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Expression of Blood Group Antigens H-2, Le^y, and Sialylated-Le^a in Human Colorectal Carcinoma

An Immunohistochemical Study Using Double-labeling Techniques

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In this study, double-labeling immunohistochemistry was used to gain insight into the coexpression or interrelationship between blood group antigens (BGA) that are differentiation antigens in the normal colon, and BGA that are sequential moieties in the same synthetic pathway. Paired-wise Sialylated-Le^a/Le^y and H-2/Le^y was studied. The Sialylated-Le^a and Le^y are synthesized from type 1 and type 2 backbones, respectively. In the normal colon, the Le^y and Sialylated-Le^a are expressed by cells at the base and surface of the crypt, respectively, representing undifferentiated and differentiated enterocytes. The H-2 is considered oncofetal in nature, and is considered to be the immediate precursor in the synthesis of Le^y. In individual cancers, Sialylated-Le^a and Le^y were detected in different cancer cells within the same malignant glands, separately in different glands, and in different subcellular compartments of the same cell. Both H-2 and Le^y were coexpressed in the same individual cells in 92% of cancers expressing both these BGA. In 50% of the cancers, the H-2 and Le^y also were expressed separately in different malignant glands within individual tumors. These findings indicate that, in colorectal cancers, differentiation antigens (Sialylated Le^a and Le^y) are expressed by different individual cells within the same malignant gland somewhat, recapitulating the normal colon crypt. Antigens of different backbones occasionally may be expressed in the same cells but within different subcellular compartments. Precursor accumulation is common in cancers, and antigens in the same synthetic pathway are coexpressed in the same cell. The expression of H-2 and Le^y in different glands (lack of coexpression) may be explained possibly by aberrant synthesis of Le^y by an alternate pathway. (Am J Pathol 1991, 138:103–110)

Many investigators have studied colorectal cancers for their expression of blood group antigens (BGA).[1–9] Some of these studies have commented on the inter-relationship or coexpression of BGA in individual neoplasms.[1–4] Also studied has been the expression and accumulation of precursor BGA in these neoplasms. These studies have 'crudely' compared these relationships at a very gross level, however, by looking at individual slides labeled for a single different BGA and then comparing one slide with another.

In this study, we attempted to gain more information at the cytologic level regarding the coexpression, interrelationships, or presence of 'precursor' BGA in individual colorectal carcinomas. To meet these ends, we employed double-labeled immunohistochemistry with certain BGA known to represent different levels of differentiation in the normal colon crypt and stepwise sequences in a common synthetic pathway. We specifically chose to study paired wise: 1) Sialylated-Le^a and Lewis y (Le^y), as they are expressed in the normal distal colon by enterocytes at the surface and base of the crypts, respectively, and therefore are markers for mature (differentiated) and immature (undifferentiated) colonic epithelium, respectively.[3,10–13] 2) The H-2 and Le^y, as they are structurally related, differing only by a single fucose, with the H-2 being the purported immediate precursor substance for the synthesis of Le^y.[14]

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The H-2 is oncofetal in nature in that it is not expressed by the normal distal colon, but is expressed by the epithelium of fetal colon and colonic adenocarcinomas.[3,5,8]

Our findings indicate that the expression of BGA in distal colonic adenocarcinomas show that

1) Within malignant glands, individual cells express either markers of mature or immature enterocytes, indicating a caricature of the normal crypt
2) Aberrant expression of differentiation BGA as compared with the normal crypt is noted by individual separate glands expressing only either Sialylated-Le^a or Le^y
3) Individual cells can aberrantly simultaneously express both mature (Sialylated-Le^a) and immature (Le^y) markers
4) Blood group antigens that are structurally related are often coexpressed in the same cell and subcellular compartment after a logical synthetic pathway; however
5) Certain patterns of expression of H-2 and Le^y may indicate that Le^y may be synthesized by alternate pathways.

Materials and Methods

Materials

Twenty-four adenocarcinomas of the sigmoid colon or rectum were obtained from the surgical pathology files of the Thomas Jefferson University Hospital. The study materials were from formalin-fixed paraffin-embedded materials. Blood types (A, B, O) were obtained for each patient from the blood bank of the Thomas Jefferson University Hospital. Because this was a retrospective study, secretor status was not obtainable; however investigators have shown that one cannot retrospectively determine secretor status by the expression of Lewis^a or Lewis^b on red blood cells or endothelial cells in tissue sections.[6,15] The antibodies used (all monoclonal) were obtained as follows: H-2 (Bio Genex, San Ramon, CA) is an antibody of the IgM isotype. The Le^y (55-2) and the Sialylated-Lea (19-9) were obtained from the Wistar Institute (Philadelphia, PA). These antibodies are both of the IgG isotype, and have been well characterized and extensively studied.[11,16–18] The 55-2 has been shown immunohistochemically to react with colonic enterocytes at the base of the crypts[10,11,13] similar to the results of Brown et al,[3] and its epitope is considered to be the tetrasaccharide Fuc α 1-2 Gal B 1-4 Glc NAC (3-1 α Fuc), which is the determinant for Le^y.[16] Secondary antibodies used were biotinylated goat anti-mouse IgM, mu-chain–specific (Vector Laboratories, Burlingame, CA), alkaline phosphatane conjugated goat anti-mouse IgG, gamma-chain–specific (Sigma Laboratories, St. Louis, MO), and horseradish peroxidase (HRP) conjugated polyvalent goat anti-mouse immunoglobulin (Sigma). Detection of biotinylated antibodies was with a Vector mouse IgM avidin biotin complex (ABC) kit and the Detek 1-alk kit (Enzo Biochem, New York, NY). Color detection of HRP was with diaminobenzidene (DAB) (Polysciences Inc., Warrington, PA) and the color detection for the alkaline phosphatase was with the Vector red Chromagen kit #1 (Vector). The monoclonal antibody (MAb) 19-9 (Sial-Le^a) was biotinylated with Enzotin-biotinylating reagent CAT # E7-406 as per manufacturer's specifications (Enzo Biochem Inc., New York, NY). Normal mouse serum (DAKO, Santa Barbara, CA), normal goat serum (Vector) also were used.

Methods

The method of Van Der Loos et al[19] was used to simultaneously detect Le^y and Sialylated-Le^a. With this method, the Le^y (MAb 55-2) was visualized sequentially as a brown color (HRP/DAB) with an indirect immunoperoxidase technique, while the Sialylated-Le^a (19-9) was subsequently visualized as a red color (alkaline phosphate/Vector red) through biotin-labeled 19-9, followed by an alkaline phosphatase strep-avidin. A red-brown color was noted when both antigens were simultaneously present in the same cell or cell compartment. Briefly, the technique is as follows:

1) Four-micron–thick paraffin sections deparaffinized, washed in phosphate buffered saline (PBS), and blocked with 0.3% H_2O_2
2) PBS wash for 20 minutes
3) Normal goat serum applied for 20 minutes
4) Incubation with MAb 55-2 diluted 1:700 in PBS for 30 minutes, followed by a wash in PBS for 10 minutes
5) Incubation with polyvalent goat anti-mouse Ig—HRP conjugated diluted 1:10 in PBS, for 30 minutes
6) Wash in PBS for 10 minutes
7) Incubation with 0.05% DAB/H_2O_2 for 5 minutes and wash in tap water for 5 minutes
8) Wash in PBS, 10 minutes × 2
9) Incubation with normal mouse serum diluted 1:20 (PBS) for 20 minutes
10) Incubation with biotinylated 19-9 (diluted 1:2800 in PBS) for 30 minutes and wash in PBS for 10 minutes
11) Dilute the Detek 1-alk (ENZO) 1:50 in a '1×' modified PBS ('25×' stock = 200 ml deionized water + 37.6 g K_2HPO_4, 6.6 g NaH_2PO_4. H_2O, 36 g NaCl, 20 mg thimerosal. "1×" = 40 ml of '25×' stock + 960 ml of deionized water)
12) Incubate slide with Detek 1-alk for 30 minutes, followed by a wash in PBS for 10 minutes
13) Wash in predetection buffer as per kit instructions for detek 1-alk, (Enzo Biochem)

14) Incubation for 30 minutes with a Vector red chromagen kit #1 in a moist chamber in the dark
15) Wash in tap H_2O 5 minutes, counterstain with hematoxylin, dehydrate, and coverslip with Permount (Fisher Scientific, New York, NY)

The method of Valness and Brandtzaeg[20] was used to simultaneously detect H-2 and Le^y. Sequentially H-2 was detected as a brown color (HRP-DAB) using the ABC method followed by detection of the Le^y as a red color (alkaline phosphatase—vector red) through an indirect immunoperoxide technique. A red-brown color was obtained when both H-2 and Le^y were expressed simultaneously within the same cell or cell compartment. Briefly, the technique is as follows:

1) As per steps 1 through 3 above
2) Incubation with prediluted H-2, 30 minutes, followed by 10 minutes PBS wash
3) Incubation with biotinylated goat anti-mouse IgM (mu chain specific) for 30 minutes as per dilution instruction of the Vectastain kit (Vector labs)
4) Wash in PBS for 10 minutes
5) Incubate with ABC reagent, 30 minutes per Vector kit instructions followed by a 10-minute wash in PBS
6) Incubation with 0.05% DAB/H_2O_2 for 5 minutes followed by a wash in tap water for 5 minutes
7) Wash in PBS 10 minutes × 2
8) Incubate with normal goat serum, 20 minutes
9) Incubate with MAb 55-2 (Le^y) diluted 1:1400 for 30 minutes followed by a wash in PBS for 10 minutes
10) Incubation for 30 minutes with alkaline phosphatase conjugated goat anti-mouse IgG (gamma chain specific) diluted 1:40 in PBS
11) Wash in PBS for 10 minutes
12) Wash sections in predetection buffer for 5 minutes (predetection buffer as per detek 1-alk ENZO Biochem)
13) Incubation with vector red chromagen substrate kit #1 (as per kit instructions) for 30 minutes in a moist chamber in the dark.
14) Wash in tap water for 5 minutes
15) Counterstain with hematoxylin, dehydrate, and coverslip with Permount

Biotinylation of 19-9

The MAb 19-9 was biotinylated using the ENZOTIN biotinylating reagent as per manufacturer's instructions (Enzo Biochemical Inc., New York, NY).

Controls

The following controls were performed:

1) Simultaneously substituting PBS for both individual and primary MAbs
2) Substituting PBS for the second MAb in each pair
3) Substituting PBS for the second MAb in each pair and deleting the DAB staining reaction for the first MAb
4) Running each MAb singly with appropriate immunohistochemical technique and chromagen
5) For the H-2/Le^y double stain, the secondary antibodies biotinylated goat anti-mouse IgM and alkaline phosphatase conjugated goat anti-mouse IgG were reversed (eg, H-2 followed by GAM-IgG Alk Phos and Ley followed by GAM-IgM-Biotin)
6) For the Le^y/Sialylated-Le^a pair, DAB was deleted from the procedure as per step 7 of the method of Van Der Loos et al.[19]

Endogenous Alkaline Phosphatase

We initially checked for the presence of endogenous alkaline phosphatase in the paraffin-embedded tissues. Sections were treated with and without 20% acetic acid, followed by color detection reagents.[21] We found no detectable endogenous alkaline phosphatase with either method.

Results

Le^y and Sialylated-Le^a

The Le^y and Sialylated-Le^a were each expressed in 17 of 24 cancers (71%). Thirteen of twenty-four (54%) cancers expressed both Le^y and Sialylated-Le^a, 4 of 24 (17%) expressed only Le^y, 4 of 24 (17%) expressed only Sialylated-Le^a, and 3 of 24 (13%) failed to express either Le^y or Sialylated-Le^a (Table 1).

Seven of eight (88%) cancers from patients of the O blood type expressed Le^y, while 10 of 16 (63%) of cancers from patients of the A, B, or AB blood types expressed Le^y. Five of eight (63%) cancers from patients of the O blood type expressed Sialylated-Le^a and 12 of 16 (75%) of cancers from patients of the A, B, or AB blood type expressed Sialylated-Le^a (Table 1).

The Le^y and Sialylated-Le^a were both localized to the regions of the glycoalyx, apical cytoplasm, total cytoplasm, mucin vacuoles (Sialylated-Le^a only), or combinations of these four patterns. Of the 13 cases expressing both Sialylated-Le^a and Le^y, the Sialylated-Le^a and Le^y were localized to different individual cells within the same malignant gland (or cluster of cancer cells) in 3 of 13 cases (23%) (Figure 1). In 3 of 13 cases (23%), Sialylated-Le^a and Le^y were not expressed in the same malignant glands or clusters, but were each expressed by different glands. In 7 of 13 cases (54%), Sialylated-Le^a and Le^y were expressed by different cells in the same glands and individually in different malignant glands (Table 2). Therefore, in

Table 1. *Expression of LE^y and Sialylated-LE^a*

	Le^y/Sialylated-Le^a	Sialylated-Le^a	Le^y	No expression	Total
Blood Type					
A	6 (67%)	2 (22%)	0 (0%)	1 (11%)	9
B	1 (20%)	1 (20%)	2 (40%)	1 (20%)	5
O	5 (63%)	0 (0%)	2 (25%)	1 (13%)	8
AB	1 (50%)	1 (50%)	0 (0%)	0 (0%)	2
Total	13 (54%)	4 (17%)	4 (17%)	3 (13%)	24 (100%)

77% of the cancers, Sialylated-Le^a and Le^y were expressed separately by individual cells in the same malignant gland. Besides showing the above patterns, in four cases, rare focal areas of tumor appeared to express both Sialylated-Le^a and Le^y in the same individual cells. When this occurred, we noticed distinct red and brown colors juxtaposed next to each other in what appeared to be the same cell (Figure 2). This pattern was unlike the red/brown color mixing seen with H-2/Le^y (see below). The degree of tumor differentiation had no relationship to the type of expression of the pair of antigens.

Le^y and H-2

Fifty percent (50%) of the cancers expressed H-2 and 71% expressed Le^y. Fifty percent (12 of 24) of the cancers simultaneously expressed both H-2 and Le^y, 7 of 24 (29%) failed to express either H-2 or Le^y, and 5 of 24 (21%) expressed only Le^y. None of the cancers (0%) expressed only H-2 (Table 3). Six of eight cancers (75%) from patients of the O blood type expressed H-2, while 6 of 16 (38%) of the cancers from patients of the A, B, and AB blood types expressed H-2. Seven of eight (88%) of cancers from patients of the O blood type expressed Le^y, while 10 of 16 (63%) of cancers from A, B, and AB patients expressed Le^y (Table 3).

The Le^y and H-2 were localized to the regions of the glycocalyx, apical cytoplasm, total cytoplasm, or combinations of these three patterns. Eleven of the twelve (92%) cancers that simultaneously expressed both H-2 and Le^y showed coexpression of these tumor antigens in the same identical tumor cells. This was clearly evidenced by the presence of a distinct red brown color (mixing of brown and red chromagens) when compared with the color of each antigen stained individually (Figure 3). Five of the twelve (42%) cases showed only identical expression of both Le^y and H-2 in individual cells throughout the entire tumor. Six of twelve (50%) showed identical expression of both Le^y and H-2 in focal areas of the tumor, while other areas of the tumor expressed either H-2 or Le^y alone in distinct and separate malignant glands (Figure 4). Only 1 of 12 (8%) cases failed to show coexpression of both antigens in individual tumor cells. In this case, the expression of Le^y and H-2 were present in separate glands (Table 4).

There was no relationship to the type of staining pattern between the pair of antigens or to the degree of differentiation of the tumor.

Results of Controls

Our extensive use of controls showed that all color reactions were real and there was no unwanted interaction

Figure 1. *(Top left) High-power view of tumor, simultaneously double label staining for Le^y and Sialylated-Le^a. Note that within an individual malignant gland, individual separate cells express only Le^y or Sialylated-Le^a. The Le^y is expressed as a brown color (arrow head) while the Sialylated-Le^a is expressed as a red color (arrow). 400× Le^y, indirect – HRP, DAB, and Sialylated-Le^a-biotinylated alk-phosphatase strepavidan, Vector red.*

Figure 2. *(Top right) High-power view of tumor simultaneously expressing Sialylated Le^a (red) and Le^y (brown) within different subcellular compartments of the same cells. The Sialylated Le^a is expressed in the region of glycocalyx, (faint red staining), while Le^y is expressed within the cytoplasm (brown). (compare with Figure 3D). 400× Le^y, indirect-HRP, DAB, and Sialylated-Le^a-biotinylated alk-phosphatase, strep avidan, Vector red.*

Figure 3. *(Middle)* **A:** *Low-power view of colonic adenocarcinoma showing expression of H-2 and Le^y. Arrows indicate area shown in* **B, C,** *and* **D** *(DAB-Hematoxylin counterstain ×40).* **B:** *High-power view of arrowed area in* **A.** *There is expression of H-2 in the region of both the apical cytoplasm and the total cytoplasm, as evidenced by the chromogen showing a brown granular color. Compare with* **C** *and* **D** *(×400 H-2, ABC, HRP, DAB).* **C:** *High-power view of arrowed area in* **A.** *There is expression of Le^y in the region of both the apical cytoplasm and total cytoplasm, as evidenced by the chromogen showing a fine red granular color. Compare with* **B** *and* **D.** *(×400 Le^y Indirect-Alkphos, Vector Red).* **D:** *High-power view of arrowed area in* **A.** *This figure is of the simultaneous double staining of H-2 and Le^y. Note the identical localization of both H-2 and Le^y, as evidenced by the red-brown color of the chromogen mixing as compared with* **B** *and* **C,** *showing each antigen singly stained brown and red, respectively. (×400 H-2, ABC, HRP, DAB and Ley, Indirect-Alkphos, Vector red).*

Figure 4. *(Bottom right) High-power view of tumor simultaneously double-labeled staining for H-2 and Le^y. Note that each antigen is expressed separately among different malignant glands. The H-2 is expressed as a brown color, while the Le^y is expressed as a red color. Compare with Figure* **3D** *(×400 H-2, ABC, HRP, DAB, and Le^y, Indirect Alkphos, Vector red).*

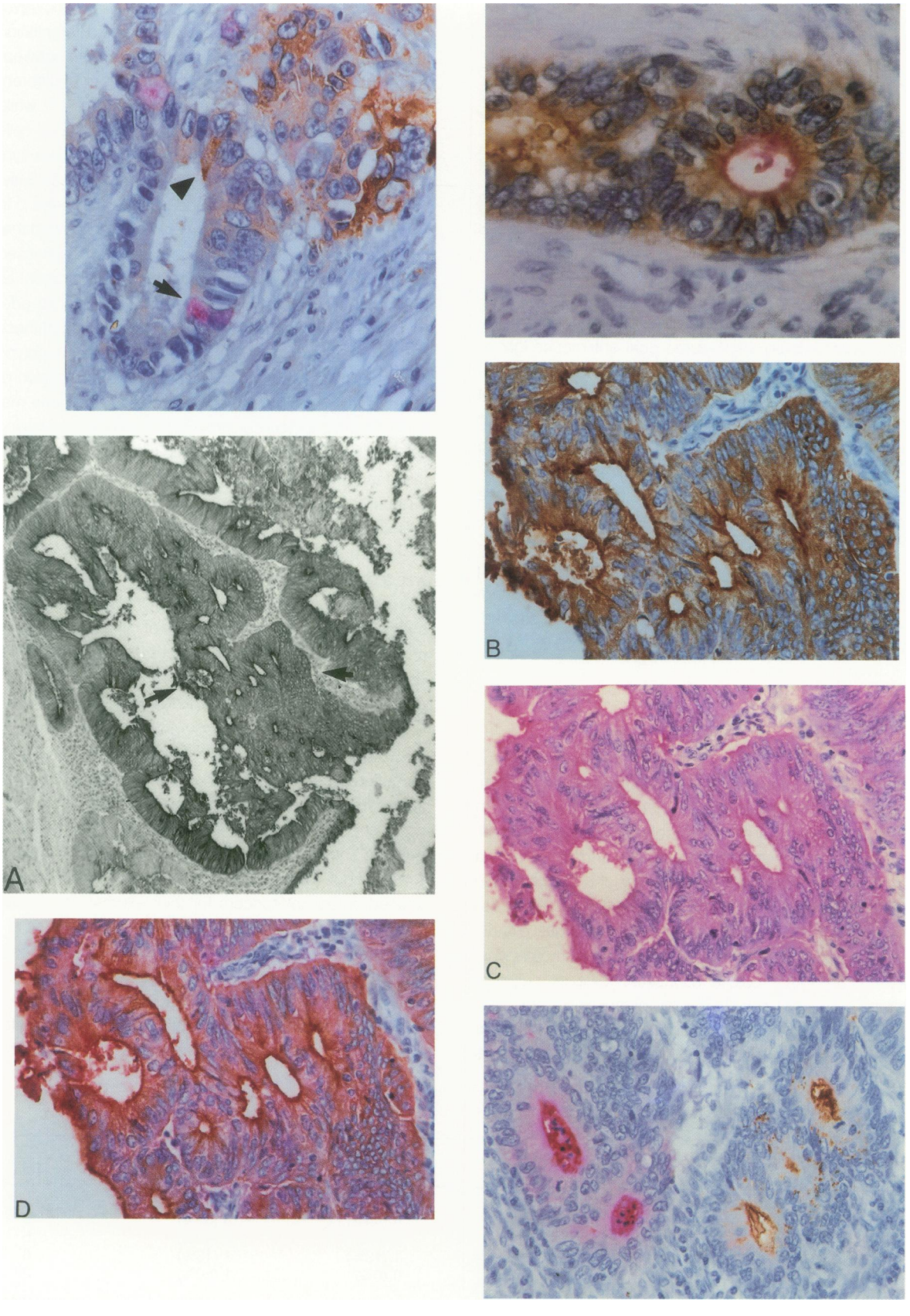
A
B
C
D

Table 2. *Pattern of Expression of Le^y and Sialylated-Le^a in Tumors Expressing Both Antigens*

	DC*	SG*	DC + SG*	Total
Le^y/Sialylated-Le^a	3 (23%)†	3 (23%)	7 (54%)†	13

* DC = Le^y and Sialylated-Le^a expressed by different cells within the same gland. *SG = Le^y or Sialylated-Le^a expressed only in separate glands. *DC + SG = Le^y and Sialylated-Le^a expressed by different cells of the same gland plus expression of either Le^y or Sialylated-Le^a (but not both) in separate glands.

† In four cases we noticed Sialylated-Le^a and Le^y were expressed in the same cell; however, they appeared to be localized to different subcellular compartments, as evidenced by separate red and brown colors juxtaposed next to each other.

between different antibodies in different sequences of antigen detection (eg, biotinylated goat anti-mouse IgM of the H-2 staining sequence reacting undesirably with the Le^y and therefore causing spurious mixing). We found that the secondary antibodies used to detect H-2 and Le^y had to be heavy chain specific to prevent spurious mixing of chromogens. In the study of double labeling with Le^y and Sialylated-Le^a, as both were of the IgG isotype, we found it necessary to biotinylate one of the antibodies (19-9) to obtain complete color separation.

The normal mouse serum is essential as a blocking reagent in the methods used to detect Le^y and Sialylated-Le^a, to prevent unwanted reactions between the secondary goat anti-mouse Ig and the biotinylated 19-9.

Discussion

In this study, by using double-labeled immunohistochemistry, we were able to elucidate at the cytologic level in distal colorectal cancers the coexpression or interrelationship between differentiation BGA and BGA from a common synthetic pathway. The Le^y and Sialylated-Le^a BGA were expressed by different individual cells of the same malignant glands, or separately by individual glands. In no instance did we find color mixing of chromagens, as with the H-2/Le^y pair. In four cases, however, individual tumor cells were simultaneously expressing both Sialylated-Le^a and Le^y, but probably in different subcellular compartments. The Le^y and Sialylated-Le^a are both well-studied tumor-associated antigens[3,10,11,13,16,22,23]; however these antigens can also be considered to be differentiation antigens that are expressed by the normal colorectal epithelium. The Le^y is a marker for 'immature or undifferentiated' cells that sit at the base of the crypt,[3,10,11,13,22] while Sialylated-Le^a is expressed by mature differentiated cells at the crypt surface.[10-13] Both of these antigens are synthesized through a different pathway from type 1 (Sialylated-Le^a) and type 2 (Le^y) blood group backbones.[14] In a previous study of colonic adenomas,[10] we showed that these benign neoplasms (adenomas) often expressed Sialylated-Le^a and Le^y in the same crypt compartment as the normal mucosa. This indicated that colorectal adenomas recapitulated the normal crypt distribution of these differentiation antigens. Studies with hyperplastic polyps showed similar results.[11] In our study, the expression of Le^y and Sialylated-Le^a in separate cells in the same malignant gland could be construed as cancer still maintaining (albeit abnormal) a caricature of the normal crypt. Similarly this pattern of expression may indicate that within an individual malignant gland some cells express markers of differentiated (Sialylated-Le^a) enterocytes, while other cells express markers of immature enterocytes (Le^y). Also noted was a pattern in which individual glands expressed either Le^y or Sialylated-Le^a, but not both. Here we see separate malignant glands showing features of either differentiated or undifferentiated cells, but not both. This indicates a deviation of the normal spatial relationship of these antigens or 'aberrant' expression. Itzkowitz et al,[24] in a study of stage-specific embryonic antigen-1 (SSEA-1) and carcino embryonic antigen (CEA) using double-labeling immunofluorescence, noted that within malignant glands some areas expressed SSEA-1 or CEA and others did not. They noticed that when both antigens were coexpressed, they rarely did so in the same part of the gland, similar to our data with the Le^y/Sialylated-Le^a. Gong et al[4] studied colorectal cancers for their expression of Sialylated-Le^a (19-9) and SSEA-1. The SSEA-1 like Le^y is a differentiation antigen in that it is expressed at the base of the crypt in the normal colon. Both antigens were expressed by the majority of tumor cells; however these authors did not comment about coexpression or interrelationships of expression. Ernst et al[1] in a study that

Table 3. *Expression of Le^y and H-2*

	Le^y/H-2	H-2	Le^y	No expression	Total
Blood Type					
A	2 (22%)	0 (0%)	4 (44%)	3 (33%)	9
B	3 (60%)	0 (0%)	0 (0%)	2 (40%)	5
O	6 (75%)	0 (0%)	1 (13%)	1 (13%)	8
AB	1 (50%)	0 (0%)	0 (0%)	1 (50%)	2
Total	12 (50%)	0 (0%)	5 (21%)	7 (29%)	24 (100%)

Table 4. *Pattern of Expression of LE^y and H-2 in Tumors Expressing Both Antigens*

	ID*	ID + S†	S‡	Total
Le^y/H-2	5 (42%)	6 (50%)	1 (8%)	12 (100%)

*ID = Identical distribution of Le^y and H-2 in the same individual cells; †ID + S = Identical distribution of Le^y and H-2 in the same cells plus expression of either Le^y or H-2 (but not both) in separate glands; ‡S = Le^y or H-2 expressed only in separate glands.

compared the interrelationship of expression of BGA, found no statistical relationship between Sialylated-Le^a and Le^y; however they made no comment about cytologic relationships. Mollicone et al[25] used double-labeling immunofluorescence to study the expression of blood group antigens in the normal duodenum. They noted that type 1 backbone (Le^a) antigens and type 2 backbone (Le^x) antigens were expressed in different subcellular locations within the same individual cells. These findings are of interest, as they compare with our findings of the coexpression of Sialylated-Le^a and Le^y in different subcellular compartments of the same cell.

At the cytologic level, 92% (11 of 12) of the tumors that expressed both H-2 and Le^y expressed both these tumor antigens in the same cell compartment of the same individual cells, as evidenced by color mixing (red-brown color). This seems logical when one considers the biochemical structure and synthetic pathway of these antigens.[3,14,16,23] The H-2 is a monofucosylated structure on a type 2 backbone, while the Le^y is a difucosylated structure on a type 2 backbone. Most investigators believe that the Le^y is synthesized directly from the H-2 molecule by the addition of an alpha 1-3 fucose.[14] The finding of H-2 and Le^y together speaks for accumulation of the precursor BGA H-2. Yuan et al[5] found that 29 of 32 cancers of the A, B, or AB blood type expressed H-2 along with the appropriate BGA. Their figures of serial sections stained for a single BGA showed both coexpression and heteroexpression of H-2 and A or B BGA. Itzkowitz et al[7] studied tumors for expression of Tn and T BGA. The former is the precursor substance for the synthesis of T BGA. In 15 of 24 cases, both Tn and T were expressed, in 6 of 24 only Tn was expressed, and in 2 of 24 only T was expressed. Our data shows that precursor accumulation in colorectal cancers is common. Unlike other studies, by our use of double-labeling immunohistochemistry we have convincingly shown that both the precursor BGA (H-2) and the final BGA (Le^y) are expressed in the same cells and cellular compartments.

In 6 of 12 (50%) cases in which both Le^y and H-2 were expressed, in addition to the mixing pattern, we also noted that Le^y and H-2 were separately expressed by different individual malignant glands (Figure 4). In these cases, the finding of H-2 in separate glands from Le^y may indicate that we are detecting the H-2 antigen at a time before terminal fucosylation or this may actually represent different tumor clones. Another explanation might be that colorectal cancers may synthesize Le^y not by the H-2 precursor, but by another pathway, possibly directly from Lewis^x.[25] In five cases Ley was the only antigen expressed; however, we never found H-2 expressed by itself, findings similar to those of Brown et al.[3] Those tumors that are H-2(−)/Le^y(+) are recapitulating the expression of these antigens as seen in the normal crypt of the distal colon.

Six of eight (75%) of the tumors from patients of the O blood type expressed H-2 substance compared with 6 of 16 (37.5%) of the patients of A, B, and AB blood type. An explanation for this may be genetic in nature. In patients of the O type, H-2 is a final glycoprotein, while in A, B, or AB patients, it is a precursor for A and B substance.[14] Theoretically these patients of the latter blood types may have converted H-2 to A or B substance in those tumors negative for H-2.

Brown et al[3] noted, comparing patients of the O blood type with A blood type, that tumors of the former expressed Le^y and H-2 over a much greater percentage of tumor surface (cells) area than tumors of the latter group. In our study, however, we found no such difference. Similar to previous investigators, we noted that the patient's blood type had no significant effect on whether a tumor was positive or negative for Le^y.[6,22]

Our findings regarding colorectal adenocarcinomas as they relate to expression of BGA show that

1) Markers for differentiated and undifferentiated enterocytes are expressed by different cells within individual malignant glands, somewhat recapitulating the 'normal crypt distribution'.
2) Individual glands within the same tumor may express either mature or immature BGA, indicating aberrant expression (as compared with the normal crypt) of Sialylated-Le^a and Le^y and heterogeneity from one gland to another.
3) Precursor accumulation of BGA occurs frequently; however, both penultimate and terminal BGA are often coexpressed by the same individual cells and within the same cell compartment.
4) The expression of H-2 and Le^y in separate glands within the same tumor may indicate that Le^y may be synthesized through an alternate pathway and not always directly from H-2 substance.
5) Blood group antigens of different backbones may be simultaneously synthesized in different compartments of the same individual cell.

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References

1. Ernst CS, Shen J-W, Litwin S, Herlyn M, Koprowski H, Sears HF: Multiperameter evaluation of the expression in situ of normal and tumor associated antigens in human colorectal carcinoma. J Natl Cancer Inst 1986, 77:387–395
2. Enblad P, Glimelius B, Busch C, Ponten J, Pahlamn L: Antigenic heterogeneity and individuality in adenocarcinomas the rectum and their secondaries. Br J Cancer 1987, 55: 503–508
3. Brown A, Ellis IO, Embleton MJ, Baldwin RW, Turner DR, Hardcastle JD: Immunohistochemical localization of Y hapten and the structurally related H type 2 blood group antigen on large bowel tumors in normal adult tissues. Int J Cancer 1984, 33:727–736
4. Gong E, Hirohashi S, Shimosato Y, Watnabe M, Ino Y, Teshima S, Kodaria S: Expression of carbohydrate antigen 19-9 and stage specific embryonic antigen one in non-tumorous and tumorous epithelia of the human colon and rectum. J Natl Cancer Inst 1985, 75:447–454
5. Yuan M, Itzkowitz SH, Palekar A, Shamsudan AM, Phelps PC, Trump BF, Kim YS: Distribution of blood group antigens A, B, H, Lewis A, Lewis B, in human normal, fetal, and malignant colonic tissue. Cancer Res 1985, 45:4499–4511
6. Cordon-Cordo Lloyd KO, Sakamato J, McGrody ME, Old LG, Lenemed MR: Immunohistochemical expression of blood group antigens in normal gastrointestinal tract and colon carcinoma. Int J Cancer 1986, 37:667–676
7. Itzkowitz SH, Yuan M, Montgomery CK, Kjeldsen T, Takahashi HK, Bigbee WL, Kim YS: Expression of Tn, Sialosyl-Tn, and T antigens in human colorectal cancer. Cancer Res 1989, 49:197–204
8. Cooper HS, Hassler WE: Blood group substances as tumor antigens in the distal colon. Am J Clin Pathol 1978, 69:594–598
9. Schoentag R, Primus FJ, Kuhns W: ABH and Lewis blood group expression in colorectal carcinoma. Cancer Res 1987, 47:1695–1700
10. Ruggierio F, Cooper HS, Steplewski Z: Immunohistochemical study of colorectal adenomas with monoclonal antibodies against blood group antigens (Sialosyl-Lewis A, Lewis A, Lewis B, Lewis X, Lewis Y, A, B, H). Lab Invest 1988, 59: 96–103
11. Cooper HS, Marshall C, Ruggierio F, Steplewski Z: Hyperplastic polyps of the colon and rectum colon in immunohistochemical study with monoclonal antibodies against blood group antigens (Sialosyl-Lewis a, Lea, Leb, Lex, Ley, A, B, H). Lab Invest 1987, 57:421–428
12. Bara J, Zabaleta EH, Mollicone R, Nap M, Burtin P: Distribution of GICA in normal gastrointestinal and endocervical mucosa and in mucinous ovarian cysts using antibody 19-9. Am J Clin Pathol 1986, 85:152
13. Cooper HS, Steplewski Z: Immunohistologic study of ulcerative colitis with monoclonal antibodies against tumor associated and/or differentiation antigens. Gastroenterology 1988, 95:686–693
14. Lloyd OK: Blood group antigens as markers for normal differentiation and malignant change in human tissues. Am J Clin Pathol 1986, 87:129–139
15. Limas C: Detection of urothelial lewis antigens with monoclonal antibodies. Am J Pathol 1986, 125:515
16. Blasczyk TM, Blasczyk-Thurin M, Thurin J, Hindsgaul O, Karlsson KA, Steplewski Z, Koprowski H: Y and blood group B type 2 glycolipid antigens accumulate in human gastric cancer cell line as detected by monoclonal antibody. J Biol Chem 1987, 262:372–379
17. Magnani JL, Nilsson B, Brockhaus M, Zopf D, Steplewslu Z, Koprowsli H, and Ginsburg V: A monoclonal antibody defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto -n- fucopentose II. J Biol Chem 1982, 257:14365–14369
18. Koprowski H, Steplewski Z, Mitchell K, Herly D, Herlyn M, Fuhrer P: Colorectal carcinoma antigens detected by hybridoma antibodies. Somat Cell Mol Genet 1979, 5:957–971
19. Van Der Loos CM, Van Den Dord JJ, Das PK, Houthoff JJ: Use of commercially available monoclonal antibodies for immunoenzyme staining. Histochem J 1988, 20:409–413
20. Valnes K, Brandtzaeg P: Paired indirect immunoenzyme staining with primary antibodies from the same species. Application of horseradish peroxidase and alkaline phosphatase as sequential labels. Histochem J 1984, 16:477–487
21. Ponder BA, Wilkinson MM: Inhibition of endogenous tissue alkaline phosphatase with the use of alkaline phosphatase conjugates in immunohistochemistry. J Histochem Cytochem 1981, 29:981–984
22. Abe K, Hakomori SI, Ohshiba S: Differential expression of difucosyl type 2 chain (Lewis Y) defined by monoclonal antibody AH6 in different locations of colonic epithelia, various histological types of colonic polyps, and adenocarcinomas. Cancer Res 1986, 46:2638–2644
23. Hannsson GC, Zoph D: Biosynthesis of the cancer associated Sial-Lea antigen. J Biol Chem 1985, 260:9388–9392
24. Itzkowitz SH, Shi ZR, Kim YS: Heterogeneous expression of two oncodevelopmental antigens, CEA and SSEA-1, in colorectal cancer. Histochem J 1986, 18:155–163
25. Mollicone R, Bara J, LePendu J, Oriol R: Immunohistologic pattern of type 1 (Le^a, Le^b) and type 2 (X, Y, H) blood group related antigens in the human pyloric and duodenal mucosa. Lab Invest 1985, 53:219–227