Alterations in Glycosyltransferase Levels in Mouse Erythroleukemia Cells during Erythroidifferentiation and Cell Growth

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ABSTRACT

Mouse erythroleukemia (MEL) cells contained galactosyltransferase (GT), fucosyltransferase (FT), and N-acetylglucosaminyltransferase activities and only a small amount of sialyltransferase activity. Dimethyl sulfoxide-treated MEL cells, undergoing erythroidifferentiation, contained twice as much GT, 20 to 50% less FT, and approximately the same amount of N-acetylglucosaminyltransferase activities as did untreated cells. When log-phase MEL cells were used as the starting culture, GT activity remained constant during log phase but decreased to 30 to 60% of the log-phase level as the cells reached stationary phase. Under these conditions, FT activity increased more than two-fold as the cells reached stationary phase. When stationary-phase MEL cells were used as the starting culture, GT activity increased as the cells entered log phase and decreased as the cells returned to stationary phase. FT activity decreased initially and then increased as the cells reached stationary phase. Whether log-phase or stationary-phase MEL cells were used as the starting culture, cells maintained in dimethyl sulfoxide always contained more GT and less FT activity than did untreated cells. Since the changes in GT and FT activities were dependent on the concentration of dimethyl sulfoxide used and occurred prior to the onset of hemoglobin synthesis, it is possible that they are related to erythroidifferentiation in MEL cells.

INTRODUCTION

Friend virus-induced MEL cells are neoplastic cells that can be induced to differentiate in vitro along the erythroid pathway by treatment with DMSO (6) or other chemical agents (15). On culture with inducers, MEL cells are characterized by a reduction in proliferative capacity (6, 8) and many morphological and biochemical changes similar to those observed during normal, erythropoietin-regulated erythropoiesis (10, 15). These include a number of alterations in surface membranes, such as the increase in certain mouse erythrocyte antigens (11), changes in membrane permeability (e.g., Ref. 7), changes in agglutinability by various lectins (5, 19), an increase in the synthesis of spectrin (4), and a decrease in the glycosidically bound sialic acid content (3). Changes in membrane structure and/or function may be important in MEL cell erythroidifferentiation, and many proteins involved in these changes are glycoproteins (15).

Glycosyltransferases are a group of enzymes responsible for the biosynthesis of the oligosaccharide portion of glycoproteins (21). It has been suggested that the carbohydrate moiety of glycoproteins and glycosyltransferases are involved in many cell membrane recognition phenomena (23). These include cellular adhesion and cell-cell interaction, the immune response and antigenicity, and the selective binding of enzymes, hormones, and toxins. These activities are also altered in neoplastic cells (16, 25). In view of the potential significance of glycosyltransferases, we have studied the alteration of glycosyltransferase levels of MEL cells during DMSO-induced erythroidifferentiation and at different growth phases.

MATERIALS AND METHODS

Cell Culture. MEL cells of clone DS-19 (17) were grown in Glasgow’s minimal essential medium supplemented with penicillin (50 units/ml), streptomycin (5 μg/ml), and 15% (v/v) heat-inactivated fetal bovine serum as described (6, 22). Cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air. Cell numbers were determined by a Model 5725 Z1 Coulter Counter (Coulter Electronics, Hialeah, Fla.) or with the aid of a hemocytometer. Cell viability was judged by trypan blue exclusion. Unless otherwise stated, DMSO treatment was carried out in the same medium by the addition of 1.8% DMSO (v/v) to exponentially growing MEL cells seeded at 5 × 10⁴ cells/ml. The percentage of differentiated cells (containing enough hemoglobin to be detected) in a culture was measured by a benzidine-hydrogen peroxide cytological staining reaction (18).

Preparation of Cell-free Extract. After 5 days of growth, unless otherwise stated, cell cultures were centrifuged at 250 × g for 15 min at 4°C. Both cell pellets and culture media were stored at −30°C at least overnight. The cell-free extracts were prepared by thawing cell pellets and adding cold 0.2% (v/v) Triton X-100 in Hanks’ medium to yield 10⁷ cells/ml. The mixture was briefly vortexed. No intact cells remained as determined by microscopic examination. Protein concentration was determined according to the method of Lowry et al. (14) with bovine serum albumin as a standard.

Optimal GT and FT activities were obtained when cells were solubilized in 0.1 to 0.5% Triton X-100. Homogenization of MEL cells with a Dounce homogenizer resulted in a preparation with much less GT and FT activities. However, if this preparation was assayed in the presence of 0.1% Triton X-100, these activities were similar to those obtained using the 0.2% Triton X-100 treatment alone. Therefore, homogenization was omitted.

Glycosyltransferase Assay. Details for the assays of ST, GT, FT, and NGT activities have been described earlier (13). The assay mixture for sialyltransferase contained: 10 μl of 0.25
m sodium phosphate buffer, pH 7.0, with 50 mM MgCl₂; 10 µl of desialylated fetuin (50 mg/ml) (2); 30 nmol of CMP-[¹⁴C]-sialic acid (2 cpm/pmol); and 50 µl of cell extract from 2 x 10⁶ cells. The assay mixture for GT contained: 10 µl of 0.75 m sodium cacodylate, pH 7.0, with 0.19 m MnCl₂; 10 µl of fetuin free of sialic acid and galactose (50 mg/ml) (19); 6.5 nmol of UDP-[³H]galactose (13.6 cpm/pmol); and 25 µl of cell extract from 2.5 x 10⁶ cells. The assay mixture for FT contained: 10 µl of 0.375 m Tris-HCl, pH 6.8, with 0.12 m MnCl₂ and 1.2 mm ATP; 10 µl of ovalbumin (50 mg/ml); 2 nmol of UDP-N-acetyl[⁶⁻³H]glucosamine (47 cpm/pmol); and 50 µl of cell extract from 2 x 10⁶ cells. Final volumes of all assays were made to 80 µl with serum-free medium. Incubations were carried out at 37°C for 1 hr for the GT and FT assays and for 2 hr for the ST and NGT assays. The reactions were stopped by adding 1 ml of 10% trichloroacetic acid, and the mixture containing the glycosylated product was poured on to Millipore filters (HAWPO2500; Millipore Corp., Bedford, Mass.). The filters were washed 3 times with 1-ml aliquots of 10% trichloroacetic acid, put into vials with 7.5 ml of Aguasol, and counted in a Beckman LS-250 liquid scintillation spectrometer (Beckman, Mountaineide, N. J.).

All glycosyltransferase assays were performed within the linear range of both the amount of cell extract and the incubation time. For example, GT activity was proportional to the amount of extract up to 6 x 10⁶ cells/assay and linear with time up to 1.5 hr; therefore, most GT assays were performed with cell extract from 2.5 x 10⁶ cells and an incubation time of 1 hr.

Components of the culture medium and fetuin (Spiro method) were from Grand Island Biological Company, Grand Island, N. Y. UDP-galactose, UDP-N-acetylgalactosamine, ovalbumin, DMSO, benzidine dihydrochloride, and Triton X-100 were from Sigma Chemical Company, St. Louis, Mo. Aqasol, CMP-[¹⁴C]sialic acid, UDP-[³H]galactose, and UDP-N-acetyl[⁶⁻³H]glucosamine were from New England Nuclear, Boston, Mass.; and GDP-[¹⁴C]fucose was from Amersham, Chicago, Ill.

RESULTS

MEL cells were cultured with or without 1.8% DMSO for 5 days. More than 80% of DMSO-treated cells were differentiated as judged by their being stained in the benzidine-hydrogen peroxide reaction, whereas less than 2% of the untreated cells were differentiated. Cell extracts prepared from untreated MEL cells contained GT, FT, and NGT activities and a small amount of ST activity (Table 1). Cell extracts prepared from DMSO-treated MEL cells contained twice as much GT and 20 to 50% less FT activity compared to cell extracts prepared from untreated cells. DMSO, at concentrations of 2% or higher, resulted in a decrease in the percentage of benzidine-positive cells and an increase in cell death. GT activity, like the percentage of benzidine-positive cells, increased linearly with increasing DMSO concentration up to 2% and then decreased, whereas FT activity decreased linearly with increasing DMSO concentrations up to 1.5%. These results suggest that the alterations of GT and FT levels in MEL cells were associated with the extent of differentiation induced by DMSO.

The proliferative capacity of differentiated MEL cells is decreased as compared to undifferentiated cells (6, 8). Therefore, it was of interest to measure GT and FT levels in DMSO-treated and untreated MEL cells at different stages of growth. Cells were maintained in exponential growth for 2 or 3 days by diluting the cells daily to 10⁶ cells/ml. The doubling time, under these conditions, was about 10 hr (Chart 2B). At Day 0, the cells were reseeded at 4.5 x 10⁵ cells/ml in medium with or without 1.8% DMSO. Glycosyltransferase activities in MEL cell culture medium were probably due to release from live cells rather than from lysis of dead cells, since more than 95% of the cells in both untreated and DMSO-treated cultures were alive, as judged by trypan blue uptake.

Chart 1 shows the effect of growing cells for 5 days in various concentrations of DMSO on GT and FT activities. The percentage of benzidine-positive cells increased linearly with DMSO concentration up to 1.8%, without any significant effect on final cell density. DMSO, at concentrations of 2% or higher, resulted in a decrease in the percentage of benzidine-positive cells and an increase in cell death. GT activity, like the percentage of benzidine-positive cells, increased linearly with increasing DMSO concentration up to 2% and then decreased, whereas FT activity decreased linearly with increasing DMSO concentrations up to 1.5%. These results suggest that the alterations of GT and FT levels in MEL cells were associated with the extent of differentiation induced by DMSO.

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that, there was a linear increase in the percentage of benzidine-positive cells (6) in DMSO-treated cultures but not in untreated cultures. Extracts from untreated and DMSO-treated cells contained more protein in log phase than in stationary phase. DMSO-treated cells contained an amount of protein either equal to or slightly smaller than that in untreated cells (Chart 2A). Generally, cells in log phase contained higher GT activity than did stationary-phase cells, whether the activity was calculated per cell or per mg of protein (Chart 3A). DMSO treatment resulted in an increase of GT activity in cells both in the log phase and the stationary phase. For FT activity, the situation was reversed. Stationary-phase cells contained more FT activity than did cells in log phase, and DMSO treatment decreased FT activity of cells in both phases (Chart 3B). These results suggest that MEL cells contained different amounts of GT and FT activity at different growth stages and that DMSO altered the GT and FT levels independently of cell growth.

To further support the above conclusions, cells in stationary phase were used in the initial cultures. Cells were maintained in stationary phase for 2 to 3 days and then reseeded at 6.5 × 10⁶ cells/ml in medium with or without DMSO. A lag in growth was seen at Day 1 in the untreated culture which had at this time a doubling time of 21 hr (Chart 4B). By the second day, the log-phase multiplication rate resumed, and the culture had a 12-hr doubling time. A final cell density of 3 × 10⁶ cells/ml was reached at Day 5. In DMSO-treated cultures, there was a transient lag in multiplication rate, and a 16-hr doubling time was noted on the second day. The cell density at plateau was only 1.5 × 10⁶ cells/ml. The percentage of benzidine-positive cells in DMSO-treated cultures initiated from stationary-phase MEL cells was lower than that initiated from log-phase cells.

without added DMSO. The untreated cells, after a slight lag, grew exponentially for nearly 2 days with a doubling time of 12 hr. The cultures with DMSO grew exponentially for nearly 3 days with a doubling time of 13 hr. Both types of culture reached plateau stage at Day 4 with a cell density of approximately 3 × 10⁶ cells/ml. No significant difference in the percentage of benzidine-positive cells was detected in DMSO-treated cultures compared to untreated cultures at Day 1. After growth, differentiation, and protein concentration in cultures initiated with cells in log phase. Cells were diluted daily to a density of 10⁶ cells/ml for 2 or 3 days and then at Day 0 were seeded at 4.5 × 10⁵ cells/ml with or without added 1.8% DMSO. In A, the protein concentration of extracts prepared with 0.2% Triton X-100 of untreated (O) and DMSO-treated (D) cells was determined at the times indicated as described in "Materials and Methods." In B, the cell density of untreated (O) and DMSO-treated (D) cells and the percentage of benzidine-positive cells of untreated (O) and DMSO-treated (D) cells were determined at the times indicated as described in "Materials and Methods."
As expected, the amount of protein per cell was low in the starting, stationary-phase cultures and reached a peak at a time when the cells had the highest multiplication rate (Chart 4A). Cells that reached stationary phase at Day 5 had the same reduced amount of protein per cell as did those at stationary phase at Day 0. The GT activity levels, calculated per cell or per mg protein, in untreated cells in log phase were higher than those of both starting and final stationary-phase cells (Chart 5A). DMSO induced an additional increase in GT activity as the cells passed from stationary phase into log phase. This DMSO-induced increase in GT activity was maintained throughout the subsequent stationary phase.

In untreated MEL cultures initiated from cells in stationary phase, FT activity per mg protein was initially high (Chart 5B). It decreased during log phase and then increased in the subsequent stationary phase. In DMSO-treated cultures, FT activity, calculated on the basis of the number of cells extracted or on the mg of protein used, followed the same pattern, but the decrease in FT activity during log phase was more pronounced, and it was maintained throughout the subsequent stationary phase. In untreated cultures, FT activity, when determined on the basis of number of cells extracted, increased initially, decreased in log phase, and increased in subsequent stationary phase. When Day 1 and 2 values of FT activity calculated on the basis of number of cells and of mg protein were compared, an apparent discrepancy was noted (Chart 5B). We do not have an explanation for this discrepancy at present. These results were reproducible in 4 separate experiments.

DISCUSSION

This report demonstrated that in MEL cells DMSO induced an increase in cellular GT activity and a decrease in FT activity.

These changes occurred a day earlier than the onset of hemoglobin synthesis, and they were dependent upon the concentration of DMSO and on the length of DMSO exposure. Similar treatment of human hepatoma cells and Chang cells (derived from normal human liver) with 1.5% DMSO did not result in changes in glycosyltransferase activities or synthesis of hemoglobin. These results suggest an association between DMSO-induced erythrodifferentiation and alteration in glycosyltransferase activity of MEL cells. It was reported (5) that the sensitivity to agglutination by several plant lectins and the number of receptors for them were changed during the induced erythrodifferentiation of MEL cells. For example, the number of receptors for ricin, which binds specifically to a galactose moiety on the cell membrane, increased 3-fold in DMSO-treated MEL cells compared to untreated cells. Changes in glycosyltransferase levels with development in vivo have been reported. For example, in intact undifferentiated rat intestinal crypt cells, GT, FT, and NGT activities were higher and ST activity was lower than in differentiated mature villus cells (26). In another report (20), collagen GT and collagen glycosyltransferase activities in developing chick embryos increased from the 4th to the 16th day of growth and decreased thereafter.

The results reported here demonstrated that MEL cells in log growth contained higher levels in GT activity and lower levels of FT activity than did cells in stationary phase. Glycosyltransferases also have been related to cell growth in other studies. The growth rate of transformed mouse fibroblasts but not untransformed fibroblasts was reduced by dibutyryl cyclic adenosine 3':5'-monophosphate (12), and dibutyryl cyclic adenosine 3':5'-monophosphate also markedly reduced the cell surface ST and GT activities of transformed but not untrans-
formed fibroblasts (24). Moreover, it was shown (1) that the levels of ST, GT, FT, and NGT activities in both the plasma membrane and the microsomal fractions of mouse fibroblasts expressed a "peak pattern" with maximal activities only during the late-G1-early-S phases of the cell cycle. These data suggested that dividing mouse fibroblasts contained higher levels of ST, FT, and NGT activities than did undividing fibroblasts.

Another interesting aspect of cell growth is cell density-dependent growth control, which is lost in malignant cells (16). It is possible that cells growing at different cell densities may contain different levels of glycosyltransferase activities. Our results did not discriminate between whether the alterations in glycosyltransferase activity levels were related to cell density changes or to changes in the number of cells entering the cell division cycle. It is not known how or if the levels of glycosyltransferases affect cell growth. It was observed that the length of surface carbohydrates on normal cells increased as they reached confluency (9). Therefore, the levels of glycosyltransferases might affect cell growth by their capability to synthesize different types of surface carbohydrates. However, glycosyltransferase may play a more direct role in regulating cell growth. Shur and Roth (23) have proposed an enzyme-substrate binding mechanism of surface galactosyltransferase in regulating cell-cell interaction.

REFERENCES

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