Sialosyl-Tn (STn) is a mucin-associated carbohydrate antigen that is not expressed by most normal epithelial cells but becomes expressed in several types of adenocarcinomas, where it is often associated with a poor prognosis. Little is known about the regulation of the STn phenotype in tumor cells and the immune response to STn antigen. In the present study, we established clonal cell lines in which virtually all of the cells were STn positive (designated LS-C) or STn negative (designated LS-B). These two cell lines, derived from a single parental cell line, LS174T, have the same total protein electrophoretic profiles but carry markedly different oligosaccharide structures on their mucin; the mucin from LS-C cells has only the Tn and STn structures, whereas LS-B cell mucin lacks these simple structures and carries more complex oligosaccharides. These results indicate that lack of STn expression by cells can be due to the lack of STn synthesis rather than inaccessibility of antibodies to bind to STn by steric hindrance. Both clones were similar in their growth rates, response to γ-interferon, and sensitivity to lysis by lymphokine-activated killer cells. These cells may be important models for understanding the regulation of glycosylation at the cellular level and for further studies of tumor biology and immune response to STn antigen.

INTRODUCTION

Many cancer-associated antigens defined by monoclonal antibodies are carbohydrate epitopes. Some carbohydrate epitopes, such as blood group ABH and Lewis antigens, reside on both glycoprotein and glycolipid molecules. Others, however, appear to be restricted to glycoproteins (1). An example of the latter is the mucin-associated carbohydrate antigen, STn.

The STn antigen, defined by several mAbs (2–5), is expressed by very few normal tissues but becomes expressed in many types of human adenocarcinomas including colon, breast, pancreas, ovary, stomach, and lung (6–10). The expression of STn antigen by cancers of the colon (11), stomach (12), and breast (13) has been associated with a poor prognosis. In addition, STn expression in the serum of patients with ovarian and gastric cancer has also been correlated with an adverse clinical outcome (14, 15). These data suggest that STn antigen may play an important role in the pathobiology of cancer cells.

To elucidate the biochemical basis of the STn phenotype by colon cancer cells and to test the role of STn antigen in tumor cell biology, it would be important to study cell lines that either completely express or completely lack the antigen. Because such cell lines do not exist, we conducted the present study to isolate STn+(+) and STn(−) clones from the parental LS174T cell line and analyze their mucin oligosaccharides and biological characteristics.

MATERIALS AND METHODS

Cell Culture

Human colon cancer cell line LS174T was obtained from American Type Culture Collection (Rockville, MD). This cell line and its clonal derivatives described herein were fed with DMEM supplemented with 10% fetal calf serum, 50 units/ml penicillin, and 50 μg/ml streptomycin and incubated at 37°C in 7.5% CO₂.

Establishment of STn(+) and STn(−) Clones

After screening many available human colon cancer cell lines we found that most of them barely express STn antigen in culture. However, we noted that the LS174T cell line did contain a subpopulation of cells that expressed STn. To select STn(+) and STn(−) cells from this cell line, a two-step procedure was used wherein cells were first enriched for STn expression and then cloned by limiting dilution.

Step 1: Enrichment of STn(+) Cells. LS174T cells were trypanized and 5 × 10⁶ cells were resuspended in 1 ml of mouse monoclonal antibody TKH2 (hybridoma culture supernatant), which specifically recognizes STn antigen (2). The cell suspension was incubated at 4°C for 30 min and washed twice with DMEM by centrifugation. After cells were resuspended in DMEM, magnetic polystyrene beads coated with sheep anti-mouse IgG antibody (Dynabeads M-450; DYNAL, Inc., Great Neck, NY) were added, with a bead:cell ratio of 3:10 (bead:STn-positive cell ratio was about 3:1 based on the observation that positive cells in the parental line comprised approximately 10%). The suspension was again incubated at 4°C for 5 min. Cells which bound to the beads were collected by a magnet and washed with DMEM according to the manufacturer’s protocol. The collected cells together with the bound beads were placed in culture and incubated under standard conditions.

Step 2: Cloning of STn(+) and STn(−) Cells. Cell cultures enriched for STs expression in Step 1 were trypanized and the concentration of cells was adjusted so that each well of a 96-well plate received one cell. Wells were checked under a microscope 24 h after seeding and those with a single cell were marked. Single cell colonies were grown, expanded, and screened for STn antigen expression. Screening was done by immunocytochemistry on cells grown in chamber slides (Nunc Inc., Naperville, IL) using mAb TKH2. One STn-negative and one highly positive clone were selected and a second round of cloning by limiting dilution was performed from these lines using the same method to ensure their clonality. The resulting clones were named LS-B (STn negative) and LS-C (STn positive).

Immunohistochemical Analysis

The monoclonal antibodies 1E3 and TKH2, were kindly provided by Dr. S. Hakomori (The Biomembrane Institute, Seattle, WA). Hybridoma producing monoclonal antibody CA19-9 was purchased from the American Type Culture Collection. The biotinylated lectins peanut agglutinin (Arachis hypogaea) and Vicia villosa agglutinin were obtained from Sigma Chemical Co. (St. Louis, MO).

For apomucin peptide expression, the following antibodies were used: SM3, which recognizes the tandem repeat region of the MUC1 gene product (16); and the CTI rabbit polyclonal antiserum, which recognizes the cytoplasmic tail region of MUC1 peptide, a region that is conserved between species and is poorly glycosylated (17). These antibodies were generously provided by Drs. Joyce Taylor-Papadimitriou and Sandra Gendler (Imperial Cancer Research Fund, London, United Kingdom). The CCP58 and M3.3 monoclonal antibodies recognize the tandem repeat region of the MUC2 and MUC3 mucins, respectively.
Analysis of [3H]Glucosamine-labeled Mucin Oligosaccharides

Mucin was prepared from both the spent medium and cell lysates of LS-B and LS-C cells. The spent medium was saved and cleared of cells by centrifugation at 5000 × g. Ammonium sulfate was added to the supernatant to 50 and 75% saturation sequentially; the precipitates at both concentrations were collected and dissolved in 10 mM Tris-HCl buffer, pH 8 (spent medium).

The cell layers were washed with and scraped into phosphate-buffered saline. The cells were pelleted, frozen at -80°C, and resuspended in 0.1 M sodium phosphate buffer (pH 7.2) containing 1 mM EDTA, 2 mg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. The cell suspension was then sonicated for 30 min and cleared by centrifugation at 100,000 × g for 1 h (Triton extract).

The spent media and Triton extracts were loaded onto a Sepharose CL-4B column (1.6 x 90 cm) and eluted with 10 mM Tris-HCl buffer, pH 8 (column buffer). The void volume fractions were collected, dialyzed against water, and lyophilized. The lyophilized material was dissolved in an appropriate volume of column buffer and subjected to CsCl density gradient centrifugation for 48 h at 30,000 rpm (Beckman SW40 rotor) with a starting density of either 1.39 or 1.47 g/ml. After centrifugation, 1-ml fractions were collected and their glucose content was determined by counting an aliquot in a liquid scintillation counter.

Analysis of Total Cellular Proteins

Protein constituents of LS-B and LS-C cells were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing and non-reducing conditions.

Metabolic Labeling of LS-B and LS-C Cells with [3H]Glucosamine

LS-B and LS-C cells were each grown in 10-cm tissue culture dishes and subconfluent cultures were labeled by incubating the cells for 24 h in low-glucose DMEM supplemented with 5% fetal calf serum and 5 µCi/ml of [3H]glucosamine (DuPont New England Nuclear).

Isolation/Purification of Mucin

Isolation/purification of mucin was determined by counting an aliquot in a liquid scintillation counter.

LAK Cell Sensitivity

Lymphocytes, isolated from peripheral blood of a normal healthy individual by Ficoll-Paque, were cultured for 5 days in RPMI-5 and recombiant IL-2 (100 units/ml) at a concentration of 1.5 × 10^6 cells/ml. The IL-2-activated cells were washed with RPMI-5 and counted. The target cells (LS-B and LS-C cells) were seeded in the 96-well plates (5 × 10^3 cells/well) and cultured for 2 days at 37°C. The cells were then labeled in the wells by replacing the medium with 50 µl of RPMI-5 containing 10 µCi of [3H]glucosamine. After incubation at 37°C for 4 h, the labeling medium was removed and the wells were washed 3 times and fed with 100 µl/well of fresh RPMI-5. To each well, 100 µl of LAK cell suspension of various concentrations were added to make different effector:target ratios and the plates were incubated at 37°C. After 4 h of incubation, 100 µl of supernatant from each well were harvested for determination of [3H]counts released into the medium.

RESULTS

Establishment of LS-B and LS-C Cell Lines

Immunohistochemical staining of parental LS174T colon cancer cells with mAb TKH2 revealed that 10–15% of the cells expressed the STn antigen. After enrichment using the magnetic bead approach, approximately 60% of cells were STn positive. From this culture, single cell colonies were grown by limiting dilution, expanded, and screened for the expression of STn. A second round of limiting dilution cloning was performed using these clonal lines to ensure the clonality of the lines. Consequently, one STn-positive line (clone LS-C) and one STn-negative line (clone LS-B) were selected. Fig. 1 illustrates that essentially all of the LS-C cells express STn, whereas fewer than 1% of LS-B cells express the antigen.

Expression of Carbohydrate Antigens by LS-B and LS-C Cells

Table 1 lists the expression of several carbohydrate and apomucin peptide immunodeterminants in LS-B- and LS-C-cultured cells. LS-C cells expressed Tn and STn antigens but not TF or SLeα. In contrast, LS-B cells expressed TF and SLeα but not Tn or STn epitopes. Despite these differences in carbohydrate antigen expression, both cell lines expressed apomucin determinants quite similarly; there was no cx peptide immunodeterminant in LS-B- and IS-C-cultured cells. IS-C cells expressed TF and SLeα but not Tn or STn epitopes. Despite these differences in carbohydrate antigen expression, both cell lines expressed apomucin determinants quite similarly; there was no cx peptide immunodeterminant in LS-B- and IS-C-cultured cells. IS-C cells expressed TF and SLeα but not Tn or STn epitopes. Despite these differences in carbohydrate antigen expression, both cell lines expressed apomucin determinants quite similarly; there was no cx peptide immunodeterminant in LS-B- and IS-C-cultured cells. IS-C cells expressed TF and SLeα but not Tn or STn epitopes. Despite these differences in carbohydrate antigen expression, both cell lines expressed apomucin determinants quite similarly; there was no cx peptide immunodeterminant in LS-B- and IS-C-cultured cells. IS-C cells expressed TF and SLeα but not Tn or STn epitopes. Despite these differences in carbohydrate antigen expression, both cell lines expressed apomucin determinants quite similarly; there was no cx peptide immunodeterminant in LS-B- and IS-C-cultured cells. IS-C cells expressed TF and SLeα but not Tn or STn epitopes. Despite these differences in carbohydrate antigen expression, both cell lines expressed apomucin determinants quite similarly; there was no cx peptide immunodeterminant in LS-B- and IS-C-cultured cells. IS-C cells expressed TF and SLeα but not Tn or STn epitopes. Despite these differences in carbohydrate antigen expression, both cell lines expressed apomucin determinants quite similarly; there was no cx peptide immunodeterminant in LS-B- and IS-C-cultured cells. IS-C cells expressed TF and SLeα but not Tn or STn epitopes. Despite these differences in carbohydrate antigen expression, both cell lines expressed apomucin determinants quite similarly; there was no cx peptide immunodeterminant in LS-B- and IS-C-cultured cells.
Table 1 Expression of carbohydrate and apomucin epitopes on LS-B and LS-C cells

<table>
<thead>
<tr>
<th>Antigen</th>
<th>mAb/lectin</th>
<th>LS-B</th>
<th>LS-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate epitopes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tn</td>
<td>1E3; VVA</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>STn</td>
<td>TKH2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TF</td>
<td>PNA</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SLe*</td>
<td>CA19-9</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Mucin peptide epitopes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC1</td>
<td>SM3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MUC2</td>
<td>CT1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MUC3</td>
<td>CCP58</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AS3</td>
<td>AS7S</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* (-) no expression; (+) weak expression; (++) moderate expression; (+++) strong expression.

Fig. 1. Immunocytochemical staining of LS-B (a) and LS-C (b) cells using mAb TKH2 reactive with STn antigen. LS-B cells form broad, flat colonies which lack STn expression. LS-C cells grow as clusters, with essentially all cells positive for STn expression.

Fig. 2. Polyacrylamide gel electrophoresis (9% acrylamide) of total cellular proteins from Triton extracts of LS-B (Lane 1) and LS-C (Lane 2) cells. The gel was stained with Coomassie brilliant blue. Ordinate, molecular weight markers.
Characterization of Oligosaccharides Derived from Cell-associated Mucins of LS-B and LS-C Cells. Mucins were also purified from Triton X-100 extracts of \[^{3}H\]glucosamine-labeled LS-B and LS-C cells. The Triton extracts were fractionated on a Sepharose CL-4B column (Fig. 7, a and b) and the void volume fractions were pooled and further purified by CsCl gradient centrifugation (Fig. 7, c and d). The radiolabeled materials from LS-C cells were separated into two peaks (Fig. 7d); the major one had a density of 1.43 g/ml, which is the same as the mucin of its spent medium counterpart, and the minor one had a density of >1.56 g/ml, which corresponds to the material in AS75. The material from LS-B cells, on the other hand, gave a profile similar to that of spent medium-derived mucin from the same cell line, having a peak density of 1.49 g/ml (Fig. 7c). However, the peak included the material with the density of 1.54 g/ml or higher, which might be contaminated by proteoglycan, as was the case with AS75-derived peak. In an attempt to further separate the peak, the void volume fraction was subjected to CsCl gradient centrifugation with higher density range. As shown in Fig. 7e, the peak was actually separated into two peaks. Subsequent heparitinase and chondroitinase treatment of all the peaks revealed that the major peaks of both cell lines were resistant to the treatment and that the minor peak with higher density of each cell line was proteoglycan (data not shown).

The \[^{3}H\]glucosamine-labeled oligosaccharides linked to the purified cell-associated mucins were also analyzed. Although not shown, the elution patterns of the oligosaccharides derived from the cell-associated mucins of both cell lines were almost identical to their counterparts derived from the respective spent medium (see Fig. 5, a and b). In particular, the major peak from LS-C cell-associated mucin showed the same chromatographic behavior as its medium counterpart in the subsequent neuraminidase digestion and after Dionex column chromatography (data not shown), proving it to be sialosyl α-2,6-N-acetylgalactosamine.

Biological Characteristics of LS-B and LS-C Cells

Growth Rates in Vitro. LS-B and LS-C cells were plated in T-25 flasks (5 × 10^4 cells/flask) and cultured. Cell numbers were determined after 2, 4, 7, 9, and 13 days of culture (Fig. 8a). There was no significant difference in the growth rate between LS-B and LS-C cells.
MODEL STn(+) AND STn(−) CELL LINES

Fig. 4. Chondroitinase ABC and heparitinase digestion. The peak fractions from CsCl density gradient centrifugation derived from AS50 and AS75 of LS-B spent medium were digested by the enzymes and applied to a Sepharose CL-4B column. The material purified from AS50 fraction (○) was resistant to the digestion whereas the one from AS75 of the same spent medium (■) was almost completely digested.

The doubling time of 26 h and the saturation density of approximately 2 × 10⁷ cells/25 cm² were similar for both cell lines.

LS-B and LS-C Cells as Targets of Cytotoxicity. In order to investigate whether the different glycosylation of mucins furnishes colon cancer cells with different levels of susceptibility to cytotoxic cells, cytotoxicity by IL-2-activated lymphocytes was assessed on the two cell lines. As shown in Fig. 8b, although LS-C was a bit more susceptible than was LS-B to lysis, both cell lines were sensitive to LAK cytotoxicity with a minimum difference for all of the effector:target ratios tested.

Growth Inhibition by IFNγ. In order to test their sensitivity to IFNγ, LS-B and LS-C cells were grown in the presence of different concentrations of IFNγ and growth rates were determined. As shown in Table 2, 25 units/ml of IFNγ inhibited the growth of LS-B cells by 37.3% and LS-C cells by 35.4%. At 100 units/ml, the inhibition was 75.4 and 75.1%, respectively, indicating that the two cell lines are equally sensitive to IFNγ.

DISCUSSION

Many tumor markers in clinical use are carbohydrate immunodeterminants found on glycoconjugates. There are several reasons why STn has attracted particular interest: (a) it may be a useful diagnostic marker since it is rarely expressed in normal tissues but is widely expressed in a variety of adenocarcinomas (6–10); (b) STn has potential use as a prognostic marker for patients with colorectal, gastric, breast, and ovarian cancer (11–15); (c) STn holds therapeutic promise, and a synthetic STn-based immunoconjugate is being used for cancer immunotherapy (23). To elucidate the role of STn antigen in tumor biology, however, it would be important to study model cells that either all express or lack the antigen. Such cells would also facilitate studies of the biosynthetic mechanisms underlying STn expression in cancer cells. The use of cell lines which express STn antigen in only a subpopulation of cells would provide indefinite and misleading results.

We herein report the establishment of clonal cell lines from LS174T colon cancer cells. The LS-B clone expresses STn in fewer than 1% of cells, whereas virtually 100% of LS-C cells express STn. To date, these cell lines have maintained their respective STn(−) and STn(+) phenotypes for more than 50 passages. Because these clones were derived from a cell line of a single patient, they contain the same genetic background, thereby offering the best model system for comparative studies on the effects of STn antigen expression. This is confirmed by the fact that the two cell lines share an identical pattern of cellular proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The STn epitope has thus far been identified only on mucin-type glycoproteins. We therefore isolated mucins from LS-B and LS-C cells and analyzed the carbohydrate chains attached to them. It was demon-
N-acetylgalactosaminitol and N-[3Hjacetylgalactosaminitol, respectively. (b) The major oligosaccharide was also analyzed by high performance anion exchange chromatography on dianex G-50 column (C). Arrows 1 and 2, the elution positions of [3H]sialosyl N-acetylgalactosaminol and N-[3H]acetylgalactosaminol, respectively. (b) The major oligosaccharide was also analyzed by high performance anion exchange chromatography on a Dionex BioLc system fitted with a pulsed amperometric detector. Arrow, elution position of authentic sialic acid α-2,6-N-acetylgalactosaminitol; •, radioactivity.

Fig. 6. Confirmation of sialosyl-Tn structure in IS-C-secreted mucin. (a) The oligosaccharides derived from LS-C-secreted mucin were separated by Sephadex G-50 column (C). The major oligosaccharide (bar) was digested with neuraminidase and the digest was rechromatographed on the same column (C). Arrows 1 and 2, the elution positions of [3H]sialosyl N-acetylgalactosaminol and N-[3H]acetylgalactosaminol, respectively. (b) The major oligosaccharide was also analyzed by high performance anion exchange chromatography on dianex G-50 column (C). Arrows 1 and 2, the elution positions of [3H]sialosyl N-acetylgalactosaminol and N-[3H]acetylgalactosaminol, respectively. (b) The major oligosaccharide was also analyzed by high performance anion exchange chromatography on a Dionex BioLc system fitted with a pulsed amperometric detector. Arrow, elution position of authentic sialic acid α-2,6-N-acetylgalactosaminitol; •, radioactivity.

strated that oligosaccharides released from LS-B mucins (derived from both spent medium and cell extract) lack the STn structure, whereas the major oligosaccharide chains released from LS-C mucin were sialosyl-GalNAcol (STn) and GalNAcol (Tn). Thus, negative staining of LS-B cells by mAb TKH2 is due to the lack of the antigenic structure, not inaccessibility of the antibody to bind to the STn antigen. It should be noted that we pursued only one STn(+) and one STn(−) clone for these in-depth biochemical studies. Whether the biochemical and immunochemical profiles reported in the present study are representative of other STn(+) and STn(−) clones is not known. A critical question that arises is why the mucins synthesized by the two clonal lines are glycosylated differently. There are at least two possible reasons: (a) LS-B and LS-C cells may express different species and levels of glycosyltransferases and/or glycosidases. For example, it is possible that LS-C cells may not express the glycosyltransferase necessary for adding galactose and/or N-acetylglucosamine to the core N-acetylgalactosamine for further oligosaccharide elongation (24); (b) the two cell lines might express different apomucin polypeptides which might become differentially glycosylated. We previously reported that LS-B and LS-C cells lack MUC1 but express MUC2, MUC3, and very low levels of MUC4 mucin genes at similar levels, suggesting that both are synthesizing a similar amount of these three species of mucin (25). Since other mucin genes have been described recently (26, 27) and more are likely to be discovered, we cannot exclude the possibility that the two cell lines are indeed expressing different apomucins yet to be identified. However, it should be noted that even when different apomucins are expressed, they may carry similar carbohydrate epitopes (28). This highlights the complexities involved in studying which carbohydrate determinants are carried by which apomucins.

LS-B and LS-C cells proliferated in vitro at nearly identical rates. However, tumor cells in vivo grow under the influence of many soluble factors, as well as immunological effector cells, which may either augment or suppress their growth. It is possible that different tumor cell phenotypes will induce a different array of factors and effector cells in vivo. Therefore, different outcomes of patients with STn(−) or STn(+) tumors may be the result of susceptibility to effector molecules and/or cells encountered in the host. Since natural killer and LAK cells have been implicated in tumor cell killing, we studied the sensitivity of LS-B and LS-C to LAK cell cytotoxicity. The two cell lines were equally sensitive to LAK cell killing but this finding is not surprising since LAK cells kill tumor targets in an antigen-nonspecific fashion.

Treatment of nude mice carrying human colon cancer cells by interferon has completely suppressed lung metastasis and significantly extended survival time (29). More recently, a type I interferon-resistant variant of a poorly differentiated human colon cancer cell grew at twice the rate of the parental line and also produced liver metastasis at a higher frequency, suggesting the role of interferon in growth inhibition of tumor cells in the host (30). We found that LS-B and LS-C cells were equally susceptible to the antiproliferative effect of γ-interferon. This result, however, still does not preclude the possibility that γ-interferon will differentially alter the growth of the cell lines in vivo by working through modulation of other host immune mechanisms such as enhancement of cytotoxic T-cells and expression of major histocompatibility complex molecules on the tumor cells (31).

Previous biochemical studies using normal colonic tissue and colon cancer tissue have analyzed oligosaccharide structures of colonic mucin. In both rats and humans, normal colonic mucin typically carries oligosaccharides which are 2–12 sugar residues long and mainly based on core type 3 structures (32–34). In contrast, the oligosaccharides of colon cancer mucin are fewer and shorter (35), with chain lengths of only 2–8 residues and core oligosaccharide types 1, 3, 4, and 5 (36, 37).
According to those biochemical studies, both normal and cancerous mucin carry STn disaccharide (32–34, 36). However, many immunochemical investigations using monoclonal antibodies have demonstrated that normal colonic epithelial cells are negative for STn. Colon cancer cells often become positive for this antigen, but even in the STn-positive tumors, staining is typically heterogeneous. It has therefore been suspected that in normal colonic epithelium, the access of antibodies to STn antigen is hindered by neighboring longer oligosaccharide chains, and with malignant progression STn becomes exposed due to incomplete glycosylation. While this might be the
case, it is also possible that a subpopulation of tumor cells is able to synthesize STn, whereas others are not. This possibility cannot be tested by analyzing mucin oligosaccharides released from tissue extracts or cell lines of mixed cell populations as performed in previous studies. Our data using clonal cell lines indicate that the absence of STn expression by a given cell may in fact be due to a lack of synthesis of the STn structure rather than masking. The fact that a clone of HT29 colon cancer cells also appears to lack the STn structure supports the hypothesis that some cancer cells are not capable of synthesizing STn (37). The LS-B and LSC cells should therefore be an excellent system to elucidate the regulatory mechanisms of mucin oligosaccharide biosynthesis. LS174T cells have been useful for studies of colonic mucin. The mucin from xenograft tumors of LS174T cells has been characterized biochemically as a threonine-rich sialomucin (38). The LS180 cell line, from which the LS174T line was derived, has been used as an immunogen to produce monoclonal antibodies that react with cancer-associated mucin antigens. One of these mAbs, MLS 102, reacts only with colon cancers, whereas MLS 103 reacts with both normal and cancerous colon tissues (39). The MLS 102 antigen consists primarily of Tn and STn structures with a few longer oligosaccharides. In contrast, the MLS 103 antigen lacks Tn and STn but has longer oligosaccharides (40). It is interesting that the oligosaccharide profile of LS-C mucin is quite similar to that of the MLS 102 antigen (except for the lack of longer oligosaccharides in LS-C) and that of LS-B mucin is similar to the profile of the MLS 103 antigen. The MLS 102 antigens were identified using affinity chromatography but it is not clear whether they are mucin-type glycoproteins (40). Furthermore, since LS180 cells are not clonal, it is not clear whether the two antigens are being synthesized by a given cell or a mixture of cells. Therefore, the use of the LS-C and LS-B clones may help to elucidate differences in glycosylation at the cellular level. Moreover, the simplicity of carbohydrate structures on LS-C mucin indicates that it is analogous to ovine submaxillary mucin (41). Since mucins from ovine, bovine, and porcine submaxillary glands have been useful tools for studying glycosylation pathways (24), LS-C mucin may be an important source of human mucin for such analyses.

Another important potential use for LS-C cells is as a target for testing reactivity of anti-STn antibodies. Until now, solid phase immunosassays have used ovine submaxillary mucin or synthetic STn linked to keyhole limpet hemocyanin or human serum albumin to measure anti-STn titers. Although this approach is useful, the use of LS-C cells or mucin would enable conclusions to be drawn concerning immune recognition of native cancer-associated STn produced by human cells. This may have important utility for monitoring the human anti-STn response in patients receiving STn-based immunotherapy (23, 42, 43).


Use of Model Cell Lines to Study the Biosynthesis and Biological Role of Cancer-associated Sialosyl-Tn Antigen

Shunichiro Ogata, Anli Chen and Steven H. Itzkowitz