ABH alterations previously described in colon cancer tissues and appear antigens are probably not the true A or B antigens.

Classically, ABH blood group determinants reside on either a type 1 (Galβ1→3GlcNAc-R) or type 4 (Galβ1→3GalNAc/R) chains. 6

Thus, the colon cancer cell lines used in this study exhibit all of the ABH alterations previously described in colon cancer tissues and appear to be useful experimental models for studying the molecular events involved in cancer-associated ABH expression.

INTRODUCTION

Blood group ABH substances are carbohydrate structures, which are formed by the sequential addition of specific monosaccharides to the carbohydrate side chains of glycolipids and glycoproteins (1, 2). They are expressed not only in blood cells (3) but also in a variety of epithelial cells (4). Their expression is under genetic control, where the primary gene products are glycosyltransferases. Several studies have demonstrated cancer-associated alterations in ABH antigen expression in human colon cancer tissues. However, the mechanism(s) responsible for these alterations has not been elucidated. Therefore, experiments were conducted using established colon cancer cell lines (four type O, three type A, and two type B) to examine ABH antigen expression by immunocytochemistry and correlate this with activities of ABH biosynthetic (glycosyltransferase) and degradative (glycosidase) enzymes. The products of the glycosyltransferase enzymes were characterized by high performance liquid chromatography and paper chromatography, and substrate affinities (apparent Kₘ values) of the cancer cell-derived glycosyltransferases were analyzed.

The present data demonstrate: (a) all cell lines except H-498 (blood type A) expressed the appropriate ABH glycosyltransferase as well as all three glycosidases; (b) product characterization and substrate dependence experiments suggested that the cancer cell-derived ABH glycosyltransferase enzymes had properties that were similar to those of the ABH enzymes in human serum; (c) H-498 cells exhibited A antigen deletion with accumulation of H precursor substance, most likely due to insufficient A transferase activity; (d) SW1417 cells (blood type B) demonstrated B antigen deletion without precursor accumulation, despite adequate levels of B transferase and low α-galactosidase activity; and (e) weak incompatible A antigen expression occurred in LoVo (type A) and SW1116 cells. However, since these cells lacked incompatible A or B transferase activity, these incompatible antigens are probably not the true A or B antigens.

Thus, the colon cancer cell lines used in this study exhibit all of the ABH alterations previously described in colon cancer tissues and appear to be useful experimental models for studying the molecular events involved in cancer-associated ABH expression.

MATERIALS AND METHODS

Materials. UDP-D-[3H]galactose (specific activity, 17 Ci/mmol) was purchased from New England Nuclear (Boston, MA), UDP-N-acetyl-D-galactosamine (specific activity, 23 mCi/mmol) was purchased from ICN (Irvine, CA), and GDP-L-[14C]fucose (specific activity, 308 mCi/mmol) was obtained from Amersham (Arlington Heights, IL). Phenyl β-galactoside, 2′→fucosyllactose, p-nitrophenyl galactoside, p-nitrophenol α-fucoside, p-nitrophenol-α-N-acetylgalactosaminide, and biotinylated isoelectin I-B, from Bandeiraea simplicifolia were purchased from Sigma Chemical Co. (St. Louis, MO). Dowex 1-X8-formate (100–200 mesh) was obtained from Bio-Rad Laboratories (Richmond, CA). Histostain-S.P. kit was purchased from Zymed Laboratories (South San Francisco, CA).

Cell Culture. Human colon adenocarcinoma cell lines LS174T, SW1417, LoVo, SW1116, HT29, and SW480 were obtained from the American Type Culture Collection (Bethesda, MD). The HM7 and LM12 cell lines are high- and low-mucin variant clones established from the LS174T cell line in our laboratory (21). The H498 cell line was recently established from a blood type A individual and was generously provided by Dr. A. Gazdar, National Cancer Institute. All cell lines were grown in Dulbecco’s modified Eagle’s medium, with high glucose, penicillin (50 units/ml), streptomycin (5 µg/ml), and 10% heat-inactivated fetal bovine serum, as described (18). All of the cell lines were tested for Mycoplasma contamination using the Mycoplasma T.C. II rapid detection system (Gen-Probe, Inc., San Diego, CA) and were found to be negative. Cell cultures were incubated at 37°C in a
humidified atmosphere of 5% CO₂ in air. Heat treatment (56°C for 1 h) of the fetal bovine serum destroyed almost all the glycosyltransferase activity of the fetal bovine serum.

After the cells were confluent, the medium was decanted and the cells were washed 3 times with PBS and scraped from plates into 8 ml of PBS with a rubber policeman. The cells were centrifuged at 3000 rpm for 10 min and the cell pellet was resuspended in 2 ml of 20 mM-cocadylate buffer (pH 6.8) and sonicated for 2 min. Aliquots (1 ml) were frozen (−70°C) immediately, until further analysis. Protein content was determined by the method of Lowry et al. (22).

Glycosyltransferase Assays. The details for glycosyltransferase assays have been described earlier (23, 24). The assay mixture for α₁,3GalNAc transferase (A transferase) contained 410 μM 2'-fucosyllactose, 27.9 μM UDP-[³²P]α-galactose (30,000–50,000 cpm), reaction cocktail (0.1 M sodium cacodylate-HCl buffer, pH 6.8; 200 μM ATP; 20 mM MnCl₂; 0.1% Triton X-100), and cell homogenate (25–100 μg). The assay mixture for α₁,3GalNAc transferase (B transferase) contained 410 μM 2'-fucosyllactose, 17–32 nM UDP-[³²P]galactose (50,000–100,000 cpm), reaction cocktail, and cell homogenate (25–75 μg). The reaction mixture for α₁,2-fucosyltransferase (H transferase) contained 39 mM phenyl-β-galactoside, 1–1.8 μM UDP-[³²P]fucose (30,000–40,000 cpm), reaction cocktail, and cell extract (100–200 μg). Final volumes of all assays were made up to 100 μl. All glycosyltransferase assays were performed within the linear range of both the amount of enzyme (cell homogenate) and incubation time. Results shown are the median of triplicate tubes for A transferase and B transferase and single assays for H transferase. Incubations were carried out for 37°C for 4 h. The reactions were stopped by being placed on ice. The reaction mixtures were then applied to Dowex 1-x8 (100–200 mesh) formate columns (0.5 x 2 cm) and the products were separated by washing each column with 2 ml water. The product of α₁,2-fucosyltransferase enzyme was further separated by descending paper chromatography (24). An aliquot of each eluate was mixed with ACS II scintillation fluid and counted in a scintillation counter. The values obtained without acceptor were taken as endogenous enzyme activity and were subtracted from the total incorporation to give the acceptor-dependent activity.

Characterization of A and B Tetrasaccharides. For characterization of A tetrasaccharide, cell homogenate (HT29 cell line), as well as normal A blood type serum, was incubated with UDP-[³²P]acetyl-D-galactosamine (23). The product was well separated from GDP-fucose and free L-fucose, as judged by using reference sugars, which were detected with silver nitrate reagent (25).

Glycosidase Assay. All glycosidase assays were performed as described previously, with 2 mM p-nitrophenylglycoside in 0.1 M acetate, pH 4.0 (23). These reactions measure the ability of cell homogenates to release any α-GalNAc, α-galactose, or α-fucose residue, including those of the blood group A, B, and H substances, using p-nitrophenylglycoside substrates. Glycosidases were also assayed under the conditions of the glycosyltransferase assays (pH 6.8) with 2 mM p-nitrophenylglycosides but without nucleotide sugars.

Immunochemistry. The monoclonal antibodies used in this study have been used by us previously (9) and were generously provided by Dr. R. Murray Ratcliffe, ChembioMed, Ltd. (Edmonton, Alberta, Canada). The anti-A and anti-B monoclonal antibodies recognize their respective epitopes on either type 1 or type 2 chains, whereas the anti-H monoclonal antibody recognizes only H type 2 antigen.

Cells were seeded onto chamber slides (Intermountain Scientific Co., Bountiful, UT) at a density of 3 x 10⁴ cells/chamber and allowed to attach overnight. The cells were then washed 3 times with buffer (PBS/1% bovine serum albumin), fixed in acid-ethanol for 20 min at −20°C, and washed again 3 times with buffer. In pilot experiments, the acid-ethanol fixation was found to give lower background staining than formaldehyde or glutaraldehyde fixation. Endogenous peroxidase activity was blocked by incubating cells with 1% hydrogen peroxide in methanol for 10 min, followed by three washes with buffer. Normal rabbit serum (10%) was applied for 10 min and blotted off. Primary antibody (2 μg/ml) was added for 60 min, the cells were washed 3 times with buffer, and biotinylated rabbit anti-mouse IgG + IgA + IgM (10 μg/ml) was applied for 20 min. The cells were then washed, reacted with streptavidin-peroxidase conjugate (10 μg/ml) for 30 min, washed again, and incubated with diaminobenzidine for 10 min. Slides were counterstained with 1% methyl green, rinsed in tap water, dehydrated, and mounted. For lectin binding the primary antibody was omitted and the secondary antibody was replaced by biotinylated isolentin I-B₄ of B. simplicifolia.

Negative controls consisted of substituting normal mouse IgM for primary antibody and PBS for the primary and secondary antibodies. In all cases, when primary antibody was omitted, a completely negative staining pattern was obtained. Cells were scored for antigen expression based on percentage of positive cells as well as staining intensity: weak (+), moderate (++), strong (+++).

RESULTS

Table 1 shows the expression of A antigen and α-N-acetyl-d-galactosaminyltransferase (A transferase) and α-N-acetylgalactosaminidase activities in the nine colonic adenocarcinoma cell lines examined. Of the three cell lines derived from blood type A individuals, only two, HT-29 and SW480, showed substantial A transferase activity as well as A antigen expression (Fig. 1, a and b). When unlabeled UDP-GalNAc (40 μM) was added, there was a 2- to 3-fold decrease in net [¹⁴C] incorporation for HT-29 and SW480 and A transferase was still undetectable in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Expression of A antigen, α₁,3-N-acetylgalactosaminyltransferase (A transferase), and N-acetylgalactosaminidase in human colonic adenocarcinoma cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line</td>
<td>A antigen expression (% of positive cells)</td>
</tr>
<tr>
<td>LS174T</td>
<td>O</td>
</tr>
<tr>
<td>HT-29</td>
<td>O</td>
</tr>
<tr>
<td>SW1116</td>
<td>O</td>
</tr>
<tr>
<td>SW480</td>
<td>A</td>
</tr>
<tr>
<td>H-498</td>
<td>A</td>
</tr>
<tr>
<td>LoVo</td>
<td>A</td>
</tr>
<tr>
<td>SW1417</td>
<td>B</td>
</tr>
<tr>
<td>5 (++)</td>
<td>35.9 ± 15.7</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean ± SE, five experiments except for H-498 (four experiments).
* Mean ± SE, four experiments.
* Numbers in parentheses, staining intensity scored as weak (+), moderate (++), and strong (+++).
ABH ANTIGENS IN ADENOCARCINOMA CELL LINES

the other cell lines. Interestingly, the H-498 cell line did not express any A antigen, thereby representing an example of blood group A antigen deletion (Fig. 1c). This antigenic deletion most likely results from an absence of A transferase activity in these cells (Table 1). Very weak expression of A antigen was also found in SW1116 (blood type O) and LoVo (blood type B), representing incompatible A antigen. However, since there was very little or no activity of A transferase in any of the O or B blood type cell lines, it is likely that this weak incompatible A antigen represents an A-like or cross-reacting substance. α-N-Acetylgalactosaminidase activity measured at pH 4.0 was similar in all of the cell lines, regardless of blood type (Table 1). Under the conditions of the A transferase assay (pH 6.8), α-N-acetylgalactosaminidase was undetectable.

Using HT-29 cells as a prototype blood type A cell line, the enzyme kinetics of A transferase were examined with different concentrations of the acceptor, 2'-fucosyllactose, and the donor, UDP-GalNAc. The apparent $K_m$ for 2'-fucosyllactose was 0.050 mM (Fig. 2b), which is comparable to the $K_m$ of the A enzyme in human serum ($K_m = 0.03–0.16$ mM) (26–28). The fucosyl moiety of 2'-fucosyllactose was required for acceptor activity. When the nine cell lines were tested for transfer of N-acetylgalactosamine to lactose, there was less than 0.4 pmol/mg/90 min in each. The apparent $K_m$ for UDP-GalNAc of the HT-29 A transferase was 0.032 mM (Fig. 2a), which is also similar to that of the serum enzyme ($K_m = 0.05–0.09$ mM) (26–28). The apparent $K_m$ of the SW480 enzyme for UDP-GalNAc was 0.024 mM (data not shown). The A transferase of HT-29 cells was inhibited by UDP-galactose, with 65% inhibition at 50 μM UDP-galactose in the presence of 64 μM UDP-[14C]-GalNAc. However, there was no detectable transfer of galactose to 2'-fucosyllactose by the A transferase of HT-29 cells.

In order to establish the product of the A transferase, reaction mixtures were passed through Dowex 1-X8 columns, deionized, and separated by gel filtration on Sephadex G-15 columns. There was a peak of radioactivity corresponding to tetrasaccharide size which was formed only in the presence of 2'-fucosyllactose. This peak was further examined by amine-phase HPLC (Fig. 3). The tetrasaccharide-size peak was chromatographically similar to the product formed by the A transferase of human serum, which has been previously characterized as GalNAcα1,3[Fucα2Galβ1,4Glc] (29).

Table 2 shows the expression of B antigen and α1,3-galactosyltransferase (B transferase) and galactosidase activities in human colonic adenocarcinoma cell lines. Of the two blood group B cell lines, only LoVo cells showed B antigen expression (Fig. 4a) as well as B transferase activity. When unlabeled UDP-galactose (2 μM) was added, there was a 2-fold decrease in net incorporation of 3H in SW1417 and LoVo cells, and B transferase was still undetectable in the other cell lines. Interestingly, the SW1417 cell line had a comparable level of B

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Fig. 1. Blood group A antigen expression in blood type A cell lines. a, SW-480. All of the cells express the antigen strongly on cell membranes and in cytoplasm. × 62. b, HT29. Antigen expression is localized more in the cytoplasm with a granular staining pattern. Some cells are negative. × 62. c, H498. None of the cells express the A antigen. × 50.

Fig. 2. Substrate dependence of A transferase. A homogenate of HT-29 (20 μg protein) was incubated with varying concentrations of UDP-[14C]GalNAc (A) and 2'-fucosyllactose (B). Linear regression analysis of $V$ versus $V/S$ plots (inset) was used to determine apparent $K_m$ and $V_{max}$ values.
Fig. 3. Characterization of A transferase and B transferase products. Homogenates of HT-29, incubated with UDP-[3H]GalNAc and 2'-fucosyllactose (B), and SW1417, incubated with UDP-[1-14C]galactose and 2'-fucosyllactose (C), were deionized on Dowex 1 formate, subjected to gel filtration on Sephadex G-15, and then analyzed by HPLC as described in “Materials and Methods.” Arrows, left to right, show the elution positions of N-acetylgalactosamine, galactose, and 2'-fucosyllactose.

Table 2 Expression of H antigen, α1,3-galactosyltransferase (B transferase), and galactosidase in human colonic adenocarcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Blood type</th>
<th>B antigen</th>
<th>B transferase (pmol/mg/90 min)</th>
<th>α-Galactosidase (nmol/galactose/16 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS174T</td>
<td>O</td>
<td>60 (+++)</td>
<td>1.4 ± 1.1*</td>
<td>177 ± 13.1</td>
</tr>
<tr>
<td>HM-7</td>
<td>O</td>
<td>40 (+++)</td>
<td>0.2 ± 0.1</td>
<td>256 ± 14</td>
</tr>
<tr>
<td>LM-12</td>
<td>O</td>
<td>NT</td>
<td>0.2 ± 0.1</td>
<td>146 ± 19</td>
</tr>
<tr>
<td>SW1116</td>
<td>O</td>
<td>30 (+)</td>
<td>0.3 ± 0.3</td>
<td>114 ± 15.6</td>
</tr>
<tr>
<td>HT-29</td>
<td>A</td>
<td>0.3 ± 0.1</td>
<td>69.6 ± 9.8</td>
<td></td>
</tr>
<tr>
<td>SW-480</td>
<td>A</td>
<td>40 (+)</td>
<td>0.3 ± 0.3</td>
<td>120.6 ± 11.6</td>
</tr>
<tr>
<td>H-498</td>
<td>A</td>
<td>10 (+)</td>
<td>0.2 ± 0.1</td>
<td>150.6 ± 9.8</td>
</tr>
<tr>
<td>LoVo</td>
<td>B</td>
<td>70 (+++)</td>
<td>8.8 ± 1.9</td>
<td>141.6 ± 17.9</td>
</tr>
<tr>
<td>SW1417</td>
<td>B</td>
<td>5 (+)</td>
<td>9.4 ± 1.5</td>
<td>70.6 ± 12.5</td>
</tr>
</tbody>
</table>

* MAb, monoclonal antibody; NT, not tested.  
† Mean ± SE, six experiments except for LoVo (five experiments).  
‡ Mean ± SE, four experiments.

Fig. 4. Blood group B antigen expression. a, LoVo cells stained with monoclonal anti-B antibody. Cells that react are stained strongly in the cytoplasm but also on cell membranes. × 62. b, H-498 cells stained with B. simplicifolia I-B lectin. Staining is more intense and more prevalent than with monoclonal anti-B antibody. × 62. c, H-498 (blood type A cell line) stained with monoclonal anti-B antibody. There is faint, granular, cytoplasmic staining. × 62. d, H-498 cells stained with B. simplicifolia I lectin. Intense granular staining is shown. × 62.

transferase activity but did not react with anti-B antibody (Fig. 2b), thereby demonstrating B antigen deletion. This antigenic deletion is not apparently due to excess degradation because, if anything, α-galactosidase activity in SW1417 cells was lower than in the other cell lines. Weak incompatible B antigen expression was noted in SW1116 and H498 cell lines (Fig. 4c). However, since there was little or no B transferase activity in the blood type O and A cell lines, this incompatible antigen expression must represent a B-like or cross-reacting substance. Using B. simplicifolia isolectin I-B4, which is specific for α-galactosyl residues (30), LoVo was very strongly stained (Fig. 4b), but there was also strong staining of LS174T, HM-7, SW-480, and H-498 (Fig. 4d). Galactosidase activity at pH 4.0 was present in all of the cell lines, regardless of blood type antigen expression, but no α-galactosidase could be detected at pH 6.8.

The enzyme kinetics of the SW1417 B transferase was examined with different concentrations of 2'-fucosyllactose and UDP-galactose. The apparent K_m for 2'-fucosyllactose was 0.24 mM (Fig. 5b), which is comparable to the serum B transferase (K_m = 0.05 mM) (31). As for the A transferase, the fucosyl moiety of 2'-fucosyllactose was required for B transferase acceptor activity. The nine cell lines all showed at most 2 pmol/mg/90 min transfer of galactose to lactose. Using UDP-galactose, the B transferase of SW1417 colon cancer cells showed an apparent K_m of 0.0021 mM (Fig. 5a), which is lower than that of the serum enzyme (K_m = 0.01 mM) (31). The B transferase of LoVo cells had an apparent K_m for UDP-galactose of 0.0010 mM (data not shown). The B transferase of SW1417 cells was inhibited by UDP-GalNAc, with 65% inhibition at 10 μM UDP-GalNAc in the presence of 5 μM UDP-[3H]galactose. However, there was no detectable transfer of N-acetylgalactosamine to 2'-fucosyllactose by the B transferase of SW1417 cells.

The B-specific tetrasaccharide product was characterized in a manner similar to that of the A tetrasaccharide (Fig. 3). A single peak of tetrasaccharide size containing [3H]galactose was formed only in the presence of 2'-fucosyllactose. This tetrasaccharide product was chromatographically similar to that formed by the serum B transferase, which has previously been characterized as Galα1-3[Fucα1-2]Galβ1-4Glc (32).

Expression of H antigen and α1,2-fucosyltransferase (H transferase) and fucosidase activities in colonic adenocarcinoma cell lines is shown in Table 3. All of the blood group O cell lines expressed H antigen as well as H transferase activity. The H antigen expression was less prevalent in LS174T and its derivatives (HM-7, LM-12) than in SW1116 cells (Fig. 6). Of the A and B cell lines, only H-498 reacted with anti-H antibody. This represents accumulation of the precursor H substance in this cell line, which is also deficient in A transferase activity.

There were significant amounts of H transferase activity in all
Table 3  Expression of H antigen, α-1,2-fucosyltransferase (H transferase), and fucosidase in human colonic adenocarcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Blood type</th>
<th>H antigen expression (pmol/mg/90 min)</th>
<th>H transferase (nmol/mg/16 h)</th>
<th>α-Fucosidase (nmol/mg/16 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS174T</td>
<td>O</td>
<td>10 (+)</td>
<td>492.9</td>
<td>2484 ± 345*</td>
</tr>
<tr>
<td>HM-7</td>
<td>O</td>
<td>10 (+)</td>
<td>452.4</td>
<td>5404 ± 450</td>
</tr>
<tr>
<td>LM-12</td>
<td>O</td>
<td>5 (+)</td>
<td>414.1</td>
<td>6059 ± 505</td>
</tr>
<tr>
<td>SW1116</td>
<td>O</td>
<td>80 (+)</td>
<td>241.8</td>
<td>1776 ± 94</td>
</tr>
<tr>
<td>HT-29</td>
<td>A</td>
<td>372.3</td>
<td>998 ± 77</td>
<td></td>
</tr>
<tr>
<td>SW-480</td>
<td>A</td>
<td>211.9</td>
<td>657 ± 44</td>
<td></td>
</tr>
<tr>
<td>H-498</td>
<td>A</td>
<td>20 (+)</td>
<td>513.1</td>
<td>3988 ± 218</td>
</tr>
<tr>
<td>LoVo</td>
<td>B</td>
<td>399.1</td>
<td>1536 ± 110</td>
<td></td>
</tr>
<tr>
<td>SW1417</td>
<td>B</td>
<td>855.6</td>
<td>1439 ± 202</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE, four experiments.

Fig. 6. Blood group H antigen expression. LS174T cells stained with monoclonal anti-H antibody. Cells are focally positive, with staining of cell membranes. × 50.

of the cell lines, irrespective of the blood type. α-Fucosidase activity at pH 4.0 did not correlate with H antigen expression but was higher than α-galactosidase or α-N-acetylgalactosaminidase in all the cell lines. The activities of α-fucosidase at pH 6.8 were 1 to 3% of the activities at pH 4.0 (10 to 38 nmol/mg/16 h).

The enzyme kinetics of H transferase was examined with respect to different concentrations of acceptor, phenyl-β-galactoside, and donor, GDP-fucose. The apparent Kₘ for phenyl-β-galactoside was 8.65 mM (Fig. 7), which is similar to the Kₘ of the human serum enzyme (33). The apparent Kₘ for GDP-fucose was 39.7 μM. This is lower than the GDP-fucose Kₘ of the human tracheal enzyme (34). Because it has been suggested that ABH antigen expression is growth dependent (35), the doubling times of seven of the cell lines were measured (21). The doubling times of the two cell lines that failed to express the expected antigen (H-498, 42 h; SW1417, 28 h) were within the range of those that did express the appropriate antigen (LS174T, 25 h; HT-29, 30 h; LoVo, 35 h; SW480, 38 h; SW1116, 74 h).

In order to establish the product of H transferase, the reaction mixtures were passed through Dowex 1-X8 columns and then separated by descending paper chromatography. As shown in Fig. 8, the reaction product from the fucosyltransferase reaction was clearly separated from free fucose and phenyl-β-galactoside. This product was chromatographically similar to the product formed by the H fucosyltransferase of human serum, which has been previously characterized as Fucα1-2Galβ-p phenyl (24).

DISCUSSION

Our findings can be summarized as follows. (a) Except for the blood type A H-498 cell line, colon cancer cell lines derived from blood type A or B individuals expressed the appropriate A or B gene-encoded glycosyltransferase. This was associated with expression of the relevant antigen by each cell line, except H-498 and SW1417, which manifested deletion of the A and B antigens, respectively. (b) All cell lines, regardless of ABO type, expressed the H gene-encoded fucosyltransferase. The H antigen was found on all blood type O cell lines. However, H-498 cells also expressed H antigen, thereby representing accumulation of precursor substance in a cell line which is deficient in A transferase activity. (c) In general, the activity of the three α-glycosidases did not correlate with blood group antigen expression. (d) Demonstration that the measured A, B, and H glycosyltransferase activities represented the action of the true gene-encoded A, B, and H transferases was confirmed by HPLC, revealing the predicted tetrasaccharide from the A and B transferase reactions, and by paper chromatography, revealing the expected disaccharide from the H transferase reaction. Furthermore, substrate affinities of the colon cancer-derived A, B,
and H glycosyltransferases for the saccharide acceptors and nucleotide sugar donors were similar to those of human serum A, B, and H transferases.

Immunohistochemical studies have demonstrated that normal human colonocytes express A, B, and H antigens compatible with the host's blood type in the proximal but not distal colon (4, 8-11). However, during fetal life, colonocytes along the entire length of the colon bear the appropriate ABH antigen (8, 9). Thus, there is a tight control of ABH expression that occurs with development of this organ, with an apparent loosening of this control in malignancy, as manifested by ABH reappearance in distal cancers, ABH deletion of proximal cancers, and incompatible A and B expression (9, 11-18). Very little is known about the mechanisms responsible for these alterations in colonic ABH antigen expression. A few studies have addressed this issue by measuring glycosyltransferases and glycosidases in homogenates of normal and cancerous colonic tissues (36-39). While such an approach is advantageous for comparing normal and cancerous tissues from the same individual, it is limited by the fact that nonepithelial elements, such as RBC, endothelial cells, and glycosidase-producing bacteria might be contributing to the observed results. Therefore, to obtain a better understanding of the mechanisms contributing to ABH expression in malignant colonic epithelial cells, we conducted the present study using well-characterized colon cancer cell lines established from individuals of known blood type.

No study to our knowledge has investigated mechanisms of ABH antigen biosynthesis in isolated colon cancer cells. In the present study, there were two examples of blood group antigen deletion. Because it is not known whether these cell lines were established from cancers of the proximal or distal colon, it is not clear whether the lack of expression of A and B antigens in H-498 and SW1417 is analogous to the developmental loss of antigen in normal distal colon or to the cancer-associated deletion of antigen in the proximal colon. The H-498 cell line failed to express the A antigen, most likely because of absent A transferase activity. The deletion of A antigen was accompanied by precursor accumulation of H substance in this case. The SW1417 cell line failed to express the B antigen but, unlike in H-498 cells, there appeared to be sufficient levels of the biosynthetic B transferase activity. Enhanced B-degrading enzyme activity did not seem to explain the deletion. Interestingly, in this cell line, there was no accumulation of precursor H antigen, despite very high levels of H transferase activity. This suggests that, despite adequate levels of B and H transferases, precursor synthesis might be impaired. This could occur, for example, if other glycosyltransferases, such as sialyltransferase or α1,3-fucosyltransferase, successfully competed with the H gene α1,2-fucosyltransferase for the type 1 or type 2 oligosaccharide side chain. An alternate explanation for B antigen deletion in these cells could be masking of the immunodeterminant in some way as to make it inaccessible to bind antibody.

In terms of incompatible antigen expression, Drewinko and Lichtiger (19) reported incompatible A and B antigen expression in all seven colon cancer cell lines examined. However, that study used human polyclonal anti-A and anti-B antisera applied to cell suspensions analyzed by indirect immunofluorescence, and neither glycosyltransferase nor glycosidase activities were determined. In the present study, we detected weak binding of monoclonal anti-A to SW1116 (type O) and LoVo (type B) cells, and weak monoclonal anti-B binding to SW1116 and H498 (type A) cells. However, since there was little or no A or B gene-encoded glycosyltransferase activity in these cell lines, this suggests that the antigens being detected are not the products of the true ABO gene-encoded enzymes.

Although the mechanism(s) responsible for incompatible A or B antigen expression is not known, a few possibilities exist. First, the true A or B gene-encoded glycosyltransferases might become activated in cancer cells. While this concept goes against our understanding of classical immunogenetics of blood group substances, one study did find A transferase activity in a colon cancer from a blood type O individual whose tumor tissue expressed incompatible A antigen (18). A second hypothesis to explain incompatible A or B antigen expression might be a loosening of substrate specificity of the A or B transferases. For example, under certain in vitro experimental conditions, the serum A gene-encoded transferase can synthesize the B antigen (48), and the B transferase can synthesize the A antigen (29, 32, 40).

A third hypothesis is that the incompatible antigens are really A-like and B-like structures and not the products of the gene-encoded transferases. For example, substances with terminal GalNAc residues, such as Forssman antigen (41-43) or Tn antigen (44), behave as A-like structures. However, our monoclonal anti-A antibody does not react with Forssman-positive sheep RBC (9) and has a different staining pattern, compared to specific anti-Tn reagents. A-like oligosaccharides have been identified on glycoproteins from human rectal carcinoma (45). Indeed, monoclonal antibodies raised against HT-29 cells react not only with true A substances but also with A-like substances (46). In other studies, naturally occurring antibodies which recognize A-like determinants were found in the sera of two healthy sisters belonging to a kindred at high risk for colon cancer (47).

The A antigen can be converted into an "acquired" B antigen by deacetylation of the terminal α-N-acetylgalactosamine, an uncommon event that has been reported for RBC (48). Alternatively, B-like structures with terminal α-galactose residues, such as fucose-less B antigen, might also account for incompatible B expression. The B4 isolectin of B. simplicifolia I has greater affinity for B-like Galα1,3Gal structures than for the blood group B tetrasaccharide (30). In previous studies we found that purified mucin from LS174T cells reacted strongly with B. simplicifolia I (isolectins A4 and B4) (49) and, by cytochemistry, all of the cell lines used in the present study, except HT-29 and SW1116, bound the B4 isolectin of B. simplicifolia I. This suggests the existence of B-like substances in our cell lines, although we have not been able to demonstrate transfer of galactose to lactose to form a fucosyl B antigen in these same cell lines.

The A, B, and H transferases of human colon cancer cells have not previously been characterized with respect to substrate affinity. Our results demonstrate that the Km values for the saccharide acceptors and nucleotide sugar donors of colon cancer cell ABH enzymes were very similar to those reported for these enzymes in human serum. Furthermore, using a sterile cell culture system, our data illustrate the ABH-degrading glycosidases in the colon, which have been characterized as products of enteric bacteria (50), may also be produced by colonocytes.

ACKNOWLEDGMENTS

The authors wish to thank Annabelle Friera, Frank Fearney, Robert Lagace, and Dr. Sam Ho for technical assistance and Rita Burns for manuscript editing and preparation.

4 Unpublished observations.
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