Journal of Cellular Biochemistry 44:93–105 (1990)

Alteration of Lacto-Series Glycolipid Glycosyltransferase Activities in Human Colonic Adenocarcinoma DLD-1 Cells After Culture in *N,N*-Dimethylformamide-Containing Medium

Eric H. Holmes and Thomas G. Greene

Pacific Northwest Research Foundation, 720 Broadway, Seattle, Washington 98122

Human colonic adenocarcinoma DLD-1 cells were grown under conditions which induce characteristics of differentiated cells using medium containing 0.8% *N,N*-dimethylformamide in order to study alterations in glycosphingolipid glycosyltransferase activities during this process. Analysis of biosynthetic reactions involved in lacto-series antigen synthesis revealed no changes in the specific activities of either β1→4galactosyltransferase or α1→3/4fucosyltransferase with *N,N*-dimethylformamide treatment. However, a dramatic decrease of from 14- to 20-fold in the β1→3*N*-acetylglucosaminyltransferase activity was observed in the treated cells. This enzyme catalyzes the rate-limiting step in lacto-series core chain synthesis. This is consistent with the pattern of regulation of lacto-series antigen expression found to occur during oncogenesis in human colonic mucosa (Holmes EH, Hakomori S, Ostrander GK: J Biol Chem 262:15649, 1987). Total glycolipids from untreated and *N,N*-dimethylformamide-treated cells were isolated and subjected to TLC immunostain analysis and solid phase radioimmunoassay with a series of monoclonal antibodies specific for lacto-series-based carbohydrate antigens. A decrease of about 2-fold or less in the quantity of lacto-series antigens was observed as a consequence of *N,N*-dimethylformamide treatment in both neutral glycolipid and ganglioside fractions. The results suggest that only very low levels of β1→3*N*-acetylglucosaminyltransferase activity are required for the steady state expression of significant levels of lacto-series based glycolipids and that modulation of its activity levels by *N,N*-dimethylformamide treatment in DLD-1 cells represents a convenient in vitro system for studying aspects of regulation of lacto-series antigen expression.

Key words: β1→3*N*-acetylglucosaminyltransferase, cancer-associated carbohydrate antigens, biosynthesis, glycosphingolipid

Abbreviations used: PBS, phosphate-buffered saline, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid; DMF, *N,N*-dimethylformamide; HPTLC, high-performance thin-layer chromatography; glycolipids are designated according to the recommendations of the IUPAC Nomenclature Committee, but the suffix OseCer is omitted [26].

Received May 15, 1990; accepted June 28, 1990.

Alteration in cell surface carbohydrate structures and accumulation of specific classes of carbohydrate antigens characterize the membrane phenotype of many human cancers. Altered carbohydrate expression in, for example, colonic adenocarcinomas has been regarded to be "oncofetal" in nature, i.e., structures appear during stages of normal development, are absent in adult tissues, and reappear in association with oncogenesis [1,2]. A number of studies have established the accumulation of a diverse series of oncofetally expressed lacto-series-based carbohydrate structures on glycolipids in human colonic adenocarcinomas [see for review 3,4]. Further evidence has indicated that this altered expression is associated with the activation of a normally unexpressed β1→3*N*-acetylglucosaminyltransferase resulting in increased lacto-series core chain synthesis leading to further derivatives [5]. Thus, the differentiation status of a particular cell line or tumor may have a significant impact on the nature of the carbohydrate structures expressed. The effect of differentiation inducers on cell surface carbohydrate expression might then be expected to shed light on this process.

Normal intestinal epithelial cells undergo characteristic morphological and functional changes during differentiation. Growth of colonic adenocarcinoma cells in culture in the presence of certain polar solvents such as *N,N*-dimethylformamide (DMF) has been shown to induce characteristics of differentiated cells and to decrease or eliminate tumorigenicity. Treatment of DLD-1 and HCT-15 cells with DMF was reported to result in a decreased clonogenicity in soft agar and a very low tumorigenicity in nude mice. Removal of DMF from the culture medium resulted in a reversion to the original growth characteristics [6]. These conditions have presented a reversible mechanism to study aspects of enterocyte differentiation. The results presented in this paper describe the effect of DMF on expression of biosynthetic enzymes involved in lacto-series glycolipid synthesis in DLD-1 cells and the resultant effect on cell surface glycolipid composition. The results indicate that this system generally mimics the mechanism previously described in vivo [5] to regulate antigen production and represents a useful system for studying these changes.

EXPERIMENTAL PROCEDURES

Materials

Human colonic adenocarcinoma DLD-1 cells and monoclonal antibody producing cell lines BE-2, NS-19-9, and 1B2 were obtained from the American Type Culture Collection, Rockville, MD. GDP[^{14}C]fucose (268 mCi/mmol) and UDP[^{14}C]galactose (303 mCi/mmol) were obtained from Amersham, Arlington Heights, IL. UDP[^{14}C]*N*-acetylglucosamine (51 mCi/mmol) was obtained from American Radiolabeled Chemicals, St. Louis, MO. Unlabeled UDPGlcNAc, UDPgalactose, *p*-nitrophenyl-α-L-fucoside, and *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide were obtained from Sigma, St. Louis, MO. Unlabeled GDPfucose was prepared using the method of Ginsburg [7]. Lacto*neo*tetraosylceramide, nLc_4Cer was prepared by desialylation of sialosyllacto*neo*tetraosylceramide isolated from bovine erythrocytes [8]. Desialylation was performed in 1% acetic acid at 100°C for 1 h. Lc_3 was prepared from nLc_4 by overnight hydrolysis with jack bean β-galactosidase in 0.1 M citrate buffer, pH 4.5, containing 0.1% taurodeoxycholate. Lactosylceramide was isolated from human adenocarcinoma tumors [9]. Anti-Le^a and anti-Le^b affinity purified antibodies were obtained from Chembiomed, Edmonton, Alberta. Antibodies AH6 and SSEA-3 were provided by

Dr. Henrik Clausen, Biomembrane Institute, Seattle, WA. All other reagents were of the highest purity commercially available.

Methods

Growth of cells. DLD-1 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. Treatment with dimethylformamide was performed by culturing the cells in medium supplemented with 0.8% dimethylformamide. The cells were harvested and passed every 7–10 days. The cells were scraped, centrifuged, and washed with phosphate-buffered saline (PBS), and stored frozen at −80°C.

Enzyme Assays

β1→4Galactosyltransferase. β1→4Galactosyltransferase activity was determined in reaction mixtures containing 2.5 μmol HEPES buffer, pH 7.0, 30 μg Lc_3, 100 μg taurodeoxycholate, 1 μmol $MnCl_2$, 15 nmol UDP[^{14}C]galactose (15,000 cpm/nmol), 0.5 μmol galactonolactone, and 0.1 mg protein in a total volume of 0.1 ml. The reaction was conducted for 1 h at 37°C and stopped by the addition of 6 μmol of EDTA and 100 μl of $CHCl_3$:CH_3OH (2:1). The entire reaction mixture was streaked onto a 4 cm wide strip of Whatman 3mm paper and chromatographed with water overnight. The glycolipid remaining at the origin was extracted with 2–5 ml washes of $CHCl_3$:CH_3OH:H_2O, (10:5:1). The solvent was removed with a nitrogen stream and dissolved in 20 μl $CHCl_3$:CH_3OH (2:1). An aliquot, 10 μl, was removed and spotted onto an HPTLC plate (Merck, Darmstadt, West Germany) and developed in a solvent of $CHCl_3$:CH_3OH:H_2O (60:40:9) containing 0.02% $CaCl_2$ as a final concentration. Standard glycolipids were visualized by orcinol spray. Radioactive glycolipid bands were located by autoradiography, scraped from the plate, and counted by a liquid scintillation counter. One unit of activity is defined as transfer of 1 pmol of galactose per hour under the conditions of the assay. In these assays no detectable contribution by endogenous glycolipid and glycoprotein acceptors was observed.

α1→3/4Fucosyltransferase. The fucosyltransferase activity was determined in reaction mixtures containing 2.5 μmol HEPES buffer, pH 7.2, 30 μg nLc_4, 100 μg taurodeoxycholate, 1 μmol $MnCl_2$, 0.5 μmol CDPcholine, 15 nmol GDP[^{14}C]fucose (15,000 cpm/nmol), and 15–150 μg protein in a total volume of 0.1 ml. The reaction mixture was incubated for 2 h at 37°C and stopped by the addition of 6 μmol of EDTA and 100 μl of $CHCl_3$:CH_3OH (2:1). The entire reaction mixture was streaked onto a 4 cm wide strip of Whatman 3mm paper, chromatographed with water overnight, and isolated and quantitated as described above. One unit of activity is defined as transfer of 1 pmol of fucose per hour under the conditions of the assay.

β1→3*N*-Acetylglucosaminyltransferase assay. *N*-Acetylglucosaminyltransferase assays were performed in reaction mixtures containing 2.5 μmol of HEPES buffer, pH 7.2, 40 μg of lactosylceramide, 150 μg of Triton CF-54, 0.5 μmol of $MnCl_2$, 0.5 μmol of CDPcholine, 50 nmol of UDP[^{14}C]*N*-acetylglucosamine (5000 cpm/nmol), and 200–400 μg of protein in a total volume of 0.05 ml. The reaction mixture was incubated for 2 h at 37°C, terminated by the addition of 6 μmol of EDTA and 100 μl of $CHCl_3$:CH_3OH (2:1), and quantitated as described above. One unit of activity is defined as transfer of 1 pmol of GlcNAc per hour under the conditions of the assay.

α-Fucosidase. The reaction mixtures contained 30 μmol Na-acetate buffer, pH 5.0, 0.1 μmol *p*-nitrophenyl-α-L-fucoside, and 150 to 250 μg protein in a total volume of 0.2 ml. The reaction was conducted for 1 h at 37°C and stopped by the addition of 1 ml of 0.2 M Na_2CO_3. The activity was determined from the absorbance at 400 nm from the *p*-nitrophenyl product.

β-N-acetylhexosaminidase. The reaction mixtures contained 30 μmol Na-acetate buffer, pH 5.0, 0.1 μmol *p*-nitrophenyl-*N*-acetyl-β- D-glucosaminide, and 150 to 250 μg protein in a total volume of 0.2 ml. The reaction was conducted for 1 h at 37°C and stopped by the addition of 1 ml of 0.2 M Na_2CO_3. The activity was determined as described above.

Protein Determination

Protein concentrations of cell fractions were determined by the method of Lowry et al. [14] using bovine serum albumin as standard.

Extraction of Glycolipids from Cells

Glycolipids were isolated from 3 ml of packed cells from each cell line by extraction with 10 volumes of isopropanol:hexane:water (55:25:20) in a Potter-Elvehjem homogenizer followed by centrifugation at 2,500*g* for 10 min. The insoluble pellet was reextracted with 10 volumes of the same solvent followed by centrifugation. The combined supernatant fractions were concentrated to near dryness and transferred to Spectrapor 3 membrane tubing (Spectrum Medical Industries, Los Angeles, CA) and dialyzed extensively against water. The solution was removed from the dialysis bag and concentrated to near dryness and dissolved in a solvent composed of $CHCl_3$:CH_3OH:H_2O (30:60:8) and subjected to chromatography on DEAE-Sephadex A-25 according to the method of Yu and Ledeen [10] to separate neutral glycolipids from gangliosides. The neutral glycolipid fraction obtained from the passthrough of the DEAE-Sephadex column was concentrated to dryness and placed in a vacuum desiccator over P_2O_5 overnight followed by acetylation with 10 ml of pyridine and 5 ml of acetic anhydride. The acetylated glycolipid fraction was obtained by chromatography on a Fluorisil column [11]. The deacetylated neutral glycolipid fractions and the dialyzed total ganglioside fractions obtained from DEAE-Sephadex chromatography were dissolved in 1 ml of $CHCl_3$:CH_3OH (2:1). An amount of glycolipid corresponding to 20 mg packed cells was spotted for TLC as indicated.

TLC immunostaining of glycolipids. Immunostaining of glycolipids separated on HPTLC plates was performed using the procedure of Magnani et al. [12] as modified by Kannagi et al. [13]. Glycolipids were separated on an HPTLC plate (Baker) using solvent systems composed of $CHCl_3$:CH_3OH:H_2O, (56:38:10) or $CHCl_3$:CH_3OH:H_2O (60:40:9) containing 0.02% $CaCl_2 \cdot 2H_2O$. After development, the plate was dried and soaked for 2 h in 5% bovine serum albumin in PBS to block nonspecific antibody binding. The plate was then incubated in culture supernatants of the derived monoclonal antibodies or 1:1,000 diluted affinity purified commercial anti-Le^a or Le^b antibodies overnight, followed by sequential incubations with 1:1,000 diluted rabbit anti-mouse Ig antibody solution and with ^{125}I-labeled protein A solution. After extensive washes with PBS between each step and after ^{125}I-labeled protein A treatment, the plate was dried and labeled bands were detected by autoradiography.

Solid phase binding assays. Glycolipids were deposited on 96-well vinyl plates in solutions containing 3 μg cholesterol, 5 μg phosphatidylcholine, and total glycolipid corresponding to 20 mg packed cells per ml of absolute ethanol. The glycolipids were

serially diluted in ethanol containing cholesterol and phosphatidylcholine alone. Aliquots of 50 μl each were dispensed into each well and allowed to air dry. The plates were blocked with PBS containing 5% BSA for 2 h followed by incubation with antibody containing culture supernatant for 18 h. The plates were washed extensively with PBS followed by incubation with 1:500 diluted rabbit anti-mouse whole Ig (ICN Immunobiologicals) for 1 h. The plates were again extensively washed with PBS and incubated with ^{125}I-labeled protein A (90,000 cpm/well) for 1 h. The plates were washed again with PBS and the amount of ^{125}I in each well was determined in a gamma counter.

RESULTS

The effect of alteration of apparent differentiation status or growth characteristics of cells on glycosyltransferase enzyme expression and the resultant nature of the cell surface glycolipid composition has been studied using human colonic adenocarcinoma DLD-1 cells as described below.

Treatment of DLD-1 Cells With DMF in Culture

Alteration in cell growth behavior of DLD-1 cells in response to addition of DMF to the culture medium has previously been reported [6]. This condition causes the cells to adopt a more differentiated phenotype and results in a decreased clonigenicity in soft agar and loss of tumorigenicity. As previously found, addition of 0.8% DMF to the culture medium resulted in changes occurring within 4 or 5 days. Cells grown in the presence of 0.8% DMF were characterized by a slower growth rate, altered morphology, and a lower cell saturation density (results not shown). In these studies, cells were treated continuously with DMF for 3 weeks over several cell passages prior to harvest of cells for either glycolipid extraction and profile analysis or enzyme studies.

Effect of DMF Treatment of DLD-1 Cells on Glycolipid Biosynthetic and Hydrolytic Enzyme Activities

Previous results have indicated that expression of lacto-series carbohydrate antigens in colonic tissues is oncodevelopmental in nature [3,4]. Table I shows the specific activities of biosynthetic enzymes involved in lacto-series chain synthesis in crude homogenates of DMF-treated and untreated DLD-1 cells. The specific activity

TABLE I. Specific Activities of Glycosyltransferases From Human Colonic Adenocarcinoma DLD-1 Cells Grown With or Without 0.8% Dimethylformamide in the Culture Medium*

	Experiment 1		Experiment 2	
Enzyme	−DMF (pmol/h/ mg protein)	+DMF (pmol/h/ mg protein)	−DMF (pmol/h/ mg protein)	+DMF (pmol/h/ mg protein)
β1→4Galactosyltransferase	7,500. ± 200	4,900. ± 700	10,100. ± 300	10,400. ± 300
α1→3Fucosyltransferase	1,800. ± 300	1,600. ± 300	1,200. ± 300	1,800. ± 400
β1→3*N*-Acetylglucosaminyltransferase	35. ± 5	1.8 ± 0.4	35. ± 7	2.6 ± 0.6

*The reactions were conducted as described under Methods. Data are shown for two independent replicates of DLD-1 cells treated with 0.8% dimethylformamide.

of β1→4galactosyltransferase, responsible for synthesis of both the short chain precursor lactosylceramide and type 2 core nLc_4 structures, was consistently very high in two separate experiments irrespective of whether the cells were grown in the presence of DMF or not. Similarly, the α1→3/4fucosyltransferase activity determined with nLc_4 as the acceptor, although much lower than the β1→4galactosyltransferase, was highly expressed and rather constant under the two growth conditions. In contrast, the β1→3*N*-acetylglucosaminyltransferase activity, responsible for the synthesis of Lc_3, the first step in lacto-series core chain synthesis, was considerably lower than the other enzymes consistent with its catalyzing the rate-limiting step in lacto-series chain synthesis. In addition, this activity was dramatically reduced between 14- and 20-fold when the cells were grown in the presence of 0.8% DMF. Thin-layer chromatographic analysis of β1→3*N*-acetylglucosaminyltransferase reaction products from DMF-treated and untreated cells is shown in Figure 1. Strong bands corresponding to Lc_3 from transfer of [^{14}C]GlcNAc to lactosylceramide is found in two samples of untreated DLD-1 cells which is only barely detectable in samples of DMF-treated cells.

In order to determine whether the apparent alteration of β1→3*N*-acetylglucosaminyltransferase activity between the DMF-treated and untreated cells is a consequence of reduced biosynthesis or increased degradation of the labeled product in the treated cells, the activity of hydrolase enzymes was also studied. This potential is

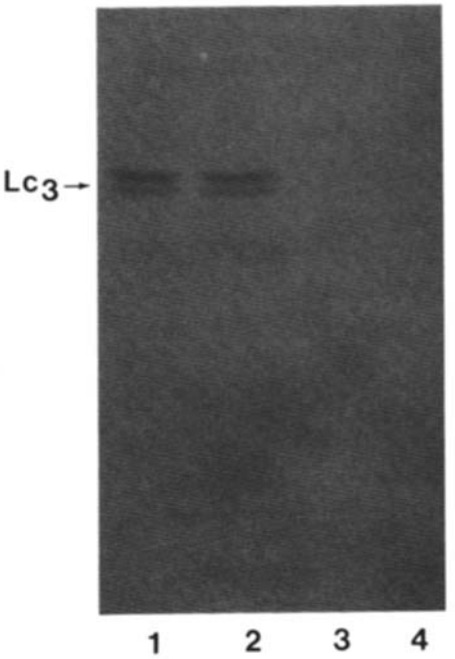

Fig. 1. Autoradiograph of TLC separated β1→3*N*-acetylglucosaminyltransferase reaction products after transfer of ^{14}C-labeled GlcNAc into lactosylceramide catalyzed by crude homogenates of DMF-treated and untreated DLD-1 cells. Lane 1, product from untreated DLD-1 cell homogenate repetition 1; lane 2, product from untreated DLD-1 cell homogenate repetition 2; lane 3, product from DMF-treated DLD-1 cell homogenate repetition 1; lane 4, product from DMF-treated DLD-1 cell homogenate repetition 2. The reactions were conducted as described under Experimental Procedures using 110 to 150 μg of homogenate protein per reaction mixture. The TLC mobility of standard Lc_3 is indicated. The plate was developed in a solvent system composed of $CHCl_3$:CH_3OH:H_2O, 60:35:8.

TABLE II. Specific Activities of Hydrolase Enzymes From Human Colonic Adenocarcinoma DLD-1 Cells Grown With or Without 0.8% Dimethylformamide in the Culture Medium*

Enzyme	Experiment 1		Experiment 2	
	−DMF (nmol/h/ mg protein)	+DMF (nmol/h/ mg protein)	−DMF (nmol/h/ mg protein)	+DMF (nmol/h/ mg protein)
α-Fucosidase	187. ± 7	271. ± 37	197. ± 12	492. ± 63
β-*N*-Acetylhexosaminidase	248. ± 33	272. ± 60	231. ± 29	488. ± 66

*The reactions were conducted as described under Methods. Data are shown for two independent replicates of DLD-1 cells treated with 0.8% dimethylformamide.

TABLE III. Stability of In Vitro Synthesized β1→3*N*-Acetylglucosaminyltransferase Reaction Products From Hydrolysis by Endogenous β-*N*-Acetylhexosaminidase in Reaction Mixtures Containing Extracts from DLD-1 Cells Grown With or Without 0.8% Dimethylformamide in the Culture Medium*

Enzyme extract	Recovery of Product (%)
Repetition 1	
−DMF	100
+DMF	97
Repetition 2	
−DMF	100
+DMF	95

*The reactions were conducted by incubating [^{14}C]GlcNAc-labeled Lc_3 (500 cpm) in the standard β1→3*N*-acetylglucosaminyltransferase reaction mixture without UDP[^{14}C]GlcNAc for 2 h. Radioactivity remaining at the origin of a 4 cm wide strip of Whatman 3mm paper after elution with water was determined and compared to the starting amount.

consistent with findings of activation of brush-border enzymes in human colonic adenocarcinoma cell lines induced to differentiate by other means [15,16]. Table II shows results of analysis of α-fucosidase and β-*N*-acetylhexosaminidase in crude cell homogenates assayed using soluble *p*-nitrophenyl hexose derivatives as substrates. A significant level of both activities was found in DLD-1 cells without DMF treatment. Cells grown in the presence of DMF had generally higher levels of these activities in both samples tested, consistent with the characteristics of more differentiated cells. In order to test the influence of increased hydrolytic activity on apparent β1→3*N*-acetylglucosaminyltransferase activity in these cell homogenates, terminally labeled [^{14}C]GlcNAc containing Lc_3 was incubated with cell homogenates and the degree of hydrolysis determined as shown in Table III. Labeled Lc_3 was highly stable after 2 h incubation irrespective of DMF treatment, with recoveries of 95–100%. These results suggest that the differing biosynthetic activities found could not be ascribable to hydrolysis of the reaction product but reduced expression of β1→3*N*-acetylglucosaminyltransferase in the DMF-treated cells. This was further supported by β1→3*N*-acetylglucosaminyltransferase assays in which *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide was added to protect the ^{14}C-labeled glycolipid product. These assays yielded results equivalent to that found in Table I (results not shown). Presumably membrane-associated or micellar substrates are poorly hydrolyzed compared to those that are soluble. In view of the dramatic reduction in β1→3*N*-acetylglucosaminyltransferase activity compared to other transferases after DMF treatment, its effect on cellular glycolipid profiles of DMF-treated and untreated cells was studied as described below.

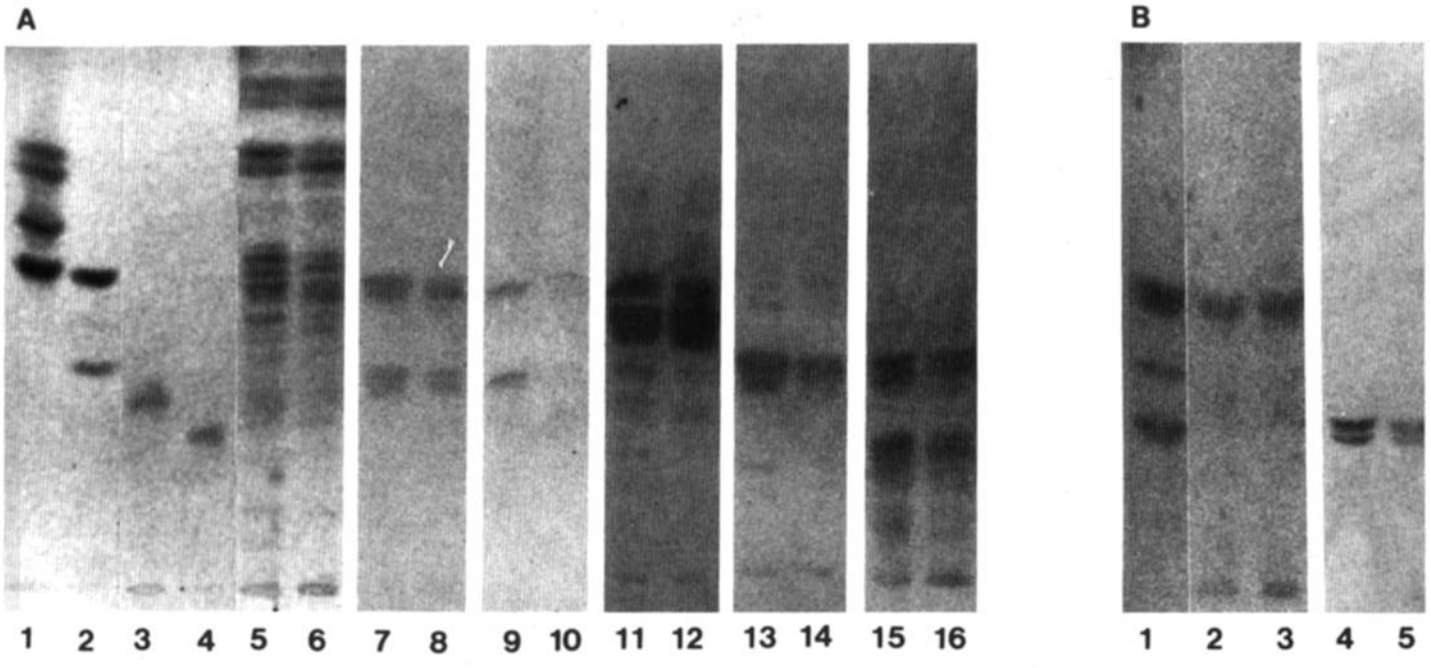

Fig. 2. Thin-layer chromatographic analysis of total neutral glycolipids and gangliosides extracted from DLD-1 cells grown in the presence and absence of 0.8% dimethylformamide. (**A**) Total neutral glycolipids. Lane 1, standard Gb_4, Gb_3, and lactosylceramide (in terms of increasing mobility); lane 2, standard nLc_6 and nLc_4 (in terms of increasing mobility); lane 3, standard $V^3FucnLc_6$; lane 4, standard $III^3V^3Fuc_2nLc_6$; lane 5, total neutral glycolipids from untreated DLD-1 cells; lane 6, total neutral glycolipids from DMF-treated DLD-1 cells. Lanes 7 through 16 show TLC immunostain results with DLD-1 total neutral glycolipids with the indicated antibodies. Lanes 7, 9, 11, 13, and 15 show results obtained with untreated DLD-1 cells. Lanes 8, 10, 12, 14, and 16 show results from DMF treated DLD-1 cells. Lanes 7 and 8, antibody 1B2; lanes 9 and 10, antibody BE2; lanes 11 and 12, anti-Le^a antibodies; lanes 13 and 14, anti-Le^b antibodies; lanes 15 and 16, antibody AH6. The glycolipids shown in lanes 1 to 6 were visualized by orcinol spray. The plate was developed in a solvent system composed of $CHCl_3$:CH_3OH:H_2O, 60:35:8. (**B**) Total gangliosides. Lane 1, standard $VI^3NeuAcnLc_6$, $IV^3NeuAcnLc_4$, and GM_3 (in terms of increasing mobility); lane 2, total gangliosides from untreated DLD-1 cells; lane 3, total gangliosides from DMF-treated DLD-1 cells; lane 4, immunostain analysis of untreated DLD-1 cell gangliosides with antibody NS19-9; lane 5, immunostain analysis of DMF-treated DLD-1 cell gangliosides with antibody NS19-9. Gangliosides shown in lanes 1 to 3 were visualized by resorcinol spray. The solvent system was composed of $CHCl_3$:CH_3OH:H_2O, 60:40:9 containing 0.02% $CaCl_2 \cdot 2H_2O$. The glycolipids corresponding to 20 mg packed cells was spotted in each case.

Glycolipid Profiles of DLD-1 Cells Grown in the Presence and Absence of DMF

Figure 2A shows the neutral glycolipid profiles derived from DLD-1 cells with and without DMF treatment. Both cell fractions showed strong orcinol positive bands corresponding to glucosylceramide and lactosylceramide (lanes 5 and 6). Bands from longer chain glycolipids migrating in the globoside and nLc_4 area were weaker in the DMF-treated cell fraction as were even slower migrating glycolipids, although the chemical quantity was not large in either cell fraction. The significant presence of globoside in both cell fractions in the tetraglycosylceramide region of the TLC plate was established by TLC immunostaining with SSEA-3 antibody which has reactivity with globoside (results not shown). Further TLC immunostain analysis with a variety of antibodies specific for lacto-series based structures is shown in lanes 7 to 16. The major glycolipid structures defined by these antibodies are given in Table IV. In each case significant expression of the particular antigen is found in DMF-treated and untreated cell fractions; however, the staining intensity is generally more intense in the untreated cell fractions, suggesting a quantitative difference in antigen levels exists in the two cell fractions. A similar result is found with total gangliosides from these cells as shown in Figure 2B. The major ganglioside in both cell fractions is GM_3, with slower bands only barely detectable. TLC immunostain analysis with the sialyl-Le^a-specific NS19-9 antibody clearly indicates weaker staining of the appropriate bands in the DMF treated fraction.

TABLE IV. Major Glycolipid Structures Referred to in This Study

Glycolipid	Structure	Antibody reactivity
Gb_4	GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer	SSEA-3
Lactosylceramide	Galβ1→4Glcβ1→1Cer	
GM_3	NeuAcα2→3Galβ1→4Glcβ1→1Cer	
Lc_3	GlcNAcβ1→3Galβ1→4Glcβ1→1Cer	
nLc_4	Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer	1B2
$IV^2FucnLc_4$ (H)	Fucα1→2Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer	BE2
III^4FucLc_4 (Le^a)	Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 4 ↑ Fucα1	anti-Le^a
$III^4IV^2Fuc_2Lc_4$ (Le^b)	Fucα1→2Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 4 ↑ Fucα1	anti-Le^b
$V^3FucnLc_6$	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 ↑ Fucα1	
$III^3V^3Fuc_2Lc_6$	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 3 ↑ ↑ Fucα1 Fucα1	
$III^3IV^2Fuc_2nLc_4$ (Le^y)	Fucα1→2Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 ↑ Fucα1	AH6
$IV^3NeuAcIII^4$ $FucLc_4$ (Sialyl-Le^a)	NeuAcα2→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 4 ↑ Fucα1	NS19-9

Relative Abundance of Various Lacto-Series Structures in DLD-1 Cells With and Without DMF Treatment

Total neutral glycolipid or ganglioside extracts from DLD-1 cell fractions described above were further studied by solid phase radioimmunoassay in order to determine the relative amounts of lacto-series glycolipids in DMF-treated vs. untreated cells. These results are summarized in Figure 3. Total neutral glycolipid and ganglioside fractions, normalized to a constant volume of packed cells, were serially diluted and tested with a variety of antibodies. Binding of antibodies to glycolipids from DMF-treated cells were lower than to untreated cells. The solid phase assay results generally reflect the qualitative results from TLC immunostaining. However, one antibody, BE2, predicted similar antigen levels based on solid phase results but suggested a decrease in the DMF-treated cells by TLC immunostaining. Presumably, this difference relates to basic behavior of BE2 in the two types of assays. Despite this, the solid phase results fit closely the TLC immunostaining results and indicate that the magnitude of the difference is variable and limited to under about 2-fold less in the treated cell fractions, depending on the antigen being tested. These results, in combination with TLC immunostaining band intensities, demonstrate a consistent, although small quantitative difference in levels of lacto-series-based glycolipids occurred in DLD-1 cells grown in the presence or absence of DMF. The magnitude of this difference is considerably less than the effect DMF treatment has on the level of β1→3*N*-acetylglucosaminyltransferase activity. This observation may have important implications on the regulation of synthesis of lacto-series glycolipid structures in human cells as discussed below.

DISCUSSION

Growth of a number of mammalian tumor cells in the presence of polar solvents is known to induce characteristics of mature, differentiated cells [6,17–19]. Application of these conditions to human colonic adenocarcinoma DLD-1 or HCT-15 cells by treatment with DMF also resulted in the reversible induction of differentiated cell growth characteristics [6]. This protocol has been used in this study to monitor the relationship between induction of differentiated cell characteristics and expression of glycosyltransferase activities associated with synthesis of lacto-series tumor-associated carbohydrate antigens expressed on glycolipids.

Analysis of these enzyme activities in DLD-1 cells grown in the presence or absence of 0.8% DMF indicated essentially no effect was induced by DMF on the specific activities of either β1→4galactosyltransferase or α1→3/4fucosyltransferase, two enzymes involved in elongation or terminal modification reactions, respectively. However, a specific reduction of 14- to 20-fold in expression of β1→3*N*-acetylglucosaminyltransferase as a consequence of DMF treatment was observed. This enzyme catalyzes the rate-limiting step in lacto-series glycolipid synthesis, and its pattern of

Fig. 3. Solid phase radioimmunoassay of total neutral glycolipid and ganglioside fractions derived from DMF-treated and untreated DLD-1 cells. The assay was conducted as described under Experimental Procedures. The results from assay of neutral glycolipid fractions with antibodies 1B2, BE2, AH6, anti-Le^a, and anti-Le^b and ganglioside fractions with NS19-9 antibody are shown. (●) antibody binding to serial dilutions of untreated DLD-1 cell fractions. (×) antibody binding to serial dilutions of DMF treated DLD-1 cell fractions.

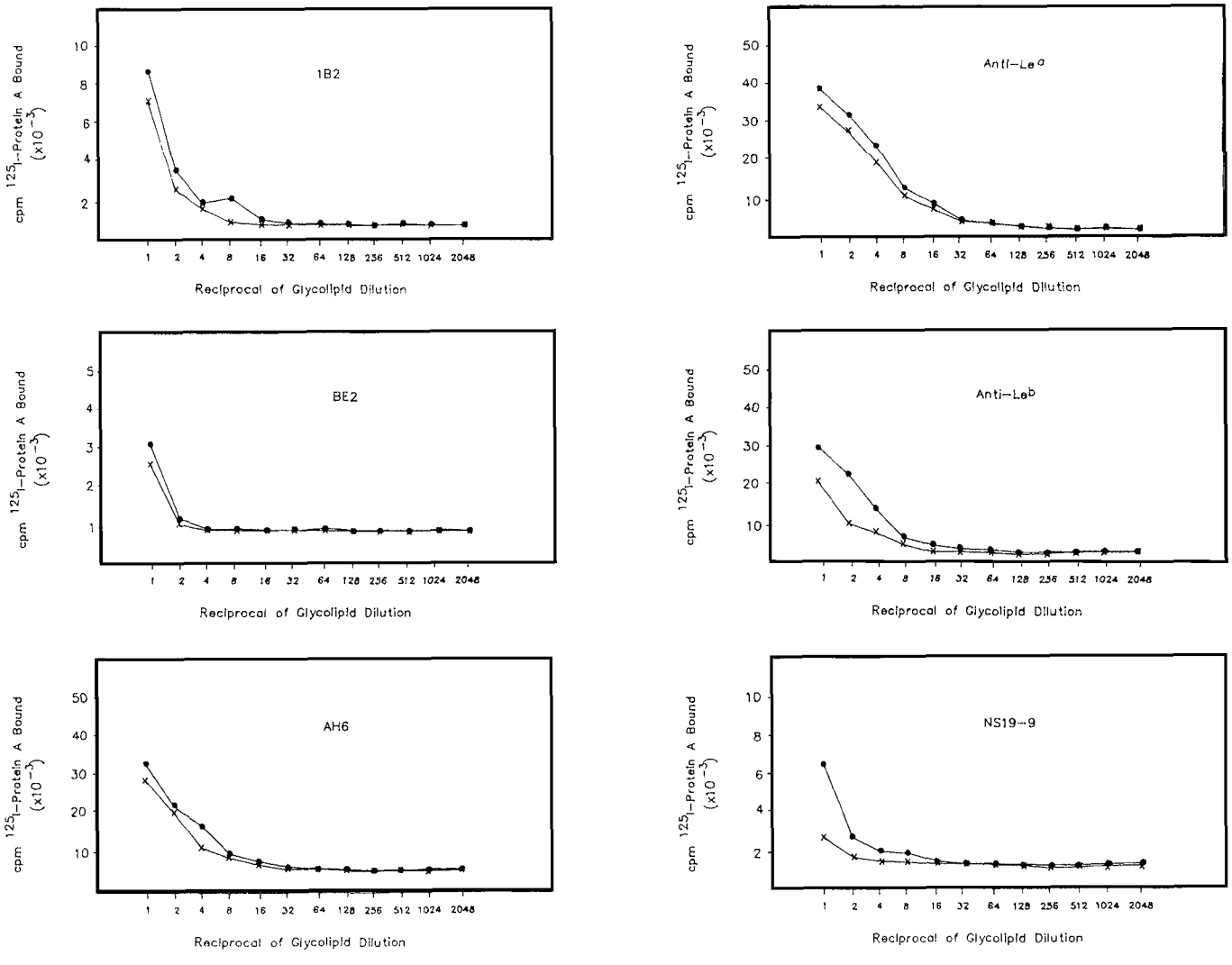
1B2
Anti-Le^a
BE2
Anti-Le^b
AH6
NS19-9
cpm ^{125}I-Protein A Bound (x10^{-3})
Reciprocal of Glycolipid Dilution
1 2 4 8 16 32 64 128 256 512 1024 2048

Figure 3.

expression in this system is identical to that previously observed for induction of lacto-series antigens in human colonic adenocarcinomas which are very low or absent in normal colonic mucosa [5]. Despite this dramatic reduction in apparent biosynthetic activity, analysis of the glycolipid profiles from these cells indicated a decrease of only about 2-fold or less in the chemical quantity of several lacto-series based glycolipid structures in cells grown in the presence of DMF. This was based on analysis of total neutral glycolipids and gangliosides extracted from cells by TLC immunostain and solid phase radioimmunoassay. In order to confirm these findings, a more detailed examination of aspects of in vitro glycolipid biosynthesis was conducted.

As a consequence of DMF treatment, an increase in activities of hydrolytic enzymes α-fucosidase and β-*N*-acetylhexosaminidase was also observed, consistent with previous observations of activation of brush-border sucrase-isomaltase enzymes with cell differentiation [15,16]. This property was analyzed with respect to its effect on apparent β1→3*N*-acetylglucosaminyltransferase activity. These results indicated that despite the increased hydrolytic activity, no significant effect on the results from the transferase assay was observed. Thus, the observed differences between the DMF-treated and untreated cells most probably reflects altered biosynthetic and not hydrolytic rates.

It is of considerable interest that a 14- to 20-fold reduction in its specific activity translates into only about a 2-fold difference in antigen amount found in cell membranes. Although the untreated cells yielded more activity, perhaps in situ intrinsic differences exist in the membrane organization of the β1→3*N*-acetylglucosaminyltransferase and acceptor lactosylceramide in the treated and untreated cells resulting in less efficient synthesis of Lc_3 in the untreated cells. Membrane organization appears to play an important role in the relative synthesis of type 1 vs. type 2 lacto-series chains under conditions of significant levels of competing transferase enzymes [20]. Alternatively, a slower flux of lacto-series precursor structures occurring in DMF-treated cells may build up to a steady-state antigen reservoir of similar magnitude as the untreated cells balanced by synthesis and turnover in each case.

An earlier study of lacto-series antigen expression in human fibroblasts after infection with cytomegalovirus also indicated increased expression correlated with β1→3*N*-acetylglucosaminyltransferase activity [21]. A significant level of lacto-series antigen expression occurred as the β1→3*N*-acetylglucosaminyltransferase activity went from an assay-based undetectable level to a very low but clearly detectable level. Despite the undetectability of this enzyme in the assay, evidence for a low amount of lacto-series antigen expression was found in the glycolipid fraction by TLC immunostain analysis. Thus, expression and accumulation of lacto-series antigens in a given system are extremely sensitive to the level of β1→3*N*-acetylglucosaminyltransferase activity present at or near the limit of detectability of the enzyme assay. Taken together, the results suggest a fine regulation of the expression of low levels of this enzyme defines the production of physiologically important antigen levels.

Similar studies of changes of carbohydrate expression on glycoproteins and glycolipids of human colorectal tumor cells as a consequence of differentiation inducers has been conducted in a number of systems [22–25]. Cell surface glycopeptides from normal colonic mucosal HCMC cells were found to lack a high molecular weight glycopeptide fraction common to three colonic adenocarcinoma cell lines [22]. Subsequently, a decrease in the amount of fucosylated polylactosaminoglycans was observed in CaCo-2 cells induced to differentiate by maintenance for extended periods

as confluent cultures [23]. Growth of HT29 cells in glucose-free medium has been found to result in differentiation permissive conditions and is associated with the expression of *N*-glycan processing reactions which are greatly reduced in cells grown in the presence of glucose [24]. Colonic adenocarcinoma SW1116 and HT29 cells induced to differentiate by butyric acid indicated a marked decrease in the synthesis of sialyl-Le^a and blood group A glycolipids, respectively, compared to untreated cells [25]. A decrease in the metabolic incorporation of [^{3}H]fucose and fucosyltransferase was also reported as a result of butyrate treatment. Thus, although modification of cell surface carbohydrate structures as a result of induction of differentiation is known to occur in cultured cells, a comprehensive view of the similarity of experimental systems or of the enzyme alterations mediating the observed changes is not available. Further work involving functional aspects of carbohydrate expression during differentiation could provide important insight into the transformed phenotype.

ACKNOWLEDGMENTS

This investigation was supported by Research Career Development Award K04 CA01343 and Grant CA41521 (to E.H.H.) from the National Cancer Institute-NIH.

REFERENCES

1. Hakomori S: Annu Rev Biochem 50:733, 1981.
2. Fukushi Y, Hakomori S, Shepard T: J Exp Med 159:506, 1984.
3. Hakomori S: Adv Cancer Res 52:257, 1989.
4. Alhadeff JA: CRC Crit Rev Oncol/Hematol 9:37, 1989.
5. Holmes EH, Hakomori S, Ostrander GK: J Biol Chem 262:15649, 1987.
6. Dexter DL, Barbosa JA, Calabresi P: Cancer Res 39:1020, 1979.
7. Ginsburg V: Methods Enzymol 8:293, 1966.
8. Chien JL, Li S-C, Laine RA, Li Y-T: J Biol Chem 253:4031, 1978.
9. Hakomori S, Nudelman E, Levery SB, Kannagi R: J Biol Chem 259:4672, 1984.
10. Yu RK, Ledeen RW: J Lipid Res 13:680, 1972.
11. Saito T, Hakomori S: J Lipid Res 12:257, 1971.
12. Magnani JL, Smith DF, Ginsburg V: Anal Biochem 109:399, 1980.
13. Kannagi R, Nudelman E, Levery SB, Hakomori S: J Biol Chem 257:14865, 1982.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951.
15. Zweibaum A, Triadou N, Kedinger M, Augeron C, Robine-Leon S, Pinto M, Rousset M, Haffen K: Int J Cancer 32:407, 1983.
16. Zweibaum A, Pinto M, Chevalier G, Dussaulx E, Triadou N, Lacroix B, Haffen K, Brun JL, Rousset M: J Cell Physiol 122:12, 1985.
17. Langdon SP, Hickman JA: Toxicology 43:239, 1987.
18. Dexter DL: Cancer Res 37:3136–3140, 1977.
19. Kimhi BW, Palfrey C, Spector I, Barak Y, Littauer UZ: Proc Natl Acad Sci USA 73:462, 1976.
20. Holmes EH: Arch Biochem Biophys 270:630, 1989.
21. Andrews PW, Gönczöl E, Fenderson B, Holmes EH, O'Malley G, Hakomori S, Plotkin S: J Exp Med 169:1347, 1989.
22. Youakim A, Herscovics A: Cancer Res 45:5505, 1985.
23. Youakim A, Herscovics A: Biochem J 247:299, 1987.
24. Ogier-Denis E, Bauvy C, Aubery M, Codogno P, Sapin C, Rousset M, Zweibaum A, Trugnan G: J Cell Biochem 41:13, 1989.
25. Liepkalns VA, Herrero-Zabaleta E, Fondaneche MC, Burtin P: Cancer Res 45:2255, 1985.
26. IUPAC-IUB Commission on Biochemical Nomenclature: Lipids 12:455, 1977.