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Dexamethasone Inhibition of DMSO-Induced Transglutaminase Activity and Differentiation of Leukemic Cells[1] (41789)

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Abstract. Treatment of the Friend erythroleukemic (FL) cell line GM979 with dimethyl sulfoxide (DMSO) or *n*-butyric acid induced erythroid differentiation. Transglutaminase (TGase) activity also increased in these treated cells. Glucocortical steroids, i.e., dexamethasone (DEX) and triamcinolone acetonide, when added to the cultured medium, inhibited the DMSO-induced hemoglobin synthesis but not *n*-butyric acid-induced hemoglobin synthesis. Similarly, these steroids inhibited DMSO-increased TGase activity but not *n*-butyric acid-increased TGase activity in intact FL cells. Neither the differentiation-inducing agents nor the steroids had any effect on TGase activity when they were directly added to cell lysates. These results support the view that the increase of TGase activity may be related to erythroid differentiation of FL cells and of its possible role of this enzyme in FL cell-induced differentiation.

Transglutaminase (TGase, glutaminyl-peptide γ-glutamyltransferase, EC 2.3.2.13) is a Ca^{2+} dependent enzyme which catalyzes the posttranslational crosslinking of proteins and the incorporation of amines into proteins. This enzyme has been reported to be important in several biological functions, including crosslinking of fibrin in blood coagulation (1) and clotting of seminal fluids (2). Intracellular enzyme activity has also been related to certain age-related processes (3–5) as well as arrest of cell division (6), receptor-mediated endocytosis (7), and triggering of B lymphocytes to form antibody (8). Recent studies from this laboratory have shown that TGase is markedly increased when the Friend erythroleukemic (FL) cell line GM 979 is treated with dimethyl sulfoxide (DMSO) or *n*-butyric acid (9). These agents also induced erythroid differentiation of this tumor cell line. A close relationship was evident between increased TGase activity and induction of hemoglobin synthesis of the treated FL cells.

The role of TGase in erythroid differentiation of FL cells is still unclear. It seems likely that high levels of activity in differentiating FL cells may be related to stabilizing cell membranes by crosslinkage of membrane proteins such as band 3 and spectrin, which have been shown to be the native substrates of TGase in human erythrocytes (3). This enzyme may also be important in regulating polyamine metabolism and the activity of L-ornithine decarboxylase (ODC). The latter is the rate-limiting enzyme in polyamine biosynthesis. For example, Haddox and Russell reported that the nuclear level of both the polyamine conjugates and TGase increase concurrently during liver regeneration (10). It has also been reported that incorporation of putrescine into ODC by purified TGase resulted in inactivation of ODC (11).

In FL cells, dexamethasone (DEX) inhibited DMSO induced but not *n*-butyric acid induced erythroid differentiation (12). This effect appeared to be due to an influence of the steroid on ODC. The effect of DMSO which stimulated ODC activity was blocked by DEX. On the other hand, DEX did not block the effect of *n*-butyric acid which in turn has no effect on ODC. Thus, it appears that DMSO and *n*-butyric acid induced differentiation may occur by different pathways (12). Since both DMSO and *n*-butyric acid increased TGase activity in FL cells, it was of interest to determine the effects of steroids on TGase activity so as to examine the role of this enzyme in FL cell differentiation. Results of the present study showed that DMSO increased TGase activity and hemoglobin synthesis were both inhibited by glucocorticoids in a dose dependent man-

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ner. Reduction of hemoglobin synthesis paralleled the reduction of TGase activity. In contrast, *n*-butyric acid increased TGase activity as well as hemoglobin synthesis was not inhibited by any of the steroids tested, further supporting the view that increased TGase activity is associated with erythroid differentiation of FL cells. These results also support the view that the role of TGase in differentiating FL cells is not related to regulation of ODC activity or polyamine biosynthesis.

Methods and Materials. *Leukemia cells.* The GM 979 cell line of FL cells was obtained from Dr. Gary Lyman, Department of Internal Medicine, University of South Florida, Tampa, Florida. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 μg/ml penicillin, and 100 μg/ml streptomycin at 37°C in a 5% CO_2 atmosphere.

Enzyme assays. TGase activity was determined by using the standard method of incorporating [^{3}H]putrescine (New England Nuclear Corp., Boston, Mass.) into *N,N'*-dimethylcasein (Calbiochem-Behring Corp., LaJolla, Calif.) in the presence of Ca^{2+} (13). One unit of enzyme activity was defined as nanomoles of putrescine incorporated per 30 min with 0.4 mg *N,N'*-dimethylcasein as acceptor substrate.

TGase specific activity staining. TGase specific fluorescence staining after electrophoresis of the cell lysate on agarose plates, including photography under UV light, was performed exactly as described (14). GM 979 cells (1×10^5/ml) were cultured with or without DMSO (280 m*M*) for 2 or 4 days, collected, and washed with Tris–HCl buffer (50 m*M* Tris, 150 m*M* NaCl, pH 7.5). The cells were then packed by centrifugation at 2000*g* for 10 min, and lysed by adding an equal volume of Tris buffer and freezing and thawing twice. Eighty microliters of cell lysates were applied to the well in agarose plates, followed by electrophoresis and activity staining with dansylcadaverine.

Hemoglobin determination. FL cells were collected and washed with phosphate-buffered saline solution (pH 7.5) and lysed by freezing and thawing 5×10^7 cells/ml. Following centrifugation at 20,000*g* for 30 min, the supernatant was collected and assayed for hemoglobin content as described (15). Human hemoglobin (Sigma Chemical Co., St. Louis, Mo.) was used as a standard.

Experimental design. Cultures of GM 979 cells, 10^5/ml, were incubated either with inducing agents, DMSO or *n*-butyric acid, or with the inducing agent plus dexamethasone (Merck Research Laboratories, West Point, Pa.), triamcinolone, progesterone (Sigma Chemical Company), or epicortisol (Research Plus Steroids, Inc., Denville, N.J.) for 4 days. The cells were then collected and assayed for TGase activity and hemoglobin content. Cell cultures without DMSO and incubated with hormones alone showed no detectable differences in cell proliferation rate and the number of cells harvested were essentially the same at 4 days. The level of TGase activity and hemoglobin was calculated in regard to units of enzyme per milligram protein or microgram of hemoglobin per milligram total protein.

Experimental results. TGase activity was present in cell lysates of untreated GM 979 cells in the range of 4–6 units/mg protein. Earlier studies showed that the activity of this enzyme increased in a dose-dependent manner when the cells were treated with DMSO at the time of culture initiation (9). Maximum increases occurred with 280 m*M* DMSO per culture. As is apparent in Table I, marked increase in TGase activity occurred over a 2- to 4-day period of culturing the FL cells with DMSO. Hemoglobin content also markedly increased over a period of 2–6 days. Treatment of FL cells with another inducing agent, *n*-butyric acid (1.4 m*M*), effectively increased TGase activity as well as hemoglobin synthesis (Table I). Increased TGase activity was also detected by TGase activity staining after electrophoresis of cell lysates on agarose plates (Fig. 1). The enzyme in DMSO-treated FL cells showed the same electrophoretic mobility as that of untreated FL cells. However, the apparent activity in DMSO-treated cells was considerably higher than in untreated cells and the activity in 4-day cultured cells was much higher than in 2-day cultured cells. This is consistent with the filter paper assay results.

The data in Table II show that the increased activity of both TGase and hemoglobin was inhibited in cultures incubated with DEX, with a dose-related inhibition. As little as 1×10^{-9} *M* DEX resulted in a consistent decrease in both TGase and hemoglobin levels

TABLE I. EFFECT OF DMSO AND *n*-BUTYRIC ACID ON INCREASE OF TGASE ACTIVITY AND HEMOGLOBIN SYNTHESIS IN FL CELLS

	Activity in cell lysates[b]			
	DMSO treatment		*u*-Butyric acid treatment	
Days after treatment[a]	TGase (u/mg protein)	Hemoglobin (μg/mg protein)	TGase (u/mg protein)	Hemoglobin (μg/mg protein)
0	4.7 ± 1.8	2.3 ± 0.4	ND[c]	N.D.
1	8.8 ± 1.2	3.7 ± 1.0	6.2 ± 2.3	4.0 ± 1.3
2	26.5 ± 3.4	19.0 ± 3.5	28.5 ± 4.5	24.5 ± 6.0
3	56.4 ± 10.1	80.3 ± 5.9	59.1 ± 3.0	102.0 ± 14.5
4	69.0 ± 7.5	122.6 ± 12.2	58.5 ± 6.4	161.8 ± 8.8
5	63.3 ± 8.4	147.2 ± 9.3	N.D.	N.D.
6	61.8 ± 4.2	168.1 ± 10.4	—	—
7	N.D.	170.0 ± 8.5	—	—

[a] GM979 cells (1×10^5/ml) were cultured with 280 m*M* DMSO or 1.4 m*M* *n*-butyric acid for the indicated length of time.
[b] Activity (mean ± SD) of TGase and hemoglobin for three cultures tested per time period.
[c] Not done.

in the DMSO-treated cells. The level of the enzyme and hemoglobin activity was reduced approximately 60% at a dose of 1×10^{-8} *M* DEX or higher. It is noteworthy, however, that increasing the concentration of DEX 10- to 100-fold failed to reduce activity lower than the base line of cultures which had not been treated with DMSO. Another glucocorticoid, triamcinolone acetonide, had similar effects on the levels of both TGase and hemoglobin activity in the DMSO-treated cultures.

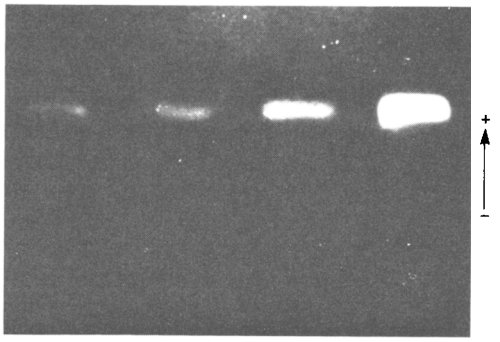

FIG. 1. Activity staining pattern of TGase in GM 979 cells treated with or without DMSO (280 m*M*). The procedure was described under Materials and Methods. Increasing activities of TGase in cell lysates are shown from left to right, line A, 2-day culture of GM 979 cells, line B, 4-day culture of GM 979 cells, line C, 2-day culture of DMSO-treated GM 979 cells, line D, 4-day culture of DMSO-treated GM 979 cells.

Two other hormones were tested, i.e., progesterone, a sex steroid, and epicortisol, the inactive stereoisomer of cortisol. These steroids were found to have little effect in altering TGase activity and hemoglobin synthesis in the FL cells. Thus, the inhibition of TGase and hemoglobin activity was glucocortical steroid specific. It is important to note that the concentrations of the hormones used in this study showed no significant effect on cell proliferation (Table II).

Hemoglobin synthesis induced by *n*-butyric acid was not inhibited in FL cells by any of the steroids tested (Table II). Similarly TGase activity in these cells also was not inhibited by the steroids. Previous studies had shown that addition of the inducing agent directly to FL lysates did not enhance TGase activity (9). As a similar control in the present study, DEX and triamcinolone acetonide did not change TGase activity in the FL cell lysates. FL cells cultured with or without DMSO or *n*-butyric acid for 4 days were lysed and the cell lysates were preincubated with the different steroids at 4 or 37°C for 30 min, followed by determination of the TGase activities. The enzyme activities in the lysates, which were preincubated at 37°C, were consistently lower than that at 0°C. No changes were detectable in either the treated samples as compared to the

TABLE II. EFFECT OF STEROID HORMONES ON TGASE ACTIVITY AND HEMOGLOBIN SYNTHESIS IN FL CELLS TREATED WITH DMSO OR *n*-BUTYRIC ACID

Inducer	Treatment[a]	Conc. (*M*)	Cell growth (10^6/ml)	Activity in cell lysates[b] TGase activity (u/mg protein)	Activity in cell lysates[b] Hemoglobin (μg/mg protein)
DMSO	None (control)		1.03 ± 0.13	53.2 ± 5.5	102 ± 15
(280 m*M*)	Dexamethasone	1×10^{-9}	1.04 ± 0.06	37.3 ± 7.1	83 ± 8
		1×10^{-8}	1.07 ± 0.09	17.0 ± 4.9	39 ± 11
		1×10^{-7}	0.98 ± 0.18	18.4 ± 5.2	29 ± 7
		1×10^{-6}	0.90 ± 0.07	20.7 ± 3.0	27 ± 10
	Triamcinolone acetonide	1×10^{-9}	0.95 ± 0.15	29.4 ± 6.7	66 ± 4
		1×10^{-8}	1.08 ± 0.08	18.1 ± 9.2	31 ± 10
		1×10^{-7}	0.96 ± 0.12	23.9 ± 4.9	31 ± 5
	Progesterone	1×10^{-7}	0.94 ± 0.10	61.2 ± 9.8	110 ± 8
		1×10^{-6}	1.10 ± 0.04	49.5 ± 10.5	96 ± 12
	Epicortisol	1×10^{-7}	0.90 ± 0.15	47.3 ± 3.9	107 ± 5
		1×10^{-6}	0.96 ± 0.09	40.2 ± 4.0	97 ± 14
n-Butyric acid	None (control)		0.92 ± 0.10	49.8 ± 7.6	118 ± 10
(1.4 m*M*)	Dexamethasone	1×10^{-9}	0.98 ± 0.13	45.2 ± 5.0	125 ± 7
		1×10^{-8}	1.00 ± 0.05	49.2 ± 6.2	110 ± 17
		1×10^{-7}	0.95 ± 0.09	56.7 ± 1.7	120 ± 3
		1×10^{-6}	0.90 ± 0.19	49.5 ± 2.8	122 ± 13
	Triamcinolone acetonide	1×10^{-9}	0.90 ± 0.22	45.0 ± 5.4	115 ± 5
		1×10^{-8}	0.97 ± 0.10	49.8 ± 1.9	118 ± 6
		1×10^{-7}	0.95 ± 0.05	50.2 ± 1.6	120 ± 4
	Progesterone	1×10^{-7}	1.02 ± 0.08	56.6 ± 4.8	108 ± 10
		1×10^{-6}	0.96 ± 0.15	51.3 ± 3.2	125 ± 3
	Epicortisol	1×10^{-7}	0.92 ± 0.07	55.8 ± 1.4	128 ± 4
		1×10^{-6}	0.99 ± 0.12	48.5 ± 2.4	120 ± 8

[a] GM979 cells (1×10^5/ml) were cultured in medium containing DMSO or *n*-butyric acid and indicated concentration of hormones.

[b] Activity (means ± SD) of TGase and hemoglobin of three determinations on Day 4 of culture.

untreated samples when they were preincubated at the same temperature (Table III).

Discussion. The *in vitro* differentiation of FL cells has become a useful model system for the investigation of the biochemical events involved in cell differentiation. Treatment of FL cells with a large variety of inducing agents, including DMSO and *n*-butyric acid, induces differentiation of these cells in culture along the erythroid pathway. The process of differentiation is characterized by a limited proliferative capacity of committed cell populations and a number of morphological and biochemical changes characteristic of erythropoiesis, i.e., accumulation of globin mRNA and globin chain synthesis, increases in heme synthesis, and appearance of erythrocyte-specific membrane antigens and membrane proteins (16). The molecular basis of these commitment processes is still poorly understood. However, some evidence has accumulated which indicated that different agents with different biological activities may induce differentiation by different pathways. Agents such as DMSO, *N,N'*-dimethylformamide, and bisacetyldiaminopentane activate ODC during a latent period of differentiation (12, 17). This activation occurs even if subsequent cell differentiation is inhibited. The stimulated ODC activity then may be inhibited by DEX and specific inhibitors of polyamine biosynthesis. These inhibitors also suppress the induction of FL cell differentiation. In contrast, inducing agents such as *n*-butyric acid and actinomycin D, which have little or no effect on ODC activity, are not inhibited by DEX and inhibitors of polyamine synthetic enzymes (12).

In the present study, increased TGase ac-

TABLE III. EFFECT OF STEROID HORMONES ON TGASE ACTIVITY IN CELL LYSATES OF FL CELLS CULTURED WITH OR WITHOUT DIFFERENTIATION-INDUCING AGENTS

Inducer	Treatment[a]	TGase activity I[b] (u/mg protein)	TGase activity II[c] (u/mg protein)
None	None	13.7 ± 2.0	11.8 ± 1.4
	Dexamethasone	13.7 ± 1.0	11.1 ± 2.1
	Triamcinolone acetonide	13.4 ± 2.4	12.1 ± 1.5
DMSO	None	74.8 ± 9.2	67.0 ± 7.1
	Dexamethasone	76.3 ± 8.6	64.6 ± 4.0
	Triamcinolone acetonide	74.1 ± 5.5	70.1 ± 4.7
n-Butyric acid	None	67.5 ± 7.0	54.7 ± 3.4
	Dexamethasone	69.4 ± 4.5	56.9 ± 5.9
	Triamcinolone acetonide	66.4 ± 3.2	56.2 ± 4.0

[a] GM979 cells (1×10^5/ml) cultured with or without DMSO or *n*-butyric acid for 4 days were lysed. The cell lysates were preincubated with steroids (1×10^{-5} *M*) for 30 min followed by determination of TGase activities.
[b] TGase activity measured after preincubation at 4°C for 30 min.
[c] TGase activity measured after preincubation at 37°C for 30 min.

tivity in differentiating FL cells was observed not only by standard filter paper method utilizing the incorporation of [^{3}H]putrescine into *N,N'*-dimethylcasein, but also by the use of a densylcadaverine-specific staining assay after electrophoresis of cell lysates on agarose plates. In addition, earlier studies showed that treatment of controls, i.e., the lymphoma cell line YAC-1 or erythrocytes with these inducing agents for the same periods of time did not enhance TGase activity in the cells (9). Furthermore, the present studies indicated that reduction of hemoglobin synthesis by DEX paralleled the reduction of TGase activity in DMSO-treated FL cells. Thus, a close relationship appeared evident between the increased TGase activity and differentiation of the FL cells.

TGase has recently been implicated as the enzyme which conjugates putrescine and possibly spermidine and spermine to glutamine residues of proteins (11, 18, 19). It has been reported that conjugated polyamines increased during rat liver regeneration. An increase in the specific activity of TGase parallel the increase of putrescine–protein conjugate (10). Such results suggests that putrescine–protein conjugates may result in the putrescine attachment to glutamine residues of ODC. The incorporation of putrescine to ODC *in vitro* resulted in a stoichiometric decrease in enzyme activity (11). These reports suggested a role for TGase in regulation of polyamine biosynthesis and/or ODC activity. Although this seems to be a plausible hypothesis, the fact that *n*-butyric acid does not induce ODC activity (12) but induces TGase activity in FL cells indicated that induction of TGase activity is independent of ODC activity. Moreover, that glucocorticoid has no effect on *n*-butyric acid induced TGase activity further suggests that the induction of TGase in FL cells may not be related to the regulation of ODC.

Brickbichler *et al.* (6) have studied a variety of normal and transformed cells in culture and reported that low levels of TGase activity are essential for proliferative growth. The continuous increase of TGase activity during exponential growth of DMSO-treated FL cells shows no inverse correlation with cell proliferation. However, FL cells once committed to terminal differentiation eventually lost their proliferative capacity. It is possible that the high levels of TGase activity in differentiating FL cells may thus be related to stabilizing cell membranes by crosslinking proteins which are essential for the irreversible arrest of cell division.

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