor-induced DNA synthesis was also suggested in 3T3 fibroblasts [7-9]. Moreover, Johnson and Davis [10] reported that IAP inhibited retinoic acid-induced expression of transglutaminase in macrophages. In this communication, we show that retinoic acid-induced differentiation of HL-60 cells is, but the Bt_2cAMP- or PMA-induced one is not, inhibited by prior exposure of the cells to IAP. The results indicate that an IAP-substrate G-protein(s) is involved not only in cell proliferation but also in cell differentiation. A possible role of IAP-substrate G-protein in the retinoic acid-induced signal transduction is discussed.

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2. MATERIALS AND METHODS

2.1. *Materials*

Bt_2cAMP was kind gift from Yamasa Shoyu Co., Ltd

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(Tokyo, Japan). [^{32}P]NAD (37 TBq/mmol) was purchased from Du-Pont New England Nuclear. Retinoic acid and PMA were from Sigma, and nitrotetrazolium blue (NTB) was from Wako Pure Chemical Industries, Ltd (Osaka, Japan). The sources of all other materials used are those described in [11,12].

2.2. *Cell culture and evaluation of morphological differentiation*

HL-60 cells (a generous gift from Professor Y. Kaziro, University of Tokyo) were cultured in RPMI 1640 medium supplemented with 5% heat-inactivated (56°C, 30 min) fetal calf serum. The cells were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air. Retinoic acid, Bt_2cAMP and/or PMA were added to the cells (5×10^5 cells/ml) at the final concentrations indicated in the specific figure legends. The cells were allowed to grow for 48 h in the presence of the reagents to promote differentiation. Where indicated, the reagents were added to cells that had been cultured for 20–24 h with IAP at the final concentration of 50 ng/ml unless otherwise specified.

For morphological evaluation of cell differentiation, the treated cells which had been washed with phosphate-buffered saline (PBS) were resuspended at 1×10^6 cells/ml in PBS containing 1 mg/ml NTB and 1 nM PMA and further incubated at 37°C for 45 min. The percentage of cells containing black formazan deposits was counted using a hemocytometer. Differential cell counting was performed on 200 to 400 stained cells from at least two preparations of each experimental point.

2.3. *ADP-ribosylation of G-protein by IAP*

Preparation of crude plasma membranes from HL-60 cells and ADP-ribosylation of cholate extract from the membrane were carried out as in [12].

3. RESULTS AND DISCUSSION

HL-60 cells were differentiated into neutrophil- or macrophage-like cells upon incubation of the cells with various reagents; the orientation of the differentiation was dependent on the inducing reagent used. For example, retinoic acid or Bt_2cAMP promoted differentiation into neutrophil-like cells. On the other hand, PMA led to macrophage-like cells. These differentiations were accompanied by an induction of NTB-reducing activity in both cell types [13], which was used as the morphological evaluation of the cell differentiation in the present study.

When HL-60 cells had been cultured with various concentrations of retinoic acid for 48 h, there was a progressive increase in the percentage of NTB-reducing cells as the concentration of retinoic acid had been increased (fig.1). The half-maximal effect was observed with about 5 nM retinoic acid under the present conditions. When the retinoic acid-induced differentiation was studied in HL-60 cells that had been cultured with 50 ng/ml of IAP for 24 h, the concentration of retinoic acid which produced the half-maximal effect was sifted to about 25 nM. A higher concen-

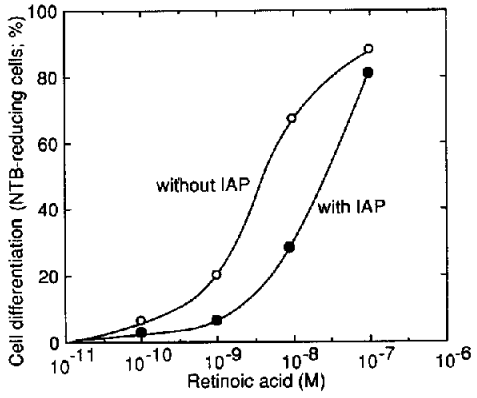

Fig.1. Inhibition by IAP, pertussis toxin, of retinoic acid-induced differentiation of HL-60 cells. HL-60 cells (5×10^5 cells/ml) that had been cultured for 24 h with (●) or without (○) 50 ng/ml of IAP were further incubated for 48 h in a medium containing various concentrations of retinoic acid. NTB-reducing cells were then counted as described in section 2.2.

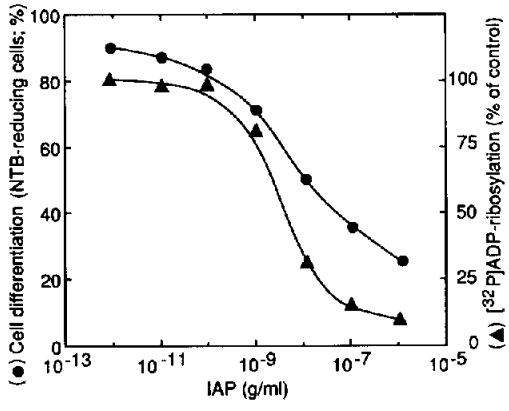

Fig.2. Inhibition by IAP of the retinoic acid-induced differentiation, as correlated with ADP-ribosylation of the 40-kDa membrane protein. HL-60 cells were first incubated for 20 h with various concentrations of IAP. Aliquots of the cells were withdrawn and subjected to IAP-catalyzed [^{32}P]ADP-ribosylation (▲) as described in section 2.3. The activities are expressed as percentages of control, amount of [^{32}P]ADP-ribosylated 40-kDa protein obtained without IAP, which was 24 pmol of ADP-ribose/mg of cholate-extracted membrane protein. The remaining cells were further cultured for 48 h with 10 nM retinoic acid, and NTB-reducing cells were then counted (●) as described in section 2.2.

tration of retinoic acid was thus required for the maximal differentiation in IAP-pretreated cells than in the control (non-treated) cells.

The IAP-induced inhibition of the differentiation was dependent on the concentration of IAP used. The degree of the inhibition was larger as the concentration of IAP had been increased (fig.2). Cholate extract of cell membranes was also prepared from HL-60 cells that had been exposed to IAP, and IAP-catalyzed [^{32}P]ADP-ribosylation of the membrane proteins was then analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. As shown in fig.2, the exposure of HL-60 cells to IAP gave rise to an inhibition of the incorporation of radioactivity into 40-kDa $G_i\alpha$ subunit: the degree of the inhibition was dependent on the concentration of IAP and well correlated with the extent of the inhibition of the cell differentiation. These results suggest that the IAP-substrate G-protein is somehow involved in the process(es) in which retinoic acid leads to the differentiation of HL-60 cells into neutrophil-like cells.

We next examined the effect of IAP on differentiation of HL-60 cells induced by the other reagents, such as Bt_2cAMP and PMA (fig.3). Unlike the case of retinoic acid-induced differentiation, IAP exerted no significant effect on either the Bt_2cAMP- or PMA-induced cell differentiation. These results suggest that the action of IAP is selective and specific for retinoic acid-induced differentiation of HL-60 cells and that the mechanism of cell differentiation induced by retinoic acid is different from that by Bt_2cAMP or PMA, which is reported to cause phosphorylation of nuclear proteins [14,15]. This might be supported by the findings that the actions of retinoic acid and Bt_2cAMP (or PMA) appeared to be synergistic for the cell differentiation (fig.4). The concentration-dependent curve of retinoic acid was markedly shifted to the left in the presence of a low concentration of Bt_2cAMP or PMA in which HL-60 cells were not differentiated by themselves (see fig.3).

We do not, as yet, know the molecular mechanisms through which IAP inhibits the retinoic acid-induced differentiation of HL-60 cells, but our data suggest that G-protein serving as the substrate of IAP is likely to be involved in an early process(es) of differentiation. It is reported that a binding protein for retinoic acid is localized in the nuclei and that retinoic acid binding to the protein is responsible for cell differentiation [16]. It is, therefore, likely that IAP somehow reduces the affinity of the binding protein for retinoic acid, since the concentration of retinoic acid required for the half-maximal effect was, but the maximal effect was not, modified by IAP. Another possibility is the existence of a membrane receptor for retinoic

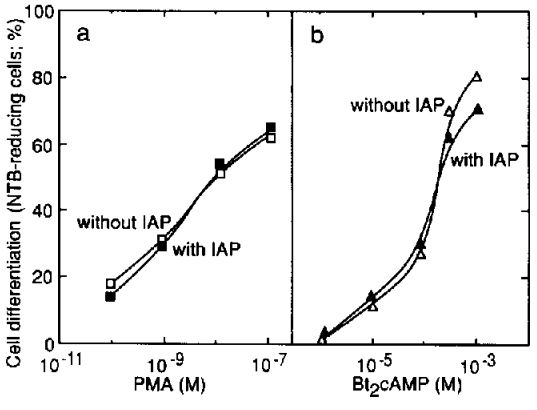

Fig.3. Effect of IAP on dibutyryl cyclic AMP- or phorbol 12-myristate 13-acetate-induced differentiation of HL-60 cells. HL-60 cells (5×10^5 cells/ml) that had been cultured for 24 h with (■,▲) or without (□,△) 50 ng/ml of IAP were further incubated for 48 h in a medium containing various concentrations of Bt_2cAMP (a) or PMA (b), and NTB-reducing cells were then counted as described in section 2.2.

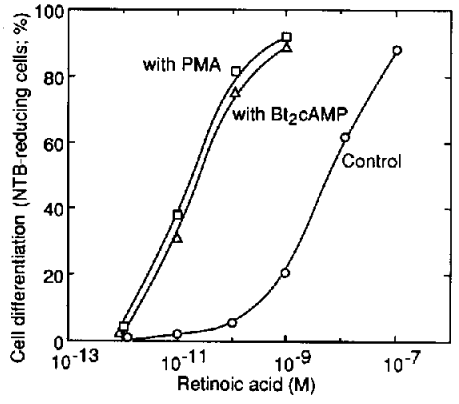

Fig.4. Effect of Bt_2cAMP or PMA on retinoic acid-induced differentiation of HL-60 cells. HL-60 cells (5×10^5 cells/ml) were cultured for 48 h without (○) or with either 0.1 nM PMA (□) or 10 μM Bt_2cAMP (△) in the presence of the indicated concentrations of retinoic acid, and NTB-reducing cells were then counted as described in section 2.2.

acid. Several receptors coupled to IAP-sensitive G-proteins have been found to be homologous to the retinoid-binding photoreceptor, rhodopsin. Thus, it is also likely that the signaling through the membrane-bound retinoic acid receptor requires G-protein(s), which is uncoupled by IAP-catalyzed ADP-ribosylation of the G-protein(s). We are currently carrying out studies to determine whether the action of retinoic acid is mediated through an as yet unidentified mechanism.

REFERENCES

[1] Collins, S.J., Gallo, R.C. and Gallagher, R.E. (1977) Nature (London) 270, 347–349.
[2] Breitman, T.R., Selonick, S.E. and Collins, S.J. (1980) Proc. Natl. Acad. Sci. USA 77, 2936–2940.
[3] Collins, S.J., Ruscetti, F.W., Gallagher, R.E. and Gallo, R.C. (1978) Proc. Natl. Acad. Sci. USA 75, 2458–2462.
[4] Olsseon, I.L., Breitman, T.R. and Gallo, R.C. (1982) Cancer Res. 42, 3928–3933.
[5] Ui, M. (1980) in: Phosphoinositides and Receptor Mechanisms. Receptor Biochemistry and Methodology, vol. 7 (Putney, J.W. ed.) pp. 163–195, A.R. Liss, New York.
[6] Fujinaga, Y., Morozumi, N., Sato, K., Tokumitsu, K., Kondo, Y., Ui, M. and Okajima, F. (1989) FEBS Lett. 245, 117–121.
[7] Murayama, T. and Ui, M. (1987) J. Biol. Chem. 262, 12463–12467.
[8] Hildebrandt, J.D., Stolzenberg, E. and Graves, J. (1986) FEBS Lett. 203, 87–90.
[9] Letterio, J.J., Coughlin, S.R. and Williams, L.T. (1986) Science 234, 1117–1119.
[10] Johnson, J.D. and Davis, P.J.A. (1986) J. Biol. Chem. 261, 14982–14986.
[11] Oinuma, M., Katada, T. and Ui, M. (1987) J. Biol. Chem. 262, 8347–8353.
[12] Katada, T., Oinuma, M. and Ui, M. (1986) J. Biol. Chem. 261, 8182–8191.
[13] Collins, S.J., Ruscetti, F.W., Gallagher, R.E. and Gallo, R.C. (1979) J. Exp. Med. 149, 969–974.
[14] Nishizuka, Y. (1984) Nature (London) 308, 693–698.
[15] Rosenfeld, M.G. and Garrieux, A. (1979) Adv. Cyclic Nucleotide Res. 11, 205–264.
[16] Sani, B.P. and Donovan, M.K. (1979) Cancer Res. 39, 2492–2496.