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Stable Expression of a cDNA Encoding a Human β1 → 3Galactosyltransferase Responsible for Lacto-Series Type 1 Core Chain Synthesis in Non-Expressing Cells: Variation in the Nature of Cell Surface Antigens Expressed

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Abstract Transient expression of a human colonic adenocarcinoma Colo 205 cell derived cDNA in cell lines which ordinarily express only neolacto-series glycolipids has resulted in the expression of a β1 → 3galactosyltransferase gene responsible for synthesis of glycolipids based upon the lacto-series type 1 core chain. Calcium phosphate transfected cells were panned on anti-IgM coated plates after initial treatment with a combination of monoclonal antibodies specific for type 1 chain terminal structures (TE-3) and a very broadly specific antibody reactive with multiple type 1 chain derivatives (TE-2). Adherent cells after panning were capable of efficiently transferring Gal in β1 → 3-linkage to the acceptor glycolipid Lc_3. Using these reagents, clones of stably transfected human colonic adenocarcinoma HCT-15 cells were produced and isolated. Parental HCT-15 cells do not express type 1 chain based antigens. The nature of the type 1 chain based antigens produced in each of these clones was analyzed by solid phase antibody binding assays. Three types of behavior were observed. Formation of type 1 terminal structures that were either exclusively sialylated or fucosylated, or a mixture of sialylated and fucosylated determinants occurred. In contrast, no difference in type 2 antigen expression between any clone and the parental cells was observed. These data suggest that coordination of subsequent reactions capable of modifying type 1 chain structures is not the same in all clones. The relationship of these results to aspects of cellular regulation of carbohydrate biosynthesis is discussed.

Key words: β1 → 3galactosyltransferase, stable expression, glycolipids, lacto-series type 1 chain, Lewis antigens

Aberrant expression of cell surface carbohydrate structures has been observed in a wide variety of cell lines following malignant transformation [see for review Alhadeff, 1989; Hakomori, 1989]. These changes include modification or loss of expression of normal cellular antigens or expression of new cell surface glycoconjugate structures [Hakomori, 1981; Stults et al., 1989]. Characteristic accumulations of lacto-series carbohydrate structures containing fucosyl or sialyl/fucosyl type 1 or 2 chains on glycoproteins and glycolipids of, for example, human colonic adenocarcinomas represent important tumor-associated markers. Evidence indicates that accumulations of these structures is largely due to enhanced levels of β1 → 3N-acetylglucosaminyltransferase activity stimulating the degree of core chain synthesis [Holmes et al., 1987]. This enzyme is responsible for synthesis of Lc_3 and is the rate limiting step in the series of reactions catalyzing the synthesis of lacto-series antigens [Andrews et al., 1989; Holmes and Greene, 1990]. Lc_3, once synthesized, is the immediate precursor for synthesis of type 1 or 2 core chains. Further modification is then accomplished by other enzymes constitutively expressed and highly organized in the ER and Golgi membranes. Thus, biosynthesis of the oligosaccharide portion of glycolipids and glycoproteins oc-

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, 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid; EDTA, ethylenediamine tetra acetate; ER, endoplasmic reticulum; glycolipids are designated according to the recommendations of the IUPAC Nomenclature Committee, but the suffix OseCer is omitted [IUPAC-IUB Commission on Biochemical Nomenclature, 1977]. The major glycolipid structures used in this study are described in Table I.

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curs due to the sequential action of specific glycosyltransferases which are localized and distributed in the order which they act [Kornfeld and Kornfeld, 1985; Roth, 1987; Pfeffer and Rothman, 1987; Trinchera et al., 1990; Young et al., 1990; van Echten et al., 1990].

Conversion of Lc_3 to the type 1 chain core structure, Lc_4, is accomplished by a $\beta 1 \rightarrow 3$galactosyltransferase which is highly expressed in human colonic adenocarcinoma Colo 205 cells [Holmes et al., 1987; Holmes, 1989]. We report here transfer of a gene for this enzyme derived from a Colo 205 cell cDNA library to two cell lines which ordinarily express only neolactoseries structures [Holmes et al., 1987; Gahmberg and Hakomori, 1975]. Stably transfected HCT-15 cell clones, which constitutively express enzymes capable of modifying type 1 core chains, were isolated and the nature of the resulting cell surface antigens expressed were investigated. The isolated clones expressed a variety of type 1 chain based cell surface carbohydrate structures. The nature of these structures and their biosynthetic regulation is discussed.

MATERIALS AND METHODS

Materials

Cell lines DLD-1, HCT-15, Colo 205, SW403, and anti-sialyl-Le^x antibody producing CSLEX-1 cells were obtained from the American Type Culture Collection, Rockville, MD. Mouse myeloma X63-Ag8 cells were obtained from Dr. Chris Grant, Pacific Northwest Res. Fdn., Seattle, WA. Polyoma virus transformed NIL cells (NILpy) and the anti-Le^y specific AH6 antibody were obtained through the courtesy of Dr. Henrik Clausen, Biomembrane Institute, Seattle, WA. *Vibrio cholerae* neuraminidase, goat anti-mouse IgM (μ-chain specific), geneticin, and UDPGal were obtained from Sigma Chemical Co., St. Louis, MO. UDP[^{14}C]Gal (303 mCi/mmol) was obtained from Amersham, Arlington Heights, IL. Lacto*neo*tetraosylceramide, nLc_4Cer, and lacto*nor*hexaosylceramide, nLc_6, were prepared by desialylation of sialosyllacto*neo*tetraosylceramide and sialosyllacto*nor*hexaosylceramide isolated from bovine erythrocytes [Chien et al., 1978]. Desialylation was performed in 1% acetic acid at 100°C for 1 h. Lactotetraosylceramide (Lc_4) and para-lacto-N-hexaosylceramide (Lc_6) were prepared from nLc_4 and nLc_6, respectively, by β-galactosidase digestion and transfer of galactose in $\beta 1 \rightarrow 3$-linkage as previously described [Holmes and Levery, 1989]. Lc_3 and nLc_5 were prepared from nLc_4 and nLc_6 by overnight hydrolysis with jack bean β-galactosidase in 0.1 M citrate buffer, pH 4.5, containing 0.1% taurodeoxycholate. IV^2FucLc_4 and III^4FucLc_4 were obtained from type "O" human meconium [Karlsson and Larson, 1979]. $IV^3NeuAcLc_4$ and $IV^3NeuAcIII^4FucLc_4$ were isolated from Colo 205 cell membranes [Holmes et al., 1987]. $III^3V^4Fuc_2Lc_6$ was prepared by transfer of fucose to Lc_6 catalyzed by Colo 205 cell fucosyltransferase as previously described [Holmes and Levery, 1989a] (Table I). Antibody TE-3 was prepared as previously described [Holmes and Greene, 1990a]. All other reagents were of the highest purity commercially available.

Methods

Immunization of animals. Biosynthetic Lc_6 was adsorbed on to Colo 205 cell membranes and used as an immunogen according to the method described [Fukushi et al., 1984] as previously reported [Holmes and Greene, 1990a].

Cell fusion and screening. Cell fusion was conducted according to a modification of the method previously described [Oi and Herzenberg, 1980] and screened using Lc_6 as the antigen [Holmes and Greene, 1990a]. An IgM isotype antibody reactive with this structure, designated TE-2, was obtained. Its characterization is described under Results. Culture supernatants were used in these studies.

Growth of cells. Cell lines DLD-1, HCT-15, Colo 205, X63-Ag8, and TE-2 hybridoma cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. SW403 cells were grown in L-15 medium supplemented with 10% fetal calf serum. NILpy cells were grown in DME medium supplemented with 10% fetal calf serum. 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.

Human cDNA library construction. Double stranded cDNA was prepared from 10 μg of Colo 205 cell poly A^+ RNA essentially according to the method previously described [Gubler and Hoffman, 1983] using the Fast Track mRNA Isolation Kit and Librarian I cDNA Library Construction System (InVitrogen). Colo 205 cDNA was sized to exclude fragments below 1,000 bp in length. These cDNAs were then ligated into

TABLE I. Glycolipid Structures Utilized in This Study

Lactosylceramide	Galβ1→4Glcβ1→1Cer
Lc_3	GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
Lc_4	Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
nLc_5	GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
Lc_6	Galβ1→3GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
nLc_4	Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
nLc_6	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
IV^2FucLc_4	Fucα1→2Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
III^4FucLc_4 (Le[a])	Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 4 ↑ Fucα1
$III^4IV^2Fuc_2Lc_4$ (Le[b])	Fucα1→2Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 4 ↑ Fucα1
$III^3IV^2Fuc_2nLc_4$ (Le[y])	Fucα1→2Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 ↑ Fucα1
$IV^3NeuAcLc_4$	NeuAcα2→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
$IV^3NeuAcIII^4FucLc_4$ (sialyl-Le[a])	NeuAcα2→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 4 ↑ Fucα1
$IV^3NeuAcnLc_4$	NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
$IV^3NeuAcIII^3FucnLc_4$ (sialyl-Le[x])	NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 ↑ Fucα1

pcDNA I vector [Aruffo and Seed, 1987] to produce a Colo 205 cDNA library of discrete size which was transformed into competent MC106/P3 *E. coli*. Plasmids from this library were used in subsequent transfections.

Transient DNA transfections. The standard $CaPO_4$-DNA coprecipitation method [Graham and van der Eb, 1973; Wigler et al., 1978] was used for transient gene transfer in HCT-15 and NILpy cells. Cells in the logarithmic phase of growth were plated at a density of 8×10^5 for the HCT-15 cell line and 5×10^5 for the NILpy line on 10 cm plates in 10 ml of complete DME medium, and the medium changed 24 h later. After 2 to 3 h in the fresh medium, 960 μl of $CaPO_4$-cDNA mixture containing 30–40 μg cDNA was added dropwise onto the surface of the medium and allowed to incubate at 37°C for 16 to 24 h. The cells were then washed with PBS and replaced with 10 ml of fresh DME medium. After 48 to 72 h, the cells were harvested, incubated with TE-2 and TE-3 antibodies, and panned on goat anti-mouse IgM (μ-chain specific) coated plates as previously described [Wysocki and Sato, 1978; Ernst et al., 1989]. Adherent cells were collected for β1 → 3galactosyltransferase assay or plasmid rescue [Hirt, 1967].

Stable DNA transfections and selection. HCT-15 cells were stably transfected by the $CaPO_4$ method with a Colo 205 cell derived plasmid subfraction capable of eliciting transient expression of β1 → 3galactosyltransferase activity. In these experiments, 30 μg of Colo 205 cell derived plasmid cDNA and 5 μg of pMCIneo plasmid (Stratagene) was used per 10 cm plate. After 72 h, 500 μg/ml of geneticin was added to the medium. One to two weeks later, geneticin resistant colonies were propagated and maintained in 200 μg/ml geneticin thereafter. These cells were panned as described earlier, but adherent cells were collected under sterile conditions and regrown for subsequent rounds of selection. In these experiments, control transfections using the pMCIneo plasmid alone or in combination with an irrelevant plasmid were conducted.

Over multiple trials, panning selection did not lead to recovery of adherent cells.

After four rounds of selection, the stably transformed HCT-15 cells were distributed into 96 well plates at an average of 2 cells/well. The wells were screened 10 days later for single clones and these were grown up and individually panned in Falcon Pro-Bind assay plates which were pre-treated as follows: The wells were coated with 75 μl of goat anti-mouse IgM (10 μg/ml) in 50 mM Tris-HCl, pH 9.5, overnight at 4°C after which the wells were washed with PBS and blocked with 1% BSA in PBS for 1 h at room temperature. The wells were washed again with PBS followed by treatment with TE-2 and TE-3 antibodies (50 μl of culture supernatant) for 1 h. After washing with PBS, cells from individual clones which were released by PBS containing 0.2% EDTA were added to each well and allowed to incubate at 4°C for 1 h. The wells were carefully washed three times with PBS and scored for % confluence of adherent cells. Colo 205 and untransfected HCT-15 cells were identically panned as positive and negative controls for cell adherence, respectively.

Ten moderate to highly adherent clones were isolated and grown up for further study. Four highly adherent clones, C11, C12, G5, and G12, and one moderately adherent clone, F6, were selected for this study.

Enzyme Assays

β1 → 3- and β1 → 4galactosyltransferase assays. β1 → 3- and β1 → 4Galactosyltransferase activity was determined in reaction mixtures containing 2.5 μmol HEPES buffer, pH 7.0, 15 μg Lc_3, 100 μg Triton CF-54, 1 μmol $MnCl_2$, 15 nmol UDP[^{14}C]galactose (15,000 cpm/nmol), 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 3 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. This solvent is capable of separating the β1 → 3- and β1 → 4-linked galactosyl products. 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.

β1 → 3N-acetylglucosaminyltransferase assays. The β1 → 3N-acetylglucosaminyltransferase activity was determined as previously described [Holmes, 1988].

α1 → 2- and α1 → 3fucosyltransferase assays. α1 → 2- and α1 → 3Fucosyltransferase activities were determined as previously described [Holmes and Levery, 1989a].

α2 → 3sialytransferase activity. α2 → 3Sialytransferase activity was determined as previously described [Holmes et al., 1986].

Solid phase binding assays. Glycolipids were deposited on 96-well vinyl plates in solutions containing 3 μg cholesterol, 5 μg phosphatidylcholine, and either 3 nmol of purified glycolipid or the total glycolipid corresponding to 5 mg of dried extracted cell residue 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-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.

TLC immunostaining of glycolipids. Immunostaining of glycolipids separated on HPTLC plates was performed using established procedures [Magnani et al., 1980; Kannagi et al., 1982].

Extraction of glycolipids from cells. Glycolipids were isolated from 0.2 ml of packed cells from each cell line by extraction with 10 volumes of isopropanol/hexane/water (55:25:20) in a bath sonicator for 10 min followed by centrifugation at 2,500g for 10 min. The insoluble pellet was re-extracted 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 [Yu and Ledeen, 1972] to separate neutral glycolipids from gangliosides. The neutral glycolipid fraction obtained from the passthrough of the DEAE-Sephadex column was concentrated to dryness followed by acetylation with 4 ml of pyridine and 2 ml of acetic anhydride. The acetylated glycolipid fraction was obtained by chromatography on a Florisil column [Saito and Hakomori, 1971]. 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).

Gangliosides were desialylated by treatment with 1% acetic acid for 1 h at 100°C, followed by removal of the solvent with a N_2 stream.

Fluorescence activated cell sorting of antibody stained cells. Tissue culture cells were released from culture dishes by incubation with PBS containing 0.2% EDTA. The cells were then centrifuged and washed twice with RPMI medium. The washed cells were aliquoted into plastic tubes for incubation with antibodies. Some samples, prior to antibody incubation, were treated with *Vibrio cholerae* neuraminidase (0.4 ml in RPMI of a 0.125 unit/ml solution) at room temperature for 1 h followed by two washes of RPMI containing 10% defined bovine calf serum (Hyclone). The cells were treated with monoclonal antibodies as culture supernatants for 45 min at 4°C followed by three washes with the above medium. The cells were then stained with a FITC-labeled rabbit anti-mouse whole Ig secondary antibody at 1:40 dilution for 1 h at 4°C and again washed three times with medium. The cells were finally fixed in PBS containing 1% paraformaldehyde for 30 min at room temperature, washed with PBS, and utilized for FACS analysis. Control cell treatments involved analysis of unstained cells and cells treated with secondary antibody only.

RESULTS

For this study, transfection of cDNA derived from the high β1 → 3galactosyltransferase expressing cell line, Colo 205, was used. This enzyme is responsible for lacto-series type 1 core chain synthesis. The cDNA was transfected into cell lines which express exclusively type 2 chain structures. The nature of the resultant antigens expressed in stably transfected clones was then investigated.

For these studies, a monoclonal antibody reactive with multiple type 1 chain based structures was derived and is initially described below.

Specificity of TE-2 Antibody

Figure 1 shows the reactivity of various neutral glycolipids and gangliosides with antibody TE-2 based upon solid phase radioimmunoassays. The antibody was found to react strongly with a variety of type 1 chain based structures containing α1 → 4fucosyl or α2 → 3sialyl/α1 → 4fucosyl substitutions. These structures included Le^a, Le^b, biosynthetic $III^3V^4Fuc_2Lc_6$ ("Le^a on Le^x" [Holmes and Levery, 1989a]), and sialyl-Le^a. Weaker but significant reactivity was found with structures lacking an α1 → 4fucosyl residue such as $IV^3NeuAcLc_4$, Lc_6, and nLc_5. No reactivity was observed with IV^2FucLc_4 or with a variety of type 2 chain based fucosylated or sialylated structures (results not shown). Complementary data was obtained from TLC-immunostain studies (results not shown). Based on these experiments, antibody binding can be summarized as follows: sialyl-Le^a ≈ Le^a ≈ Le^b > $IV^3NeuAcLc_4$ > Lc_6 > nLc_5 > Lc_4.

Dilution of antibody derived from culture supernatant in plates containing 150 pmol sialyl-Le^a, Le^a, or Lc_6 per well indicated a high titre antibody consistent with this broad antibody specificity. This antibody, along with the type 1 chain core specific TE-3 antibody, were applied as described below.

Transfection Studies and Analysis of Cell Surface Carbohydrate Antigens

Transient expression of β1 → 3galactosyltransferase activity. Thin layer chromatographic analysis of ^{14}C-Gal transfer to Lc_3 by HCT-15 and NILpy cells which were either mock transfected or transfected with an enriched Colo 205 cell cDNA library is shown in Figure 2. Autoradiography of TLC separated reaction products with Lc_3 indicated the presence of a major band corresponding to the type 2 chain β1 → 4Gal derivative, nLc_4, for both mock and positively transfected cells. In assays of the whole cell population before panning selection, a weak, faster migrating band is apparent, particularly for the NILpy cells (lanes 7 and 10). This band comigrates with standard Lc_4 (lane 3) and the

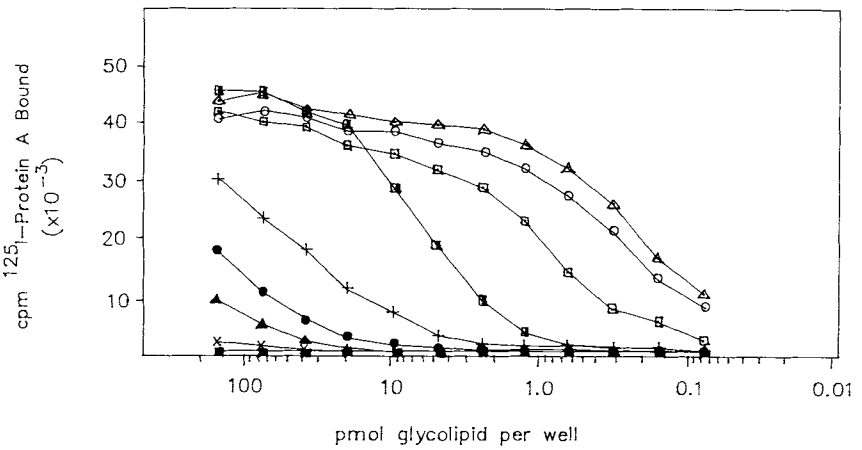

Fig. 1. Solid phase binding of antibody TE-2 to purified glycolipids. Glycolipids were serially diluted in 96-well vinyl plates and assayed for antibody reactivity as described under Materials and Methods. △—△, IV^{3}NeuAcIII^{4}FucLc$_4$; ○—○, III^{4}FucLc$_4$; □—□, III^{3}V^{4}Fuc$_2$Lc$_6$; ◨—◨, III^{4}IV^{2}Fuc$_2$Lc$_4$; +——+, IV^{3}NeuAcLc$_4$; ●—●, Lc$_6$; ▲—▲, nLc$_5$; ×——×, Lc$_4$; ■—■, nLc$_4$. Antibody binding to Gg$_4$, nLc$_6$, IV^{2}FucLc$_4$, IV^{2}FucnLc$_4$, III^{3}FucnLc$_4$, IV^{3}NeuAcnLc$_4$, VI^{3}NeuAcnLc$_6$, and VI^{3}NeuAcIII^{3}V^{3}Fuc$_2$nLc$_6$ were also tested and provided results identical to that shown for nLc$_4$. Culture supernatants were utilized as antibody source.

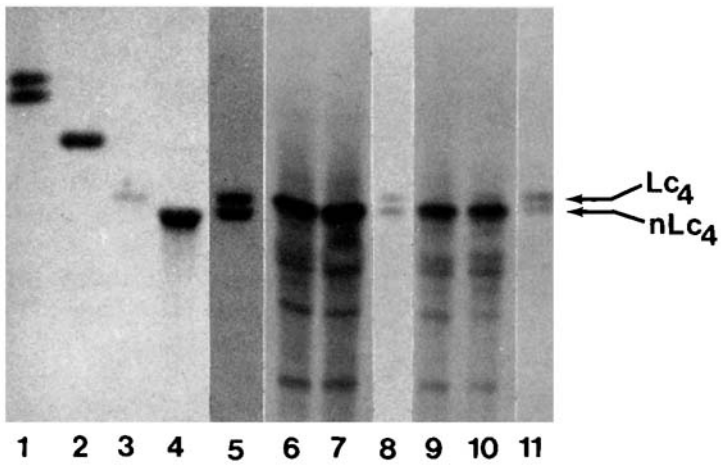

Fig. 2. Thin layer chromatographic analysis of reaction products from transfer of ^{14}C-Gal to Lc$_3$ catalyzed by HCT-15 and NILpy cell lines which were either mock transfected or transiently transfected by Colo 205 cell enriched cDNA library. **Lane 1,** standard lactosylceramide; **lane 2,** standard Lc$_3$; **lane 3,** standard Lc$_4$; **lane 4,** standard nLc$_4$. Lanes 5 to 11 show autoradiograph results from transfer of ^{14}C-Gal to Lc$_3$ catalyzed by the indicated enzyme source. **Lane 5,** Colo 205 cell homogenate; **lane 6,** mock transfected HCT-15 cells before panning; **lane 7,** transfected HCT-15 cells before panning; **lane 8,** adherent, transfected HCT-15 cells isolated after panning; **lane 9,** mock transfected NILpy cells before panning; **lane 10,** transfected NILpy cells before panning; **lane 11,** adherent, transfected NILpy cells isolated after panning. Standard glycolipids were visualized by orcinol spray. The plate was developed in a solvent system composed of $CHCl_3$:CH_3OH:H_2O, 60:40:9, containing 0.02% $CaCl_2 \cdot 2H_2O$.

faster migrating product catalyzed by Colo 205 cells (lane 5). This band has previously been identified as Lc$_4$ [Holmes, 1989]. In the case of assays using unpanned cells (lanes 6, 7, 9, and 10), slower migrating product bands are also apparent from transfer to endogenous glycolipid acceptors due to the comparatively high amount of cell extract used in these assays compared to all other tests shown. Assays of adherent, transfected cells after panning with TE-2 and TE-3 antibodies yielded TLC profiles of products for both HCT-15 (lane 8) and NILpy (lane 11) cells very similar to that found with Colo 205 cells (lane 5). In each case, the band corresponding to Lc$_4$ is present and highly expressed in comparison to the nLc$_4$ product.

Product identification. The panned, adherent cell product bands from NILpy transient

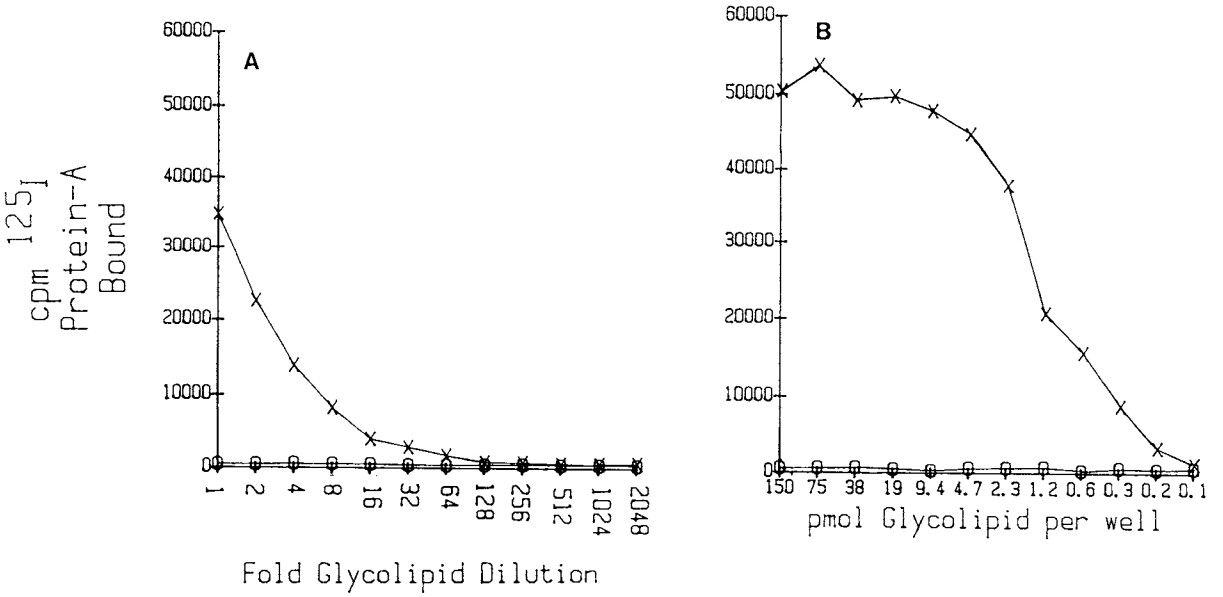

Fig. 3. Solid phase radioimmunoassay of reaction products from transfer of galactose to Lc_3 catalyzed by transiently transfected NILpy cells after panning with the anti-Lc_4 specific TE-3 antibody. The reaction products are shown in Figure 2, lane 11. **A:** Serial dilution of reaction products. ×——×, faster migrating product; O—O, slower migrating product. **B:** Serial dilution of standard Lc_4, ×——×, and nLc_4, O—O. The assays were conducted as described under Materials and Methods using TE-3 culture supernatant as the antibody source.

expression described above were isolated from the TLC plate and subjected to radioimmunoassays with the Lc_4 specific TE-3 antibody. These results are shown in Figure 3. Strong binding of the antibody was observed to the faster migrating component (panel A). No binding to the slower migrating glycolipid could be observed. Comparison with standard glycolipids (panel B) indicates the extent of antibody binding in panel A corresponds to 2 to 2.5 pmol of glycolipid product (4 to 5 pmol total transferred). Similar experiments with the nLc_4 specific 1B2 antibody yielded reciprocal results (results not shown). These results confirm the identity of the faster band as Lc_4, indicating that cDNA encoding the β1 → 3galactosyltransferase gene was expressed in these cells. Cells expressing this activity were found to be enriched in panned, adherent cells after transfection. Panning of either non-transfected or mock transfected HCT-15 and NILpy cells yielded essentially no adherent cells and was thus insufficient for detectable transferase activity.

Stable expression of β1 → 3galactosyltransferase in HCT-15 cells. HCT-15 cells were stably transfected and subjected to four rounds of panning selection. The degree of cell sticking to panning plates increased steadily with each round (results not shown).

Clonal sublines were selected from the four times panned cells. Of approximately 100 clones screened, 20% showed moderate adherence and 10% showed very high adherence after panning. Representative stably transfected clones showing high adherence, C11, C12, G5, and G12, and the moderately adherent F6 clone were selected for further study. The levels of glycosyltransferase activities associated with type 1 and 2 lacto-series antigen synthesis in these clones were tested (see Table II). Only β1 → 3galactosyltransferase activity was remarkably different from the parental HCT-15 cells.

In these experiments, non-transfected HCT-15 cells and HCT-15 cells transfected either with the pMCIneo alone or also with an irrelevant plasmid consistently yielded negative panning and cell recovery results. Thus, no endogenous cell population from HCT-15 cells is present which expresses this activity. This indicates the adherent cells from the positively transfected experiments most probably represent cells stably expressing a Colo 205 plasmid derived β1 → 3galactosyltransferase activity.

Solid phase radioimmunoassays of neutral glycolipids and gangliosides isolated

TABLE II. Specific Activity of Glycosyltransferases in HCT-15 Cells and Derived Clones*

Cell clone	β1→3N-acetylglucosaminyltransferase pmol/h/mg Protein	β1→4galactosyltransferase nmol/h/mg Protein	β1→3galactosyltransferase pmol/h/mg Protein	α1→3fucosyltransferase pmol/h/mg Protein	α1→2fucosyltransferase pmol/h/mg Protein	α2→3sialyltransferase pmol/h/mg Protein
Control	51.9 ± 3	7.87 ± 0.2	ND[a]	48.0 ± 6	13.5 ± 2	10.0 ± 1
C11	122.0 ± 12	14.0 ± 0.3	10.0 ± 3	126.0 ± 2	23.3 ± 2	9.4 ± 1
C12	64.0 ± 8	14.5 ± 0.1	7.0 ± 2	79.0 ± 2	26.0 ± 6	11.8 ± 2
F6	71.9 ± 2	12.8 ± 0.3	5.0 ± 2	115.0 ± 5	13.4 ± 1	14.6 ± 3
G5	54.0 ± 6	12.0 ± 0.1	15.0 ± 3	114.0 ± 2	51.8 ± 8	12.1 ± 2
G12	62.4 ± 2	11.3 ± 0.4	12.0 ± 3	88.5 ± 3	13.1 ± 1	11.5 ± 1

*The enzyme assays were conducted as described under Materials and Methods.
[a]ND, none detected.

from stably transfected HCT-15 cell derived clones. Figure 4 shows solid phase binding results for glycolipids extracted from parental HCT-15 cells and stably transfected clones. Analysis of neutral glycolipids with TE-2 and TE-3 antibodies indicated significant expression of only TE-2 reactive type 1 chain structures in some of the clones, i.e., C11, G5, and G12. Only very weak TE-2 binding was observed with C12 and F6. No binding was detectable with control HCT-15 cell neutral glycolipids. TE-3 binding was only slightly above background in the case of C12 neutral glycolipids. Based upon the antigen specificity for TE-2 described above, these results suggest significant expression of Le^a and/or Le^b epitopes in neutral glycolipids from C11, G5, and G12 clones. TE-2 binding to ganglioside fractions from these clones indicated high binding to G12 gangliosides and much weaker binding to C11, C12, and G5 gangliosides, from expression predominantly of the sialyl-Le^a structure. Analysis of desialylated gangliosides with TE-3 resulted in significant binding only for the C12 fraction, indicating significant expression of $IV^3NeuAcLc_4$ only for this clone. The behavior of these neutral glycolipid fractions with antibodies specific for type 2 chain structures was also tested. Significant binding to glycolipids from each cell line occurred with the type 2 core chain specific 1B2 antibody, Le^y specific AH6 antibody, and sialyl-Le^x specific CSLEX-1 antibody indicating the isolated clones were not deficient in type 2 antigen synthesis. These results are consistent with the expression of type 1 chain structures in clones stably expressing β1 → 3galactosyltransferase activity. A summary of the predominant type 1 chain structures defined by solid phase assays is shown in Table III.

FACS analysis of TE-2 binding to human colonic adenocarcinoma cells and stably transfected HCT-15 cell clones. The binding of antibody TE-2 to human cells by FACS analysis is summarized in Figure 5. As a positive control, cell lines expressing differing amounts of type 1 chain based neutral glycolipids and gangliosides were tested [Holmes et al., 1987; Holmes and Greene, 1990]. Significant antibody binding, as judged by intense fluorescent labeling, was found with DLD-1, SW403, and Colo 205 cell lines (Fig. 5, top part). These cell lines have significant amounts of type 1 chain based structures in their glycolipid fractions and also express appreciable levels of glycosyltransferase activities necessary for generation of lactoseries type 1 chain fucosylated tumor-associated antigens [Holmes et al., 1987]. Staining of HCT-15 cells was found to be negative by FACS analysis with antibody TE-2, consistent with its lack of β1 → 3galactosyltransferase activity [Holmes et al., 1987] preventing expression of type 1 chain based antigens. In each case, staining of the respective cell lines with secondary antibody alone yielded no cell staining (results not shown). Cells that had first been treated with *Vibrio cholerae* neuraminidase prior to antibody staining yielded no detectable alteration in staining intensity due presumably to the near equivalent binding of TE-2 to desialylated antigens (results not shown).

FACS analysis of HCT-15 cell derived clones stably expressing transfected β1 → 3galactosyltransferase activity is shown in the lower part of Figure 5. Significant antibody binding, similar to results for DLD-1 and SW403 cell lines, was observed for most clones with the TE-2 antibody. This behavior correlates well with the

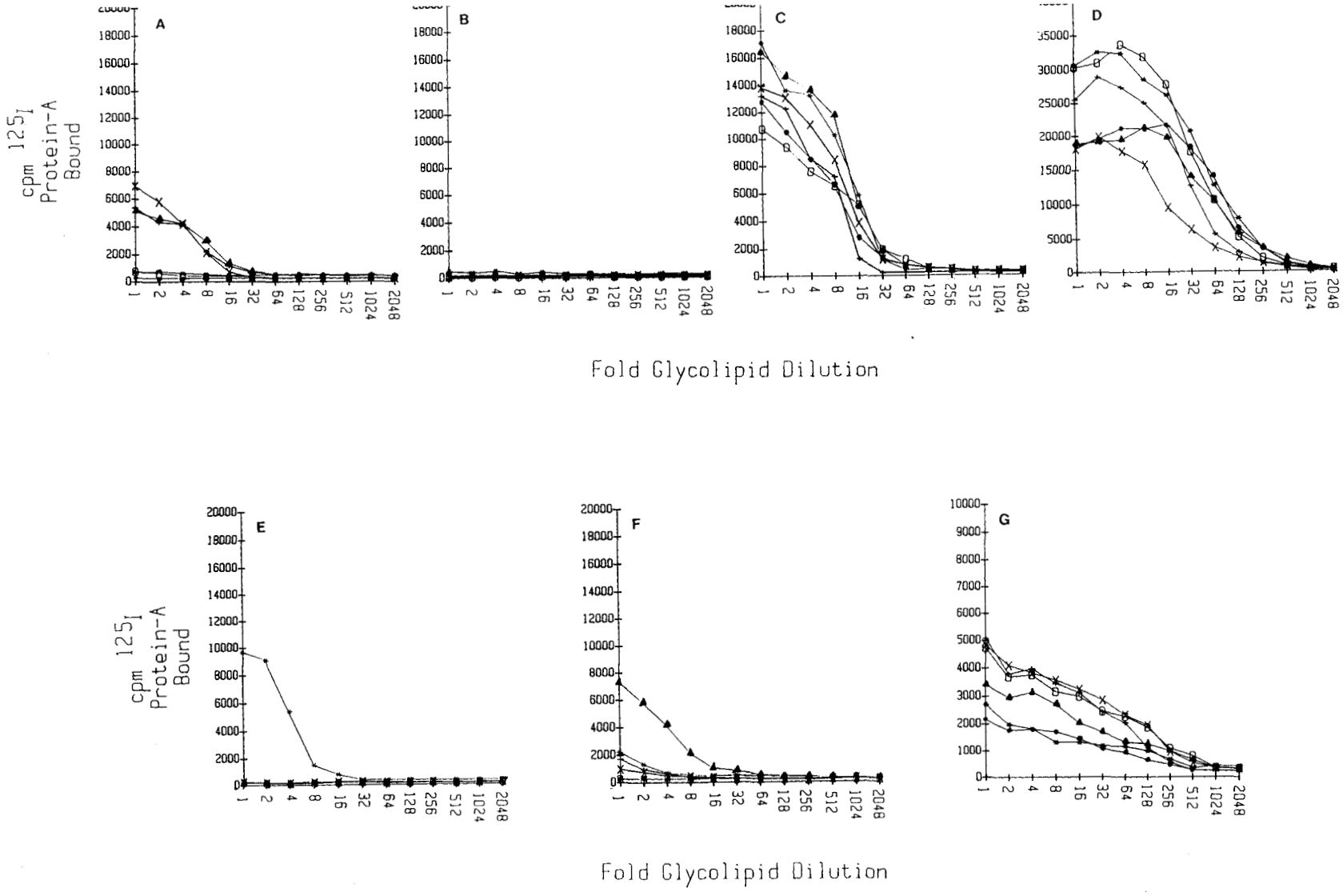

Fig. 4. Solid phase radioimmunoassays of neutral glycolipids and gangliosides isolated from stably transfected HCT-15 cell clones with the indicated monoclonal antibody. The amount of glycolipid used was normalized to the dried residue weight of the extracted cells. An amount of total glycolipid corresponding to 5 mg dried residue was dissolved in 1 ml of absolute ethanol containing 3 μg cholesterol and 5 μg phosphatidylcholine. Fifty microliters of this fraction was serially diluted as indicated. **A:** Analysis of total neutral glycolipids with antibody TE-2; **B:** analysis of total neutral glycolipids with antibody TE-3; **C:** analysis of total neutral glycolipids with antibody 1B2; **D:** analysis of total neutral glycolipids with antibody AH6; **E:** analysis of desialylated gangliosides with antibody TE-3; **F:** analysis of total gangliosides with antibody TE-2; **G:** analysis of total gangliosides with antibody CSLEX-1. The cell clones studied were ●—●, control HCT-15 cells; +——+, clone C11; *——*, clone C12; ○—○, clone F6; ×——×, clone G5; and ▲—▲, clone G12. Culture supernatant of each antibody was used in this study.

solid phase binding results (Fig. 4). TE-2 binding to clone C12 was very weak and behaved much like the parent HCT-15 cell line. The major type 1 chain species expressed in this clone is IV^{3}NeuAcLc$_4$, an antigen more weakly bound by TE-2 than its fucosylated derivative. Presumably, the FACS results for this clone are due to the poor stability of antibody binding to this antigen under conditions for FACS analysis. In each case, control cells stained with secondary antibody only were consistently negative in FACS analysis (results not shown).

DISCUSSION

Biosynthesis of the oligosaccharide portions of glycolipids and glycoproteins occurs via a nonrandom distribution of biosynthetic enzymes positioned in the ER and Golgi membranes [Kornfeld and Kornfeld, 1985; Roth, 1987; Pfeffer and Rothman, 1987; Trinchera et al., 1990; Young et al., 1990; van Echten et al., 1990]. Coordination of enzyme action during oligosaccharide biosynthesis occurs via mechanisms of cellular regulation based upon controlling the access of biosynthetic precursors to multiple competing enzymes. Consequently, the nature of the cell surface antigens expressed is frequently a function of the relative efficiency of the flow of intermediates from one enzyme to the next, as opposed to the relative levels of various enzyme activities expressed in the cell. A particular example is the almost exclusive expression of lacto-series type 1 chain carbohydrate structures in Colo 205 cells. These cells also express high levels of β1 → 4galactosyltransferase capable of formation of type 2 core chains from Lc$_3$, a common precursor of both type 1 and 2 core chains [Holmes, 1989]. Thus, identification of factors regulating glycoconjugate synthesis both at the level of gene expression and coordination of enzyme activity is of critical importance in understanding the basis of their expression, particularly, tumor-associated carbohydrate antigens.

One means to study the coordination of glycosyltransferase activities in the cell relies on introduction of enzymes not expressed by a particular cell using cDNA transfection approaches. Studies involving transfer of glycosyltransferase genes via cDNA transfection to nonexpressing parent cells have been instrumental in describing important in vivo cellular interactions. Lowe and co-workers [Lowe et al., 1990; Lowe et al., 1991] and Goelz et al., 1990 have used this approach to define the nature of a carbohydrate ligand, sialyl-Le^x, which acts as an

TABLE III. Summary of Expression of Lacto-Series Type 1 Based Antigens in Stably Transfected HCT-15 Cell Clones

Clone	Predominant type 1 chain antigen
Control cells[a]	ND
C11	Le^a or Le^b (weak sialyl-Le^a)
C12	sialyl-Lc$_4$ (weak sialyl-Le^a, Lc$_4$)
F6	weak Le^a or Le^b
G5	Le^a or Le^b (weak sialyl-Le^a)
G12	Le^a or Le^b, sialyl-Le^a

[a]Non-transfected HCT-15 cells. ND, none detected.

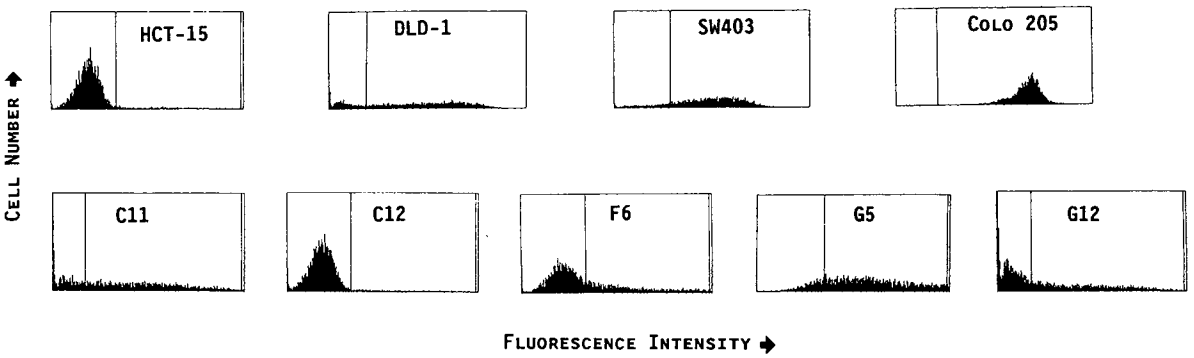

Fig. 5. Fluorescence activated cell sorting analysis of cells after staining with antibody TE-2. The fluorescence intensity vs. cell number for the indicated cell lines after staining with FITC-labeled rabbit anti-mouse whole Ig after initial treatment with antibody TE-2 is shown. The top row shows results with differing human colonic adenocarcinoma cell lines. The bottom row shows results from analysis of the indicated stably transfected HCT-15 cell clones.

adhesion receptor for neutrophils. Expression of this carbohydrate structure is an important regulator of inflammation. Other studies, using plasmids encoding an α1 → 3galactosyltransferase gene, have been used to confer expression of a terminal Galα1 → 3Gal- structure at the cell surface [Larsen et al., 1989; Joziasse et al., 1989], and have further shown that this enzyme competes with an endogenous α2 → 3sialyltransferase for the available terminal Gal containing structures [Smith et al., 1990]. Similar studies based upon gene transfer of α1 → 2fucosyltransferase (H-enzyme) [Ernst et al., 1989; Larsen et al., 1990], blood group ABH-associated enzyme [Yamamoto et al., 1990], and α2 → 6sialyltransferase [Weinstein et al., 1987] have been conducted. All of these cases involved enzymes which catalyze terminal modification reactions, many of which block further derivatization yielding end stage structures of a biosynthetic pathway.

The results described in this paper consider instead an enzyme reaction catalyzing an early step in carbohydrate antigen biosynthesis, formation of type 1 terminal structures catalyzed by β1 → 3galactosyltransferase. Transient expression studies of this enzyme indicated high levels of activity were present in cells which were selected by panning using antibodies specific for type 1 chain cell surface carbohydrates. This methodology is applicable to gene cloning experiments [Larsen et al., 1989]. Stable transfer of this enzyme into HCT-15 cells yielded clones of cells expressing levels of β1 → 3galactosyltransferase of from 5 to 15 pmol/h/mg protein when assayed with the glycolipid acceptor Lc_3. Glycolipids are generally less efficient transferase acceptors compared to oligosaccharides, often by a factor of 10 or more. Consequently, this level of enzyme expression in stable transfectants is comparable to previous reports of stable expression of α1 → 3fucosyltransferase activity in CHO cells assayed with oligosaccharide acceptors [Lowe et al., 1991].

The product of this reaction is composed of a terminal Galβ1 → 3GlcNAc-structure which can be further modified by, e.g., α1 → 3/4fucosyltransferase and α2 → 3sialyltransferase enzymes. These enzymes are constitutively expressed in many human cell lines, including HCT-15 cells and the derived stably transfected clones. Despite this, stable β1 → 3galactosyltransferase expression in these clones gave rise to differing patterns of type 1 chain products expressed at the cell surface. Three patterns of antigen expression were observed: a) formation of NeuAcα2 → 3Galβ1 → 3GlcNac- terminal structures with no evidence of subsequent fucosylation, b) α1 → 4fucosylation of non-sialylated type 1 chain termini (Le^a or Le^b antigen), and c) a mixture of α1 → 4fucosylated and α1 → 4fucosylated/α2 → 3sialylated (sialyl-Le^a antigen) type 1 chain termini.

It has previously been established that biosynthesis of sialyl-Le^a occurs by initial sialylation followed by fucosylation [Hansson and Zopf, 1985]. Further, α1 → 4fucosylated structures are not acceptors for α2 → 3sialyl transfer. Given this information, the differing fates of Galβ1 → 3GlcNAc- structures in these cells suggest that coordination of subsequent reactions is not uniform in all clones. Evidence presented suggests that this is not due to nonuniform expression of α1 → 3/4fucosyl- or α2 → 3sialyltransferase activities in the derived clones, since each expressed similar levels of these enzyme activities as well as significant quantities of gangliosides or fucosylated type 2 chain structures such as Le^y. Expression of such antigens in each clone further suggests availability of sugar nucleotide donors was not limiting. Thus, this appears to be peculiar to the newly expressed type 1 chain determinants. One explanation suggests the newly synthesized type 1 terminal structures had variable access to cellular α1 → 3/4fucosyl- and α2 → 3sialyltransferase enzymes. Perhaps the Golgi membrane distribution of these enzymes varies among the derived clones. Alternatively, the transfected β1 → 3galactosyltransferase may itself be variably distributed in Golgi membranes which may, in some cases, result in functional segregation from either the α1 → 3/4fucosyltransferase or α2 → 3sialyltransferase.

These observations support the notion that both glycosyltransferase expression and the nature of an enzyme's microenvironment in the cell are important regulators defining the specific cell surface carbohydrates expressed during, for example, tumorigenesis. Future studies will be conducted to provide a better understanding of factors involved in cellular regulation of carbohydrate biosynthesis.

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REFERENCES

Alhadeff JA (1989): Malignant cell glycoproteins and glycolipids. CRC Crit Rev Oncol/Hematol 9:37–107.

Andrews PW, Gönczöl E, Fenderson B, Holmes EH, O'Malley G, Hakomori S, Plotkin S (1989): Human cytomegalovirus induces stage-specific embryonic antigen-1 in differentiating human teratocarcinoma cells and fibroblasts. J Exp Med 169:1347–1359.

Aruffo A, Seed B (1987): Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. Proc Natl Acad Sci USA 84:8573–8577.

Chien JL, Li S-C, Laine RA, Li Y-T (1978): Characterization of gangliosides from bovine erythrocyte membranes. J Biol Chem 253:4031–4035.

Ernst LK, Rajan VP, Larsen RD, Ruff MM, Lowe JB (1989): Stable expression of blood group H determinants and GDP-L-fucose:β-D-galactoside 2-α-L-fucosyltransferase in mouse cells after transfection with human DNA. J Biol Chem 264:3436–3447.

Fukushi Y, Hakomori S, Nudelman E, Cochran N (1984): Novel fucolipids accumulating in human adenocarcinoma. II. Selective isolation of hybridoma antibodies that differentially recognize mono, di-, and trifucosylated type 2 chain. J Biol Chem 259:4681–4685.

Gahmberg CG, Hakomori S (1975): Surface carbohydrates of hamster fibroblasts. I. Chemical characterization of surface-labeled glycosphingolipids and a specific ceramide tetrasaccharide for transformants. J Biol Chem 250:2438–2446.

Goelz SE, Hession C, Goff D, Griffiths B, Tizard R, Newman B, Chi-Rosso G, Lobb R (1990): ELFT: A gene that directs the expression of an ELAM-1 ligand. Cell 63:1349–1356.

Graham FL, van der Eb AJ (1973): A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52:456–467.

Gubler U, Hoffman BJ (1983): A simple and very effective method for generating cDNA libraries. Gene 25:263–269.

Hakomori S (1981): Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. Annu Rev Biochem 50:733–764.

Hakomori S (1989): Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. Adv Cancer Res 52:257–331.

Hansson GC, Zopf D (1985): Biosynthesis of the cancer-associated sialyl-Le^a antigen. J Biol Chem 260:9388–9392.

Hirt B (1967): Selective extraction of polyoma DNA from infected mouse cultures. J Mol Biol 26:365–369.

Holmes EH, Ostrander GK, Hakomori S (1986): Biosynthesis of the sialyl-Le^x determinant carried by type 2 chain glycosphingolipids (IV^{3}NeuAcIII^{3}FucnLc$_4$, VI^{3}NeuAcV^3-FucnLc$_6$, and VI^{3}NeuAcIII^{3}V^{3}Fuc$_2$nLc $_6$) in human lung carcinoma PC9 cells. J Biol Chem 261:3737–3743.

Holmes EH, Hakomori S, Ostrander GK (1987): Synthesis of type 1 and type 2 lacto-series glycolipid antigens in human adenocarcinoma and derived cell lines is due to activation of a normally unexpressed β1 → 3N-acetylglucosaminyltransferase. J Biol Chem 262:15649–15658.

Holmes EH (1988): Characterization of a β1 → 3N-acetylglucosaminyltransferase associated with synthesis of type 1 and type 2 lacto-series tumor-associated antigens from the human colonic adenocarcinoma cell line SW403. Arch Biochem Biophys 260:461–468.

Holmes EH (1989): Characterization and membrane organization of β1 → 3- and β1 → 4galactosyltransferase from human colonic adenocarcinoma cell lines Colo 205 and SW403: Basis for preferential synthesis of type 1 chain lacto-series carbohydrate structures. Arch Biochem Biophys 270:630–646.

Holmes EH, Levery SB (1989): Preparative in vitro generation of lacto-series type 1 chain by β1 → 3galactosyltransferase from human colonic adenocarcinoma Colo 205 cells. Arch Biochem Biophys 274:14–25.

Holmes EH, Levery SB (1989a): Biosynthesis of fucose containing lacto-series glycolipids in human colonic adenocarcinoma Colo 205 cells. Arch Biochem Biophys 274:633–647.

Holmes EH, Greene TG (1990): Characterization of two monoclonal antibodies specific for lacto-series type 1 chain Galβ1 → 3GlcNAc- terminal structures. Arch Biochem Biophys 277:181–188.

Holmes EH, Greene TG (1990a): Alteration of Lacto-Series Glycolipid Glycosyltransferase Activities in Human Colonic Adenocarcinoma DLD-1 Cells After Culture in N,N-Dimethylformamide Containing Medium. J Cell Biochem 44:93–105.

IUPAC-IUB Commission on Biochemical Nomenclature (1977): "Lipids." 12:455–463.

Joziasse DH, Shaper JH, Van den Eijnden DH, Van Tunen AJ, Shaper NL (1989): Bovine α1 → 3-galactosylatransferase: Isolation and characterization of a cDNA clone: Identification of homologous sequences in human genomic DNA. J Biol Chem 264:14290–14297.

Kannagi R, Nudelman E, Levery SB, Hakomori S (1982): A series of human erythrocyte glycosphingolipids reacting to the monoclonal antibody directed to a developmentally regulated antigen, SSEA-1. J Biol Chem 257:14865–14874.

Karlsson K-A, Larson G (1979): Structural characterization of lactotetraosylceramide, a novel glycosphingolipid isolated from human meconium. J Biol Chem 254:9311–9316.

Kornfeld R, Kornfeld S (1985): Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 54:631–664.

Larsen RD, Rajan VP, Ruff MM, Kukowska-Latallo J, Cummings RD, Lowe JB (1989): Isolation of a cDNA encoding a murine UDPgalactose β-D-galactosyl-1,4-N-acetyl-D-glucosaminine α-1,3-galactosyltransferase: Expression cloning by gene transfer. Proc Natl Acad Sci USA 86:8227–8231.

Larsen RD, Ernst LK, Nair RP, Lowe JB (1990): Molecular cloning, sequence, and expression of a human GDP-L-fucose:β-D-galactoside 2-α-L-fucosyltransferase cDNA that can form the H blood group antigen. Proc Natl Acad Sci USA 87:6674–6678.

Lowe JB, Stoolman LM, Nair RP, Larsen RD, Berhend TL, Marks RM (1990): ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. Cell 63:475–484.

Lowe JB, Kukowska-Latallo JF, Nair RP, Larsen RD, Marks RM, Macher BA, Kelly RJ, Ernst LK (1991): Molecular cloning of a human fucosyltransferase gene that determines expression of the Lewis X and VIM-2 epitopes but not ELAM-1-dependent cell adhesion. J Biol Chem 266: 17467–17477.

Magnani JL, Smith DF, Ginsburg V (1980): Detection of gangliosides that bind cholera toxin: Direct binding of ^{125}I-labeled toxin to thin layer chromatograms. Anal Biochem 109:399–402.

Oi VT, Herzenberg LA (1980): Immunoglobulin-producing hybrid cell lines. In Mishell BB, Shiigi SM (eds): "Selected Methods in Cellular Immunology." New York: W.H. Freeman & Co., pp 351–372.

Pfeffer SR, Rothman JE (1987): Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. Annu Rev Biochem 56:829–852.

Roth J (1987): Subcellular organization of glycosylation in mammalian cells. Biochim Biophys Acta 906:405–436.

Saito T, Hakomori S (1971): Quantitative isolation of total glycosphingolipids from animal cells. J Lipid Res 12:257–259.

Smith DF, Larsen RD, Mattox S, Lowe JB, Cummings RD (1990): Transfer and expression of a murine UDP-Gal:β-D-Gal-α1,3galactosyltransferase gene in transfected chinese hamster ovary cells. J Biol Chem 265:6225–6234.

Stults CLM, Sweeley CC, Macher BA (1989): Glycosphingolipids: Structure, biological source, and properties. Methods Enzymol 179:167–214.

Trinchera M, Pirovano B, Ghidhoni R (1990): Sub-Golgi distribution in rat liver of CMP-NeuAc:GM_3- and CMP-NeuAc:GT_{1b} α2 → 8sialyltransferases and comparison with the distribution of the other glycosyltransferase activities involved in ganglioside biosynthesis. J Biol Chem 265:18242–18247.

van Echten G, Iber H, Stotz H, Takatsuki A, Sandhoff K (1990): Uncoupling of ganglioside synthesis by brefeldin A. Eur J Cell Biol 51:135–139.

Weinstein J, Lee EU, McEntee K, Lai P-H, Paulson JC (1987): Primary structure of β-galactoside α2,6-sialyltransferase: Conversion of membrane-bound enzyme to soluble form by cleavage of the NH_2-terminal signal anchor. J Biol Chem 263:17735–17743.

Wigler M, Pellicer A, Silverstein S, Axel R (1978): Biochemical transfer of single-copy eucaryotic genes using total cellular DNA as donor. Cell 14:725–731.

Wysocki LJ, Sato VL (1978): "Panning" for lymphocytes: A method for cell selection. Proc Natl Acad Sci USA 75:2844–2848.

Yamamoto F, Marken J, Tsuji T, White T, Clausen H, Hakomori S (1990): Cloning and characterization of DNA complementary to human UDP-GalNAc:Fucα1 → 2Gal α1 → 3GalNAc transferase (Histo-blood group A transferase) mRNA. J Biol Chem 265:1146–1151.

Young WW Jr, Lutz MS, Mills SE, Lechler-Osborn S (1990): Use of brefeldin A to define sites of glycosphingolipid synthesis: GA2/GM2/GD2 synthase is trans to the brefeldin A block. Proc Natl Acad Sci USA 87:6838–6842.

Yu RK, Ledeen RW (1972): Gangliosides of human, bovine, and rabbit plasma. J Lipid Res 13:680–686.