Selection of a Mutant Cell Line Based on Differential Expression of Glycosphingolipid, Utilizing Anti-lactosylceramide Antibody and Complement*

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A mouse mammary carcinoma mutant cell line showing a characteristic alteration of glycosphingolipid (GSL) composition, but with unchanged protein glycosylation pattern, was isolated. Parent cell line FM3A/F28-7 is characterized by the predominance of lactosylceramide (LacCer) and virtual absence of more complex GSLs. Mutant cell line FUA169 was isolated after treatment of F28-7 cells with the mutagen N-methyl-N'-nitro-N-nitrosoguanidine and incubation with anti-lacCer monoclonal antibody T5A7 and complement. FUA169 cells were characterized by a dramatic reduction in LacCer and by the presence of a major ganglioside identified as Gm3. They also showed high activity of CMP-sialic acid:LacCer 2,3-sialosyltransferase, whereas F28-7 cells had no detectable activity of this enzyme. In contrast to the difference in GSL pattern, F28-7 and FUA169 showed identical protein glycosylation patterns, as evidenced by “Western blotting” with various lectins and surface labeling with galactose oxidase/NaB3H4 and periodate/NaB3H4. Thus, FUA169 is identified as a glycosylation mutant with regard to Gm3 expression and will be useful for studies of the functional role of Gm3. Growth of FUA169 cells, relative to F28-7 cells, showed greater temperature sensitivity and greater inhibitability based on restriction of growth factors, particularly insulin.

Glycosphingolipids (GSLs)1 at the plasma membrane have been implicated as receptors for cell-to-cell recognition and various bioactive factors (1, 2), and as regulators for cell growth through possible modulation of growth factor receptor function (2, 3).

In order to assess further the functional role of cell surface GSLs, mutant cells that express clear differences in specific GSLs but are identical in glycoprotein expression are highly desirable. Mutant or variant cell lines with different patterns of protein glycosylation have been selected by Ricinus communis lectin and WGA (4–12). Some of these mutants differ in tumorigenesis, metastatic potential in vivo, and cell growth behavior (“contact inhibitability”) in vitro (10–13). A number of mAbs defining exact CHO epitopes have been established (14). These mAbs, in combination with complement, could be potentially useful in selection of mutants expressing different CHOs at the cell surface, although such trials have not been described in the literature. This paper describes an example of successful selection of a mutant cell line that differs in its GSL composition and that shows identical protein glycosylation patterns.

MATERIALS AND METHODS

Cells and Cell Culture

FM3A cell line was established from spontaneous mammary carcinoma in C57/H3 mouse (15). A subline, F28-7, used throughout this experiment was donated by Dr. Dai Ayusawa (Saitama Cancer Research Institute, Saitama, Japan). Cells were grown in suspension in DMEM supplemented with FCS (HyClone Laboratories, Logan, UT) to obtain a final concentration of 10% in a 5% CO2 incubator at 37°C. The F28-7 cells have been characterized by a novel property for animal cells of forming colonies on agar plates (2b), and this characteristic was used to select the mutant cells, as described below.

Determination of Cytolytic Effect by Anti-LacCer mAb and Complement

F28-7 cells were cultured in DMEM supplemented with 2% FCS until the cell density reached 10⁶ cells/ml. Two cell suspensions in the same culture medium with two cell population densities, i.e. 10¹⁰ and 10¹ⁱ cells/ml, were prepared. Aliquots of 10 µl of each of these cell suspensions were incubated with 10 µl of anti-LacCer mAb T5A7 (16) with three different dilutions (1:50, 1:500, and 1:5,000) and 10 µl of complement (10 units/ml) for 1 hour at 37°C. The cell suspensions were then washed in cold FCS (10% in PBS) and resuspended in cold FCS (10% in PBS). The percentage of cell lysis was calculated by trypan blue exclusion.

**The abbreviations used are: GSL, glycosphingolipid; CHO, chondrocyte; CM, chloroform/methanol; CMW, chloroform/methanol/water; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; GlcCer, glucosylceramide; LacCer, lactosylceramide (Galβ1—4Glcβ1—Cer); HPTLC, high performance thin-layer chromatography; mAb, monoclonal antibody; NTG, N-methyl-N'-nitro-N'-nitroso-guanidine; PAGE, polyacrylamide gel electrophoresis; WGA, wheat germ agglutinin; Geth, NeuAcα2—3Galβ1—4Glcβ1—Cer; Geth, NeuAcα2—3Galβ1—4Glcβ1—Cer; Geth, NeuAcα2—3Galβ1—4Glcβ1—Cer.
of complement with dilutions of 1:1 and 1:10. The concentration of the original complement solution was prepared according to the manufacturer's instruction, i.e. lyophilized material in a bottle (Gib-raltar Laboratories, Changrin Falls, OH) was dissolved in 3 ml of DMEM. Subsequently, 70 μl of culture medium was added and the cells were incubated on 96-well microtiter plates ( Falcon 3072, Becton-Dickinson, Oxnard, CA) for 18 h. Cytology and morphological changes were examined under a microscope.

Treatment of F28–7 Cells with the Mutagen NTG

Aliquots of 2 ml of cultured cells (cell density, 2 × 10^6 cells/ml) were centrifuged. The cell pellet was washed with 1 ml of culture medium and resuspended in 10 ml of culture medium in order to obtain a suspension with a cell density of 4 × 10^6 cells/ml. To this cell suspension, NTG (Aldrich) was added to obtain a final concentration of 1.5 μg/ml and cultured for 15 h. The condition for mutagenesis was a modification of the method previously described (15). Cells were centrifuged and washed twice with 10 ml of culture medium and finally suspended in 50 ml of medium and cultured for 1 week.

Selection of the GSL Mutant

The NTG-treated cells were centrifuged as described above, and 10^5 cells were washed twice with 1 ml of the medium containing 2% FCS, followed by suspension in 8 ml of the same medium mixed with 1 ml of mAb T5A7 (16) and 1 ml of complement solution prepared as described above, i.e. total volume of the incubation mixture was 10 ml. The T5A7 mAb was diluted 500-fold from ascites with DMEM. The complement solution was the original solution, prepared by dissolving lyophilized material in 3 ml of DMEM. Subsequently the mixture was cultured for 3 days, diluted 5–10-fold with medium, and spread on an agar culture plate according to the method previously described (15). The dilution was adjusted to obtain 10^5–10^6 colonies per agar Petri plate (8.7 cm diameter). The agar plate consisted of DMEM containing 10% FCS, 0.1% Bacto-peptone (Difco), and 0.5% agar (Difco). Cells spread on the plate were cultured for 14 days, at which time the surviving cells formed colonies on the agar plate. Many colonies were individually selected and recultured in liquid medium and re-treated with T5A7 mAb and complement as described above. The surviving cells were cultured on agar plates, and each clone (formed as a colony) was cultured separately in liquid medium (primary culture). Subsequently, the GSL composition of each clone was examined by HPTLC, and clones showing different GSL compositions from the parent clone were selected (secondary selection).

Determination of Cell Growth

A cell suspension was prepared from both parent and mutant cells and cultured under various medium conditions as described below.

Temperature Variation—Both the parent and the mutant cells, each having a cell population density of 10^6 cells/ml, were placed in the same medium, and the viability was tested and measured in a thermostat water bath at defined constant temperatures. Alternatively, 2-ml aliquots of the cell suspension were plated in 24-well plates (Corning Glass Work Co., Corning, NY) at different temperatures. Cell numbers were counted at defined intervals.

Variation in Serum Concentration—Both the parent and the mutant cells were cultured in DMEM containing 1.0 and 10% FCS in 24-well plates under 5% CO_2 at 37 °C. Each well received 2 ml of the cell suspension.

Insulin-dependent Cell Growth—This was tested in chemically defined serum-free medium HL-1 (Ventrex Laboratories, Portland, ME) containing transferrin, testosterone, and saturated and unsaturated fatty acids. Insulin (5 μg/ml) was added and cells were cultured at 37 °C under 5% CO_2.

Extraction and Determination of GSL Composition

Cells were harvested from fresh cultures containing 10^5 cells/ml by centrifugation at 1000 rpm for 5 min. The cell pellet from 10 ml of culture was suspended in and washed with 1 ml of CaCl_2– and MgCl_2-free phosphate-buffered saline. After centrifugation, the packed cells were combined with 0.5 ml of isopropl alcohol/hexane/water (55:25:20, v/v/v) and homogenized by sonication. The sonicate was centrifuged at 10000 rpm for 10 min. The supernatant was extracted twice with the same solvent and finally extracted three times with 0.5 ml of CM (21). All the extracts were combined, transferred to a test tube, and evaporated under air stream to dryness. The residue was dissolved in 0.5 ml of 0.1 M KCl, passed through a column of C18 alkylated porous silica gel (Bond-Elut, Analyticem International, Harbor City, CA), and washed with 2 ml of 0.1 M KCl followed by 5 ml of distilled water and finally 2 ml of CM (21). The final eluate was evaporated to dryness, and the residue was dissolved in 200 μg of CMW (21:0±0.05). The sample was analyzed on an HPTLC plate (HPTLC-Fertigplatten, Kieselgel 60, Merck AG, Darmstadt, Germany) and developed on CMW (50:40:10). The GSLs were detected by 0.2% orcinol in 2 M sulfuric acid or 0.2% resorcinol in 10 M hydrochloric acid containing 0.01% FeCl_3.

Isolation and Characterization of GSLs

The total lipid fraction, extracted from 10 ml of packed cells, was dialyzed, rotary-evaporated, and separated into neutral GSLs, monosialogangliosides, and dis- and trisialogangliosides by stepwise elution from 100 ml of DEAE-Sephadex G-25A (40–120 μl) according to a modification of the method originally described (17). The monosialogangliosides were eluted with 0.01 M, and the di- to trisialogangliosides were eluted with 0.45 M ammonium acetate in methanol (18), dialyzed at 4 °C with six changes of distilled water, and evaporated to dryness. GlCer and LacCer were separated from the neutral GSL fraction, and G_M was purified from the monosialoganglioside fraction by high performance liquid chromatography on Iatrobeads 6RS-8010 as previously described (15, 20). Each fraction was methylated (21) and analyzed by mass spectrometry and 1'H NMR spectroscopy (22).

Determination of GSL Quantity

The chemical quantity and molar ratio of LacCer and G_M ganglioside in parent and mutant cells were quantitated based on spheroginive content of each sample. Sphingosine content was determined by fluoroscinamine method after hydrolysis, as previously described (23).

Reactivity of Parent and Mutant Cells with Anti-LacCer and Anti-G_M mAbs

Cells were washed with phosphate-buffered saline containing 0.1% bovine serum albumin (called the diluent) to which 50 μl of mAb T5A7 (16) diluted 125-fold or anti-G_M mAb M2890 (24) diluted 1000-fold was added. The mAb concentration used for the analysis was 1 μg/ml. Cells were incubated at 4 °C for 30 min followed by addition of 100 μl of the diluent and centrifuged. The cell pellet was washed twice with 150 μl of the diluent, and the washed cells were mixed with 50 μl of 50-fold fluorescin isothiocyanate-diluted conjugated rabbit anti-mouse IgM (Miles Laboratories, Naperville, IL), incubated at 4 °C for 30 min, and washed with the diluent as above. The intensity of cell fluorescence was determined under a fluorescent microscope and by cytometer (FACS II, Becton-Dickinson).

Determination of Glycoprotein Glycosylation Pattern

The protein glycosylation pattern of F28–7 and FUA169 cells was analyzed by SDS-PAGE followed by Western blotting with 199-labeled R. communis lectin and 391-labeled WGA. The glycosylation pattern was also studied by cell surface labeling with galactose oxidase/Nab'BH_4 (for terminal Gal or GalNAc) and by periodate oxidation/Nab'BH_4 (for terminal sialic acid), followed by SDS-PAGE and autoradiography. These methods are essentially the same as previously described (25).

Ricin Sensitivity

Ricin (A and B chain mixture) was purchased from E. Y. Laboratories (San Mateo, CA). F28–7 and FUA169 cells were grown in DMEM containing 2% FCS plated in 96-well microtiter plates (Falcon 3072) with three different cell densities, i.e. 5 × 10^4, 5 × 10^5, and 5 × 10^6 cells/ml. To each well the ricin solution in different concentrations, i.e. 0.01, 0.02, 0.3, 0.3, 1.0, 3.0, and 10 μg/ml, was added, and the cells were cultured for 2, 4, or 6 days. Cell viability was tested after 2, 4, and 6 days, and ricin sensitivity was expressed by the minimum dose of ricin that caused death of >90% of the cell population.

Determination of CMP-Sialic Acid/LacCer 2,3-Sialyltransferase Activity of the Cell Extract

The enzyme activity was determined according to a basic procedure previously published (26). Briefly, 10 ml of packed F28–7 cells and 9 ml of packed FUA169 cells were homogenized, respectively, with 10 ml of 0.5 sucrose, 1 mM EDTA, and 50 mM HEPES buffer (pH 7.0) in a Potter-Elvehjem homogenizer. The Golgi fraction was prepared according to the method previously described (28). Either the crude
homogenate or the Golgi membrane fraction was used for enzyme assay. A complete reaction mixture was prepared as follows: 33 μl of LacCer (1.5 mg/ml) dissolved in CM (2:1) and 15 μl of Triton CF-54 (10 mg/ml) were mixed together and evaporated under an N₂ stream. 2.5 μl of 1 M cacodylate buffer (pH 5.9), 0.5 μl of 0.5 M MnCl₂, and 10 μl of 11.1 mM CMP-sialic acid (¹C-labeled at C-4 sialic acid, Du Pont-New England Nuclear) (1.8 μCi/mmol) was added to the residue. The mixture was sonicated, and 10–30 μl of crude homogenate or Golgi membrane fraction was added. The final volume of the homogenate or the Golgi membrane fraction was used for enzyme reaction mixture was adjusted to 50 μl. The reaction mixture was incubated at 37 °C for 1 h, and 6 μl of 1 M EDTA and 100 μl of CM (2:1) were then added. The extract was placed on Whatman No. 3MM filter paper and developed with water to eliminate nonreactive watersoluble sugar nucleotides. The GSLs at the origin were extracted with CM (2:1), and the extract was evaporated to dryness, transferred under an N₂ stream. The residue was dissolved in 20 μl of CM, placed on HPTLC, and developed with CMW containing 0.02% CaCl₂. The radioactive spot was transferred from CMP-¹C-sialic acid to GM₃ based on the protein content of the crude homogenate or Golgi membrane fraction, which was determined by the Lowry method using bovine serum albumin as a reference compound. Biosynthesized GM₃ labeled at sialic acid was used as substrate for sialidase activity of intact cells and cell homogenate, as described by Schengrund et al. (29).

RESULTS

GSL Composition of F28-7 Clone and Its Reactivity with mAb T5A7—This clone grown in suspension culture was characterized by the presence of a single major GSL, identified as LacCer by its TLC mobility (Fig. 1) and ¹H NMR spectrum (data not shown). A strong agglutination of the cells occurred 30–60 min after addition of the mAb T5A7, whereas no destruction of cells was observed without addition of complement (data not shown). Strong lysis (>90% of added cells) was observed on addition of complement (1:10 diluted), and the extent of the lysis correlated positively with mAb concentration (1:500 to 1:5000 dilution) and complement concentration (1:10 to 1:100 dilution), and negatively with cell density (10⁴–10⁶ cells/ml). No cytolysis was induced by addition of complement alone regardless of cell density or complement dilution (data not shown).

Isolation of GSL Mutant—Mutant FUA169 cell line was isolated after mutagenesis, followed by selection with anti-LacCer mAb and complement as described under "Materials and Methods." Through the primary selection, 1642 clones were prepared as described under "Materials and Methods." However, 96 clones survived the re-treatment with anti-LacCer and complement. Of these, 22 surviving clones exhibited characteristic GSL changes. One of these 22 clones that showed a typical depletion of LacCer and addition of GM₃ is herein called FUA169. Further study of this cell line was performed as to GSL characterization, growth characteristics, and antigenicity.

The GSL profiles of the parent F28-7 cell and the mutant FUA169 cell line are shown in Fig. 1. The major GSL in the parent cell was identified as LacCer and that of the mutant FUA169 cell was identified as GM₃. The parent cells contained only a trace quantity of GM₃ and GlcCer, whereas the mutant cells contained only a trace quantity of LacCer. The mutant also contained a significant quantity of GlcCer. The quantities of GSLs in both parent and mutant cells are shown in Table I. F28-7—not pretreated by NTG failed to yield any cell line similar to FUA169, i.e., with high GM₃ and negligible LacCer content.

Reactivity of F28-7 and FUA169 Cells with Anti-LacCer and Anti-GM₃ mAbs—The antigenicity of F28-7 cells and the mutant FUA169 cells determined by fluorescein isothiocyanate conjugates of anti-LacCer and anti-GM₃ mAb is shown in Fig. 2, a and b. As indicated in both cytfluorometry and fluorescent microscopy, F28-7 cells were strongly reactive with anti-LacCer mAb but not with anti-GM₃ mAb, whereas FUA169 cells were not reactive with anti-LacCer but displayed a strong reactivity with anti-GM₃.

Susceptibility of F28-7 and FUA169 Cells to Ricin-dependent Cell Growth Inhibition—Both F28-7 and FUA169 cells were equally susceptible to ricin-dependent growth inhibition. The same dose of ricin (in ng/ml) was required to inhibit the cell growth of both cell lines, regardless of cell density, as shown in Table II.

| F28-7 | 7.4 ± 0.5 | 35.0 ± 2.4 | 2.3 ± 0.15 |
| FUA169 | 20.0 ± 1.5 | 3.8 ± 0.25 | 33.0 ± 2.3 |

*μg equivalent to standard LacCer per 1 ml of packed cells.

![Fig. 1. HPTLC pattern of neutral GSL and ganglioside fractions of F28-7 and FUA169 cells. Samples were prepared as described under "Materials and Methods." Each fraction was dissolved in 200 μl of CM 2:1 containing 8% water, and 5-μl aliquots were spotted on HPTLC plates and developed with CM (50:40:10) containing 0.02% CaCl₂. Lane 1, standard sample of GM₃, lanes 2 and 3, monosialoganglioside fractions; lanes 4 and 5, GM₃ fractions; lane 6, references for neutral GSLs (top to bottom: GlcCer, LacCer, and GM₃, respectively); lanes 7 and 8, neutral GSL fractions; lanes 9 and 10, LacCer fractions; lanes 11 and 12, GlcCer fractions. Lanes 2, 4, 9, and 11, F28-7 cells; lanes 3, 5, 8, 10, and 12, FUA169 cells.](image1)

![Fig. 2. Reactivities of F28-7 and FUA169 cells with anti-LacCer and anti-GM₃ mAbs. Upper panel, cytfluorometric patterns of F28-7 cells (top) and FUA169 cells (bottom). Reactivities of cells with mAbs T5A7 (anti-LacCer) and M2590 (anti-GM₃) are shown. Lower panel, immunostaining pattern of F28-7 cells (a, b) and FUA169 cells (c, d) with mAbs T5A7 (a, c) and M2590 (b, d).](image2)


Table II

<table>
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<tr>
<th>Cell line</th>
<th>Density (cells/ml)</th>
<th>Ricin sensitivity*</th>
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<td>F28-7</td>
<td>5 x 10^2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5 x 10^3</td>
<td>3</td>
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<tr>
<td></td>
<td>5 x 10^4</td>
<td>3</td>
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<tr>
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<td></td>
<td>5 x 10^4</td>
<td>10</td>
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</table>

*R Minimum concentration of ricin (ng/ml) needed to inhibit cell growth when incubated for indicated period.

Fig. 3. SDS-PAGE pattern of F28-7 and FUA169 cells. Lanes 1 and 2, galactose oxidase/NaBH₄ method; lanes 3 and 4, periodate oxidation/NaBH₄ method; lanes 5 and 6, Western blotting with ¹³¹I-labeled R. communis lectin; lanes 7 and 8, Western blotting with ¹³¹I-labeled WGA; lanes 1, 3, 5, and 7, F28-7 cells; lanes 2, 4, 6, and 8, FUA169 cells.

Table III

<table>
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<tr>
<th>Enzyme source</th>
<th>Protein (µg)</th>
<th>Activity (pmol of GM₃/mg of protein)</th>
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<tr>
<td>F28-7 extract</td>
<td>410</td>
<td>&lt;1</td>
</tr>
<tr>
<td>FUA169 extract</td>
<td>410</td>
<td>123</td>
</tr>
<tr>
<td>F28-7 Golgi fraction</td>
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<tr>
<td>FUA169 Golgi fraction</td>
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<td>306</td>
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<tr>
<td>Combined F28-7/FUA169 Golgi fractions</td>
<td>see Fig. 6</td>
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</table>

*pmol of GM₃/mg of protein.

Fig. 4. Autoradiogram of sialosyltransferase product as catalyzed by the crude enzyme extract of FUA169 cells (lanes 2 and 3) and that from F28-7 cells (lanes 4 and 5). Lane 1 is the standard GM₃. Lanes 2 and 3 represent, respectively, the product synthesized by 5 and 10 µl of crude enzyme extract of FUA169 cells; lanes 4 and 5 represent, respectively, the results of incubation with 5 and 10 µl of the crude enzyme extract from F28-7 cells.

Fig. 5. Time course changes of sialosyltransferase activity of Golgi fraction. The activity was experienced on 250 µg of Golgi membrane protein.

cells and that of the F28-7 parent cells are shown in Fig. 5. No activity was demonstrated in F28-7 cells, regardless of the duration of incubation, in contrast to the increasing activity seen in FUA169 cells.

To test whether enhanced sialidase activity is responsible for the absence of detectable sialosyltransferase in the F28-7 cells, the enzyme activity of mixtures (in various proportions) of Golgi membrane fractions of FUA169 and F28-7 cells was determined. The level of GM₃ synthesis was directly correlated with the proportion of FUA169 Golgi membrane fraction added and inversely correlated with the proportion of F28-7 membrane fraction (Fig. 6). Identical results were obtained in two experiments. Thus, the absence of sialosyltransferase in F28-7 cells cannot be due to enhanced sialidase activity or to some inhibitory factor.

Sialidase activity of F28-7 and FUA169 cells, determined using GM₃ labeled by ¹³C (at C-4 of sialic acid) according to the method of Schengrund et al. (29), was 6.5 ± 0.5 and 7.4 ± 0.65 nmol, respectively, of sialic acid released per mg of cell residue per 2 h.

In Vitro Growth Properties of F28-7 and FUA169 Cells—Changes in three parameters had striking effects on the growth behavior of F28-7 and FUA169 cells: (i) temperature; (ii) serum concentration; and (iii) serum-free chemically defined medium containing insulin. Both cell lines showed a similar growth behavior at 37 °C, although FUA169 cells showed a slightly slower growth rate. In contrast, a remarkable difference in growth behavior was obvious when the two cell lines were grown at 39.5 °C. At this temperature, parent
Selection of Glycolipid Mutant after Mutagenesis

FIG. 6. \(G_M^3\) synthesis by a mixture of F28–7 and FUA169 Golgi membrane fractions. Golgi fractions prepared by the method of Senn et al. (28) were mixed in the presence of Triton X-100 detergent, and \(G_M^3\) synthesis was determined using LacCer and CMP-[\(^{14}\)C]sialic acid as substrates (see “Materials and Methods”). Lower and upper abscissas show (respectively) percent of FUA169 and F28–7 Golgi fractions, which are inversely related to each other.

FIG. 7. Temperature dependence of F28–7 and FUA169 cell growth. A, growth at 39.5 °C. B, growth at 37 °C. ○, F28–7 cells; Δ, FUA169 cells; ● (panel A only), F28–7 cells cultured in medium containing 50 μg/ml GM3, preincubated overnight at 37 °C and then switched to 39.5 °C.

F28–7 cells grew well, but growth of FUA169 cells was strongly inhibited. When F28–7 cells were preincubated overnight with 50 μg/ml GM3 at 37 °C and then switched to 39.5 °C, growth behavior mimicked that of FUA169 cells (Fig. 7A).

Both parent and mutant cells grew similarly in media supplemented with 10% FCS, although FUA169 grew slightly more slowly (Fig. 8B). However, at low serum concentration, FUA169 showed much more restricted growth than F28–7 (Fig. 8A). This trend was most pronounced under insulin-dependent cell growth conditions in chemically defined medium. Growth of FUA169 was completely inhibited and cell number slowly declined, whereas F28–7 grew normally (Fig. 8C). When F28–7 cells were preincubated in GM3-containing medium (which induced incorporation of GM3 into the cell membrane) and then grown in 1% FCS in a chemically defined medium plus insulin, growth behavior mimicked that of FUA169 cells (Fig. 8, D and F). In contrast, F28–7 cell growth was minimally inhibited by GM3 in 10% FCS (Fig. 8E).

**DISCUSSION**

Cytotoxic plant lectins have been used to select “lectin-resistant” variants, which show a clearly different glycosylation pattern and glycosyltransferase profile from the parent cells and show interesting pleiotropic phenotypes that are assumed to be due to glycosylation difference in glycoproteins (3-10). A number of mAbs have been established that are directed to defined, GSL-specific CHO structures, many of which are developmentally regulated or tumor-associated (1, 18). The majority of these mAbs, in the presence of complement, are capable of lysing cells and thus selecting specific populations of cells expressing different CHO determinants on cell surface GSL. This approach, although it has great potential applicability, has not been successfully applied to select variant or mutant cells that differ in their expression of GSLs. The mAb T5A7 reacts specifically with LacCer but not with α-acetylgalactosamyl residue (16), and the lactosyl residue is highly specific for GSLs and absent in glycoprotein CHO chains. mAb T5A7 together with complement has been applied to select the mutant of LacCer after mutagenesis of mouse mammary cancer cell line FM3A.

The mutant FUA169 was successfully isolated from T5A7-resistant cells and was clearly characterized by the presence of GM3, in contrast to the parent F28–7 cells, which contain LacCer as the major component. FUA169 lacked or showed greatly diminished quantities of LacCer as compared with the parent cells. The mutant cell line was also characterized by the clear presence of sialoxyltransferase for synthesis of GM3, in contrast to the absence of this enzyme in the parent cells. The enzyme was highly active in the crude extract of the mutant cells as well as in the Golgi membrane fraction. The absence of enzyme activity in the parent cells was not due to inhibition of this enzyme activity, because a mixture of both parent and mutant cells showed the same activity as mutant cells. Similarly, the absence of this enzyme activity in the parent cells was not due to enhanced sialidase activity.

The mutant cells were characterized by strong reactivity with anti-GM3 mAb M2590, while the parent cells lacked this reactivity. Conversely, the mutant cells lacked reactivity with T5A7, and the parent cells were characterized by strong reactivity with T5A7 mAb. However, the difference must be limited to GSLs and not seen in glycoproteins, since both the mutant and parent cells were characterized by identical glycosylation patterns with regard to cell surface-labeled Gal, GalNAc, or NeuAc, separated on SDS-PAGE and Western blotting with WGA and *R. communis* lectins. Parent and mutant cells are equally sensitive to cytotoxic *R. communis* lectin. These findings all suggest that the two cell lines have
similar glycoprotein glycosylation patterns.

The contrasting properties of mutant and parent cells, expressed in various cell biological phenotypic differences, must be due to the functional differences of G\textsubscript{M3} versus LacCer. The clear growth restriction of FUA169 cells under various growth conditions (particularly insulin-dependent growth behavior resembling those of FUA169 cells (see accompanying paper (32)). They also offer an excellent model for further study of the role of GSLs in growth regulation and in cell adhesion (see accompanying paper (32)). They also offer an excellent opportunity to study the genetic basis for encoding of sialosyltransferases.

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REFERENCES


Tyrosine kinase associated with human insulin receptor is highly susceptible to sialosylparagloboside, whereas that associated with mouse or rat insulin receptor is susceptible to G\textsubscript{M3}.