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EFFECT OF MEDIUM CHANGE ON POLY(ADP-RIBOSE) SYNTHESIS IN FRIEND ERYTHROLEUKEMIC CELLS

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SUMMARY: In Friend leukemic cells cultured in the presence of 5 mM hexamethylene bisacetamide, a potent differentiation-inducer, poly(ADP-ribose) synthesis was reduced to about one-third of that in control cells. Replacing the original culture medium with fresh medium resulted in a decrease of poly(ADP-ribose) synthesis in confluent control cultures, while cells induced to differentiate were not affected by the medium change. This is not attributable to the difference of the level of poly(ADP-ribose) synthesis in different cell cycle stages, since DNA synthesis and cell growth in differentiating cells were maintained at the same level with those of control cells. In control cultures, a medium change during the log-phase effected a prolongation in the rise of poly(ADP-ribose) synthesis. When conditioned medium was substituted during log-phase growth, poly(ADP-ribose) synthesis was stimulated in control cells. This stimulating effect was not lost by dialysis but was lost by heat-treatment or trypsin-digestion. Results suggest that poly(ADP-ribose) synthesis is regulated by some factor(s) released into the culture medium.

INTRODUCTION

Eukaryotic cell nuclei contain poly(ADP-ribose) synthetase which converts NAD^+ into poly(ADP-ribose) (1-3). No consensus regarding the activity of this enzyme during the cell cycle of various types of cells (4-8, 17) has been reached and maximal activity has been reported for G1 (4, 17), S (5), G2 (6,7) and M (7).

In contrast, cell-density dependent increase of poly(ADP-ribose) synthetase activity in the growth cycle has been observed similarly in various cell lines (9-11, 17). In $SV40_{ts}A$-transformed cells, this increase was observed at the permissive temperature, whereas at the

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restrictive temperature, the activity was low (10). We have found that poly(ADP-ribose) synthesis increases according to the rise of the density of Friend cells, and when the cells are induced to differentiate the activity remains low or gradually decreases even in the high cell-density (11, 12). Similar result was obtained by other investigators (19).

We now report results suggestive of the participation of the culture medium in poly(ADP-ribose) synthesis, growth, and differentiation of Friend cells, and discuss about the inconsistency of our results and those of Rastl and Swetly (15) who reported that poly(ADP-ribose) synthesis increased when Friend cells were induced to differentiate .

MATERIALS AND METHODS

Strain 745A Friend murine erythroleukemia cells were obtained from Dr Koyama, Cancer Institute, Tokyo. The cells were grown in Eagle's MEM containing 10% FCS, plated (1×10^5 cells/ml) in a total volume of 10 ml per plate, and maintained at 37° in a humidified atmosphere of 5% CO_2. Cells were counted with a model ZBI Coulter counter.

Poly(ADP-ribose) synthetase assay was performed as reported earlier (11). In brief, nuclei were isolated using 0.1% Nonidet P-40 and the reaction mixture (0.15 ml) contained 15 μmol Tris-HCl (pH 8.0 at 25°), 6 μmol $MgCl_2$, 300 nmol dithiothreitol, 270 nmol NAD^+, 1 μCi [^{3}H]NAD (adenine-2,8-[^{3}H], 3.1 Ci/mmol, New England Nuclear), and $1-4 \times 10^6$ nuclei. The reaction was performed at 25° and terminated by adding trichloroacetic acid to 10% (w/v). The acid-precipitable material was collected on a Millipore filter (HA, 0.45 μm), washed with 10% trichloroacetic acid and dissolved in 1 ml ethyleneglycol monoethyl ether (13). After overnight incubation at room temperature, 10 ml Triton X-100/tolouene (1:2, v/v) scintillation fluid was added.

Conditioned medium was obtained from 72 hr confluent cultures of Friend cells. DNA synthesis was assayed as previously described (14).

RESULTS AND DISCUSSION

Friend cells (1×10^5 cells/ml) were cultured for 72 hr without medium change and used for the following study. As shown in fig. 1C, cell density did not change significantly within 10 hr after medium change. In undifferentiated (control) cells, poly(ADP-ribose) synthesis slightly increased until 8 hr and then gradually decreased (fig. 1A). Medium change effected a decrease of poly(ADP-ribose) synthesis at 2-5 hr, followed by a recovery to the control level at 10 hr. These

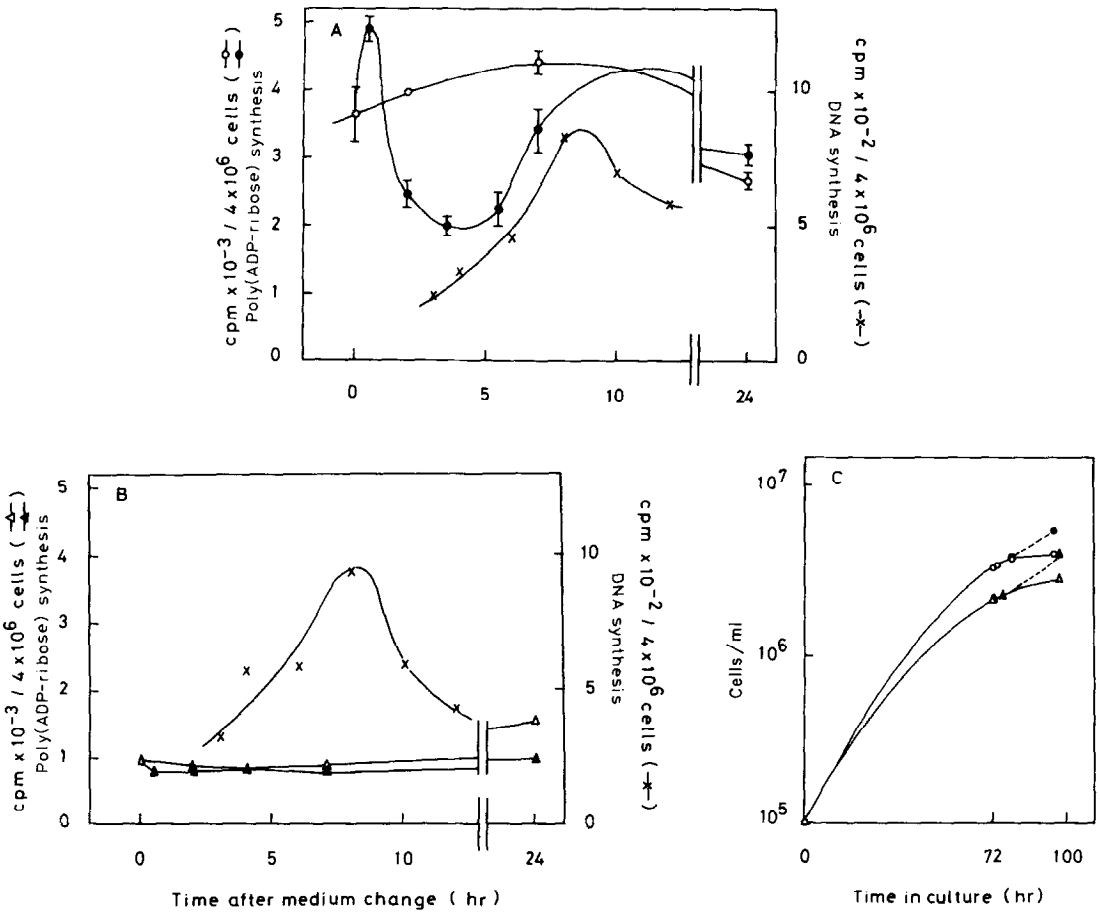

Figure 1. Effect of medium change on poly(ADP-ribose) synthesis and DNA synthesis in the confluent culture of undifferentiated (A) and differentiated (B) cells. Cell growth is shown in (C). In (B), cells were cultured for 72 hr in the presence of 5 mM hexamethylene bisacetamide beforehand. The original culture medium was replaced by identical fresh medium; the time of medium change is designated as 0 hr. Vertical bars represent ± S.E.
○, △ - medium unchanged; ●, ▲ - medium changed; X - DNA synthesis assayed by pulse-labelling the cultures for 30 min in medium containing 0.3 μCi [^{3}H]thymidine/ml (20 Ci/mmole) and 1 x 10^{-5} M thymidine. After incubation, the cells were washed with saline and trichloroacetic acid, boiled in 5% perchloric acid and the supernatant was assayed for radioactivity.

results suggest that poly(ADP-ribose) synthesis is related to the nature of the culture medium rather than to cell density itself. The decrease in poly(ADP-ribose) synthesis effected by the medium change may be due to the loss of some factor(s) released into the original culture medium during the 72 hr culture. Although the medium change

initiated DNA synthesis (fig. 1A), the lowest level of poly(ADP-ribose) synthesis preceded the peak of DNA synthesis by about 5 hr.

In striking contrast, in differentiated (hemoglobin-producing) cells, a low level of poly(ADP-ribose) synthesis was maintained throughout the experiment, irrespective of medium change (fig. 1B). This figure also shows that the enhancement of DNA synthesis was similar to that in undifferentiated cells (fig. 1A) and that the level of DNA synthesis had no effect on poly(ADP-ribose) synthesis in differentiated cells.

As shown in fig. 2B, cell density increased markedly when the culture medium was changed at 24 hr and again at 48 hr. A change of the medium at 24 hr resulted in delayed poly(ADP-ribose) synthesis increase, ascribable to the dilution of some factor(s) supportive of high-level synthesis. However, when the medium was changed at both 24 and 48 hr, the delay was rapidly corrected, possibly due to high cell density and some unknown change in cellular metabolism (fig. 2A).

The replacement with conditioned medium inhibited cell growth (fig. 3B) and resulted in enhancement of poly(ADP-ribose) synthesis

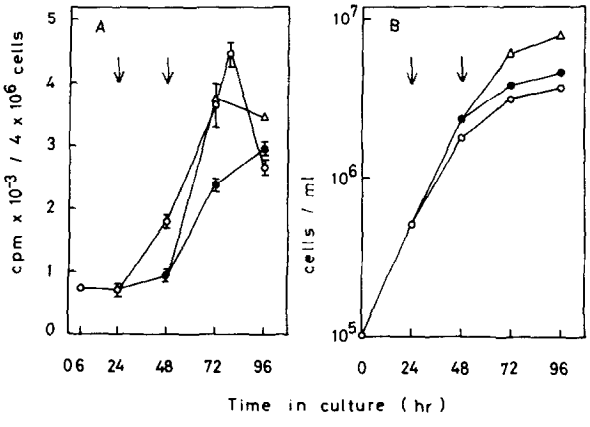

Figure 2. Effect of medium change on poly(ADP-ribose) synthesis (A) and cell growth (B) at the log-phase growth stage of undifferentiated cells. Vertical bars represent ± S.E.
○ - medium unchanged; ● - medium changed at 24 hr; Δ - medium changed at 24 hr and again at 48 hr.

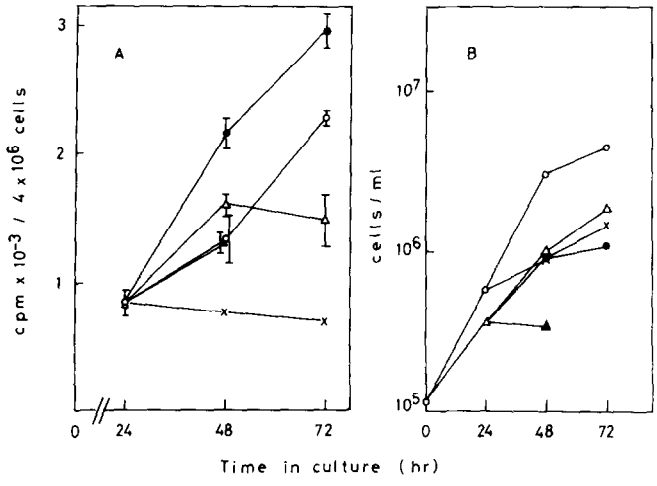

Figure 3. Effect of conditioned medium on poly(ADP-ribose) synthesis (A) and cell growth (B). Cells cultured in the absence (○,●) and presence (X,Δ,▲) of 5 mM hexamethylene bisacetamide. ○,X - medium unchanged; ●,▲ - medium replaced at 24 hr with conditioned medium. Δ -medium replaced at 24hr with fresh medium. Vertical bars represent ± S.E.

in undifferentiated cells, confirming the presence of some stimulating factor(s) in the conditioned medium (fig. 3A). Cells which had begun to differentiate exhibited a different response to the medium change. In cells cultured for 24 hr in the presence of 5 mM hexamethylene bisacetamide, which were on the way to differentiation while not yet producing hemoglobin, poly(ADP-ribose) synthesis was somewhat enhanced by the medium change (fig. 3A). This suggests that the properties of these intermediate cells are different from both undifferentiated and differentiated cells.

Preliminary characterization of the factor(s) in the conditioned medium was carried out, and it was shown that the stimulating effect was not lost by dialysis (data not shown) but was lost by heat- or trypsin-treatment (table 1). The stimulating effect was not attributable to the components in the serum, since high serum concentrations suppressed the poly(ADP-ribose) synthesis (table 1). It should also be noted that the conditioned medium used in this experimental condition never suppressed the cell growth, because it

Table 1. Heat- and trypsin-treatment of the conditioned medium

	Treatment	Poly(ADP-ribose) Synthesis (cpm/4 x 10^6 cells)	Cell growth (cells/ml)
Experiment I	Fresh medium	1764	1.8 x 10^6
	Conditioned medium	4456	2.6 x 10^6
	Conditioned medium (Heated)	1904	1.6 x 10^6
	(Trypsinized)	2730	1.9 x 10^6
Experiment II	5% Serum	1903	0.8 x 10^6
	10% Serum	1628	1.0 x 10^6
	20% Serum	1374	0.9 x 10^6

Experiment I: Conditioned medium (180 ml) obtained from 72 hr culture was concentrated to 30 ml by ultrafiltration using UM-10 membranes (Amicon Corp. Lexington, Mass). The concentrated medium was diluted to 180 ml by the addition of fresh medium, and was referred to the conditioned medium in this table. Heat-treatment was carried out at 70° for 30 min. Aggregate was removed by centrifugation. Trypsin (2mg/ml, from bovine pancreas, Sigma, St Louis) digestion was carried out at 37° for 40 min, and then the same amount of trypsin inhibitor (from soybean, Boehringer, Mannheim) was added. The mixture was incubated at 37° for 30 min, and was used as the trypsinized conditioned medium. These media were substituted for the ordinary culture medium after 24 hr culture of cells (started at 1 x 10^5 cells/ml). After the medium change cells were cultured for another 20 hr, and then assayed for poly(ADP-ribose) synthesis as described in Materials and Methods.
Experiment II: Cell cultures were started at 0.8 x 10^5/ml. After 24 hr, media containing indicated concentrations of sera were substituted. Others were the same with experiment I.

was diluted with fresh medium (table 1). This suggests that the stimulation of poly(ADP-ribose) synthesis by the conditioned medium is not due to the stop of the cell cycle such as the G1 accumulation.

We used the method of Shima et al (16) to estimate the chain length of ^{3}H-labelled poly(ADP-ribose) and found the average chain length to be 10-12 and 5-6, respectively, for undifferentiated and differentiated cells cultured for 72 hr. A detailed analysis of synthesized poly(ADP-ribose) and its acceptor protein was reported elsewhere (18).

In F4N cells, inducers, especially butyrate, have been reported to enhance poly(ADP-ribose) synthesis (15). This apparent inconsistency

with our results (11) may, at least partially, be due to the medium change which effects transient elimination of the stimulating factor(s) in the medium. If cells are cultured with daily medium change as Rastl and Swetly reported (15), the level of poly(ADP-ribose) remains low (fig. 2). Since butyrate is highly toxic in F4N cells (15), DNA fragmentation may cause the stimulation of poly(ADP-ribose) synthesis (20).

The present investigation indicated that in Friend cells, a medium change at different growth stages or differentiation stages has profound and diverse effects on poly(ADP-ribose) synthesis. In order to elucidate the regulatory mechanism(s) of poly(ADP-ribose) synthesis, we think that the response of cells to their circumstances must be further examined.

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