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Inhibition of myeloid differentiation by inhibitors of ADP-ribosylation

Ming-chi Wu, M. Rebecca Zaun and Fu-Mei Wu

Department of Biochemistry, Texas College of Osteopathic Medicine, University of North Texas, Denton, TX 76203, USA

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Inhibitors of ADP-ribosylation inhibited the myeloid differentiation of murine myelomonocytic leukemia, WEHI-3BD$^+$ cells induced by granulocyte colony-stimulating factor. Benzamide, at 2.0 mM, inhibited 50% of the WEHI-3BD$^+$ cell differentiation but had no significant effect on the proliferation. However, benzylaminododecylguanine hydrochloride and *p*-methoxylbenzylaminodecamethylene guanidine sulfate at 2.0 and 2.2 μM, respectively, inhibited 50% of proliferation but had no effect at all on differentiation. The differential effects of inhibitors provide a model to study the role of ADP-ribosylation in myeloid differentiation.

Leukemia; Cell differentiation; Colony-stimulating factor; ADP-ribosylation

1. INTRODUCTION

ADP-ribosylation is a post-translational modification process that transfers the ADP-ribose residue from NAD$^+$ to acceptor proteins. It has been suggested that this process plays an important regulatory role in cellular proliferation and differentiation [1]. The membrane-associated stimulatory (G_s) and inhibitory (G_i) components of adenylate cyclase can act as ADP-ribose acceptors for cholera toxin and pertussis toxin, respectively [2], thus activating adenylate cyclase. Chromatin proteins can be ADP-ribosylated by a nuclear ADP-ribosyl transferase which is activated by DNA strand breaks [3]. This modification of chromatin has been suggested as playing an important role in DNA repair and cell differentiation [4]. Studies on the possible role of ADP-ribosylation in myeloid differentiation are limited. Francis et al. [5] have reported that inhibitors of ADP-ribosylation such as 3-methoxybenzamide inhibit monocyte differentiation of human bone marrow progenitor cells as assayed in soft-agar for colony formation. Colon-Otero et al. [6] also reported that 3-aminobenzamide and nicotinamide caused a significant dose-response inhibition of interleukin-3 induced FDCP-1 cell proliferation. Dexter et al. [7] have used two compounds, BADGH and MBAMG [8], which are potent inhibitors of mono ADP-ribosylation to study their effect on myeloid differentiation of long-term murine bone marrow cell cultures. Their results have shown that these inhibitors can block the differentiation of committed myeloid progenitor cells. Results from these studies indicated the involvement of ADP-ribosylation in the myeloid differentiation. However, whether the effect is on the proliferation or differentiation step of the process has not been elucidated.

Correspondence address: Ming-chi Wu, Division of Biochemistry, University of North Texas, PO Box 13048, Denton, TX 76203, USA

Abbreviations: BADGH, benzylaminododecylguanidine hydrochloride; MBAMG, *p*-methoxylbenzylaminodecamethylene guanidine sulfate; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; DME-S, Dulbecco's modified Eagle's with serum; CSF, colony-stimulating factor; E-MLCM, endotoxin-treated mouse lung conditioned medium

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In this communication, we report the differential effect of various ADP-ribosylation inhibitors on the differentiation of a murine myelomonocytic leukemia cell line induced by granulocyte colony-stimulating factor. This system will provide a model to study the role of ADP-ribosylation in myeloid differentiation.

2. MATERIALS AND METHODS

2.1. *Cell culture*

Murine myelomonocytic leukemia cell line WEHI-3BD$^+$ was kindly provided by Dr Malcolm A.S. Moore of Sloan Kettering Cancer Center, New York, and was cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Cells between passage 12 and 30 were used in the experiments.

2.2. *Soft-agar assay of bone marrow stem cell differentiation*

The standard procedure of colony formation by bone marrow cells was conducted as described previously [9] to assess the effects of ADP-ribosylation inhibitors on myeloid differentiation. The ADP-ribosylation inhibitors including benzamide, 3-aminobenzamide, 3-methoxybenzamide, 5-methylnicotinamide, BADGH and MBAMG at different concentrations were added to the cultures in the beginning of the assay. The number of colonies was counted at day 5. Endotoxin-treated mouse lung conditioned medium was used as a source for GM-CSF and G-CSF. The morphology of cells in colonies was examined by staining the plates with hematoxylin according to procedures previously reported [10].

2.3. *Differentiation of WEHI-3BD$^+$ cells induced by mouse lung conditioned medium*

The WEHI-3BD$^+$ cells were seeded in 35 mm petri-dishes at 300 cells/plate containing 1 ml of 0.3% agar in DME-S (10% fetal calf and horse sera). Endotoxin-treated mouse lung conditioned medium was added as the source of G-CSF to the culture at the beginning of the assay along with ADP-ribosylation inhibitors at different concentrations. The plates were incubated at 37°C in a humidified incubator with 7% CO_2 for 7 days. Total number of colonies and number of dispersed colonies were scored separately under a dissection stereomicroscope and the differentiation activity was expressed as percent of dispersed colonies [11].

2.4. *Liquid culture assay of WEHI-3BD$^+$ cell differentiation*

Suspension cultures of WEHI-3BD cells were seeded at 1.8×10^5 cells/ml in DME medium supplemented with 10% FCS. Endotoxin-treated mouse lung conditioned medium (2000 U/ml, G-CSF), benzamide (2 mM) and MBAMG (2 μM) were added to the cultures either separately or in combination with proper control. The cells were cultured at 37°C in a humidified incubator with 7% CO_2 for 4 days. The total cell numbers were counted and cells under different treatment were washed and assayed for colony formation as described in section 2.3.

3. RESULTS

3.1. *Inhibition of colony formation by inhibitors of ADP-ribosylation*

Colony formation of bone marrow cells in a soft-agar assay was inhibited by two different groups of ADP-ribosylation inhibitors. As shown in table 1, analogs of NAD$^+$ including benzamide, 3-aminobenzamide, 3-methoxybenzamide and 5-methylnicotinamide inhibited colony formation in the millimolar range. The concentration of inhibitor that resulted in 50% inhibition of colony formation was between 1.6 mM for 3-methoxybenzamide and 6.0 mM for 3-aminobenzamide. The guanidine derivatives, BADGH and MBAMG, which are analogs of arginine residues in acceptor proteins, have shown a much more potent inhibitory activity. The concentration of BADGH and MBAMG that resulted in 50% inhibition of colony formation were 1.7 and 1.1 μM, respectively. Morphological analysis of colonies formed in the presence of ADP-ribosylation inhibitors indicated that macrophage colony formation was preferentially sensitive to the inhibitors as reported by Francis et al. [5].

3.2. *Effect of benzamide on differentiation of WEHI-3BD$^+$*

In order to study the effect of ADP-ribosylation inhibitors on differentiation of myelogenous leukemia cells, benzamide at different concentrations was added to the assay plates. The WEHI-3BD$^+$ cells form compact and tight colonies in the soft-agar plates. However, in the presence of G-CSF, the leukemia cells differentiate and form dispersed colonies [12]. The dispersed colony formation assay was used as standard assay method for G-CSF activity [12]. As shown in fig.1,

Table 1

Concentration of inhibitors at 50% inhibition of colony formation

Compound	Concentration (mM)
Benzamide	2.1
3-Aminobenzamide	6.0
3-Methoxybenzamide	1.6
5-Methylnicotinamide	3.1
MBAMG	0.0011
BADGH	0.0017

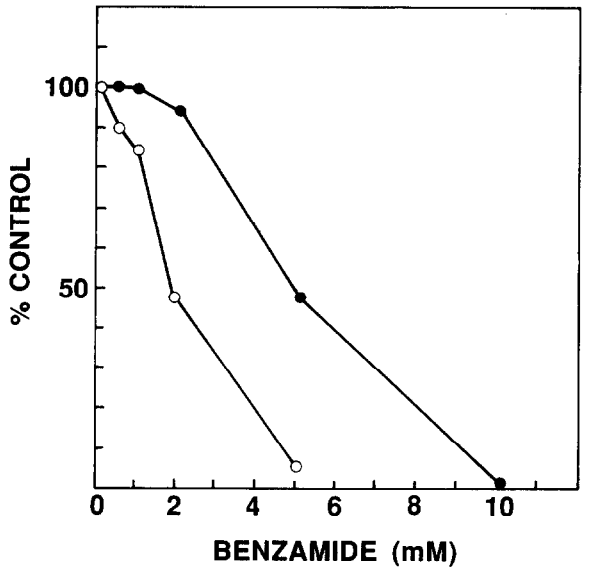

Fig.1. The effect of benzamide on colony formation. Different concentrations of benzamide were added to the soft-agar assay plates with standard amounts of E-MLCM (50 μl). The colony formation assay was conducted as described in section 2. Total number of colonies and number of dispersed colonies were scored. Percent of total colonies in control (●—●); percent of dispersed colonies in control (○—○).

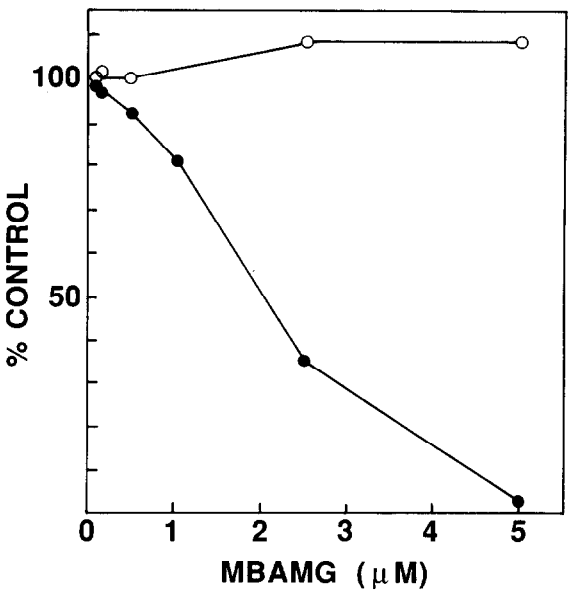

Fig.2 The effect of MBAMG on colony formation. The experiment was carried out as described in fig.1, except MBAMG at different concentrations was added instead of benzamide. Percent of total colonies in control (●—●); percent of dispersed colonies in control (○—○).

benzamide, at concentrations up to 2.0 mM, did not show significant inhibition of total colony formation. However, a 50% inhibition of dispersed colony formation was observed. There are no obvious changes in colony size (>500 cells) at this concentration of benzamide. The results indicated that benzamide at 2 mM preferentially inhibited the differentiation without significant effect on the proliferation. At a high concentration of 10 mM, benzamide completely inhibited the colony formation. Reduced colony size (<100 cells) was observed at 5 mM benzamide.

3.3. *Effect of MBAMG and BADGH on colony formation and differentiation of WEHI-3BD$^+$*

In contrast to the effect of benzamide, the arginine analogs of MBAMG and BADGH exerted a different effect on the WEHI-3BD$^+$ cells in the soft-agar assay. As shown in fig.2, MBAMG inhibited the colony formation of WEHI-3BD$^+$ cells at a much lower concentration than benzamide. It inhibited 50% of colonies formation at 2 μM and inhibited completely the colony formation at 10 μM. Although the number of colony was inhibited, the percent dispersed colonies remained about the same. There are no colony size (>500 cells) changes up to 1 mM of MBAMG, however, colony size reduced to less than 100 cells aggregates when 2.5 μM or greater MBAMG was added. This is in contrast to the benzamide inhibition where differentiation was preferentially inhibited. BADGH had the same effect as MBAMG on the colony formation of WEHI-3BD$^+$ cell (not shown).

3.4. *Differentiation of WEHI-3BD$^+$ cells in suspension culture*

As shown in table 2, when WEHI-3BD$^+$ cells were cultured in DME medium supplemented with 10% FCS, the growth was inhibited by the E-MLCM. Benzamide at 2.0 mM did not show significant inhibition as observed previously in the soft-agar assay. Based on the observation that differentiated cells could not form colonies in the soft-agar, the culture cells treated with different inhibitors and E-MLCM were washed and assayed on soft-agar as described in section 2. The number

Table 2

Effects of E-MLCM, benzamide on WEHI-3BD$^+$ cells in suspension culture

	Total viable cell number ($\times 10^6$)	Number of colonies
Control	4.2	123 ± 19
E-MLCM (2000 units/ml)	1.9	0
Benzamide (2 mM)	4.0	130 ± 21
Benzamide (2 mM) + E-MLCM (2000 units/ml)	2.0	129 ± 5

of colonies are shown in the last column of table 2. E-MLCM-treated cells, although showing only 50% growth inhibitions, could not form any colonies at all, indicating complete differentiation of the cells. Morphological study of the E-MLCM treated has indicated extensive (79%) morphological changes to mature monocyte-like cells typically with increasing cytoplasmic to nucleus ratio as reported previously [12]. However, when benzamide at 2 mM was added to the culture, cell differentiation was inhibited and the cells in the culture were able to form colonies as in the control. This result further supports that benzamide, at 2 mM, preferentially inhibited the differentiation of WEHI-3BD cells as shown in the soft-agar assay.

4. DISCUSSION

CSFs are a group of proteins required for the differentiation and maturation of macrophages and granulocytes from bone marrow stem cells [13]. Great advances have been accomplished recently largely due to the recombinant DNA techniques and their application to the cloning and expression of CSF genes [14]. However, the mechanism of action of CSF has not been fully understood. Since ADP-ribosylation is one of the regulatory reactions involved in cell proliferation and differentiation, the possible role of ADP-ribosylation in myeloid differentiation has been implicated. It is clear from the results presented here that inhibitors of ADP-ribosylation inhibit the colony formation from bone marrow stem cells as assayed on the soft-agar. Benzamide and its derivatives inhibited the colony formation in the range of 1–5 mM. The macrophage colonies were preferentially inhibited. This observation was similar to the results of Francis et al. [5] on the human marrow assay. The sensitivity of colony formation of bone marrow cells to the inhibitors was compatible in human and murine. However, the question of whether ADP-ribosylation inhibitors affect the proliferation or differentiation step cannot be answered by the bone marrow cell experiment. A murine myelomonocytic leukemia cell line WEHI-3BD$^+$, which can be induced by endotoxin-mouse lung conditioned medium to undergo terminal differentiation, was used in these experiments to study the effect of benzamide on the proliferation and differentiation. The results shown in fig.1 have indicated that benzamide at 2 mM inhibited the myeloid differentiation with minimal effect on the proliferation as shown by colony formation. Recent results from an in vivo study [15] have shown that benzamide at millimolar concentration inhibited the mono ADP-ribosyl transferase activity while the poly ADP-ribosyl polymerase was inhibited by benzamide at concentrations in the micromolar range. These data suggest that mono ADP-ribosylation may be one of the key regulatory mechanisms in myeloid differentiation. In contrast, studies on the effects of MBAMG and BADGH have indicated that these two inhibitors inhibited proliferation without any effect on differentiation (fig.2). Since MBAMG and BADGH are the analogs of arginine derivatives serving as artificial acceptors for mono ADP-ribosylation, this will suggest that mono ADP-ribosylation of arginine-protein acceptors is not involved in the myeloid differentiation. The differentiation of the WEHI-3BD$^+$ cell presented in this article provides a model system to investigate further the role of ADP-ribosylation in myeloid differentiation.

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REFERENCES

[1] Ueda, K. and Hayaishi, O. (1985) Annu. Rev. Biochem. 54, 78.
[2] Schleijer, L.S., Kahn, R.A., Hanski, E., Northup, J.K., Sternweis, P.C. and Gilman, A.G. (1982) J. Biol. Chem. 257, 20.

[3] Benjamin, R.C. and Gill, D.M. (1980) J. Biol. Chem. 255, 10493.
[4] Farzaneh, F., Zalin, R., Brill, D. and Shall, S. (1982) Nature 300, 362.
[5] Francis, G.E., Gray, D.A., Berney, J.U., Wing, M.A., Guimaraes, J.E.T. and Hoffbrand, A.V. (1983) Blood 62, 1055.
[6] Colon-Otero, G., Sando, J.J., Sims, J.L., McGrath, E., Jensen, D.E. and Quesenberry, P.J. (1987) Blood 70, 686.
[7] Dexter, T.M., Whetton, A.D. and Heyworth, C.M. (1985) Blood 65, 1544.
[8] Tait, R.M. and Nassau, P.M. (1984) Eur. J. Biochem. 143, 213.
[9] Fojo, S.S., Wu, M.-C., Gross, M.A., Purcell, Y. and Yunis, A.A. (1978) Biochemistry 17, 3109.
[10] Wu, M.-C., Cini, J.K. and Yunis, A.A. (1979) J. Biol. Chem. 254, 6226.
[11] Nicola, N.A., Metcalf, D., Matsumoto, M. and Johnson, G.R. (1983) J. Biol. Chem. 258, 9017–9023.
[12] Metcalf, D. and Nicola, N.A. (1982) Int. J. Cancer U30, 773.
[13] Metcalf, D. (1986) Blood 76, 257–267.
[14] Clark, S.C. and Kaman, R. (1987) Science 236, 1229–1237.
[15] Jacobson, M. (1987) The 8th International Symposium on ADP-Ribosylation, Fort Worth, Texas, p.30, Abstr. no.66.