Distribution of Blood Group Antigens A, B, H, Lewis\textsuperscript{a}, and Lewis\textsuperscript{b} in Human Normal, Fetal, and Malignant Colonic Tissue\textsuperscript{1}

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ABSTRACT

In humans, most blood group substances (BGS) are expressed throughout the fetal colon but are absent from the distal portion of adult colon. Cancers of the distal colon frequently reexpress BGS thereby suggesting that these antigens behave as oncofetal antigens at this organ site. We used a sensitive immunoperoxidase method with monoclonal antibodies directed against blood groups A, B, O (H), Lewis\textsuperscript{a} and Lewis\textsuperscript{b} to systematically evaluate BGS expression in fetal colon, normal adult colon from immediate autopsies of kidney donors, mucosa adjacent to cancer (transitional mucosa) and colorectal cancer tissues. In normal colon, BG-A, B, H, and Lewis\textsuperscript{b} were expressed in proximal but not distal colon, whereas Lewis\textsuperscript{a} was distributed uniformly throughout the colon. In colon cancer, and fetal colon, the proximal-distal gradient of BG-A, B, H, and Lewis\textsuperscript{b} expression was abolished because of enhanced distal expression of these antigens. In cancer tissues, three patterns of altered BGS expression emerged: (a) incompatible expression of BG-A or BG-B (over 50% of patients); (b) deletion of BGS; and (c) precursor BG-H accumulation (80% of 25 tumors). BGS staining of transitional mucosa closely resembled that of the adjacent tumor except that no examples of BGS deletion were encountered in transitional mucosa. The goblet cell secretory vacuole accounted for most of the BGS expression in normal colon, but cancer cells demonstrated differentiation-dependent antigenic expression such that well-differentiated tumors expressed BGS on cell apical membranes and glandular contents, but poorly differentiated cancers exhibited diffuse cytoplasmic staining. These findings confirm the oncofetal nature of BGS in distal colon cancer, and provide immunohistochemical evidence for a diverse repertoire of altered antigen expression in colon cancer. Further investigation is needed to elucidate the possible genetic and biochemical mechanisms involved.

INTRODUCTION

BGS\textsuperscript{3} are the major allogeneic antigens in humans. Their presence is not limited to blood cells, but they are also found in various epithelial cells. On RBC, the blood group determinants A, B, and H are found on surface glycolipids and glycoproteins, whereas in secretions, these antigens are carried by glycoproteins (1). Lewis antigens are plasma fucolipids that absorb reversibly to the red cell membrane, but their site of synthesis is uncertain.

Blood group antigens are phenotypic markers which undergo certain changes in various human tumors. The most common changes exhibited by nongastrointestinal tumors are a marked diminution or absence of BGS expression (2–4). In the case of normal human colorectal mucosa and colorectal neoplasms, BGS expression is somewhat different. The distal colon and rectum of the embryo and fetus express BGS (5), but with maturation to adulthood, BGS expression is lost from adult distal colon while being preserved in more proximal segments (6, 7). With colorectal neoplasia, the distal colon usually demonstrates a reexpression of BGS, suggesting that an oncofetal gene function has been derepressed or reactivated, in a situation analogous to that of carcinoembryonic antigen (8). Some authors have suggested that failure of colorectal cancers to express BGS may be a marker for metastatic potential (9).

Earlier investigations of BGS expression in the colon utilized a mixed cell agglutination reaction (10–13) or specific red cell adherence (14–16). Immunohistochemical techniques have also been applied, using lectins (17, 18), commercial human antisera (7, 9), and most recently, monoclonal antibodies (19–21). The purpose of the present study was to extend the observations of BGS alterations in human colorectal cancer by comparing the regional distribution of BGS in colorectal cancer, adjacent transitional mucosa, normal adult colonic mucosa, and fetal colonic mucosa. This study attempts to refine the previous literature in several ways. First, normal colonic mucosa has been obtained immediately after death rather than from nonneoplastic colonic diseases or from remote regions of colon cancer specimens, since the latter source may not represent truly normal mucosa (22). Second, transitional mucosa adjacent to cancer is systematically examined to determine whether BGS expression in this region resembles normal mucosa or cancer. Third, specific monoclonal antibodies are used in a sensitive immunoperoxidase method. Finally, 5 blood group antigens rather than just 2 or 3 are simultaneously evaluated in serial sections.

MATERIALS AND METHODS

Tissue Specimens

Primary colorectal carcinoma tissue was obtained from 32 patients undergoing surgical resection at Shady Side Hospital and the San Francisco VA Medical Center. Fourteen were from the proximal colon (2

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ocum, 11 ascending colon, and 1 transverse colon), and 18 were from the distal colon (5 descending colon, 8 sigmoid, 3 rectosigmoid, and 2 rectum). Sixteen were well differentiated, 12 were moderately differentiated, 2 were poorly differentiated, and 2 were colloid type adenocarcinomas. In 25 of the 32 surgical specimens (13 proximal and 12 distal), well-oriented mucosa immediately adjacent to the cancer was evaluated. This region is referred to as transitional mucosa.

Normal colorectal mucosa was obtained from 18 kidney donors immediately after death, according to the autopsy protocol of the University of Maryland Department of Pathology. From these 18 individuals, the following segmental sections were provided: 15 from ocum, 17 from ascending colon, 18 from transverse colon, 18 from descending colon, 16 from sigmoid, and 18 from rectum.

Fetal colonic tissue was obtained at abortion from 12 fetuses of 13–23 weeks' gestation as approved by the Human Experimentation Committee of the University of California, San Francisco. Six fetuses provided both proximal and distal colon specimens, 3 provided only proximal, and 3 provided only distal tissue.

All tissue samples were fixed in formalin or Bouin's solution and immediately after death, according to the autopsy protocol of the University of Maryland Department of Pathology. From these 18 individuals, the following segmental sections were provided: 15 from ocum, 17 from ascending colon, 18 from transverse colon, 18 from descending colon, 16 from sigmoid, and 18 from rectum.

Monoclonal antibodies with specificities for blood groups A, B, H, Lea, and Le6 were purchased from Chembiomed (Edmonton, Alberta, Canada). These antibodies are highly specific for their respective antigens on human erythrocytes and epithelial cells and do not cross-react with other blood group antigens as documented by red cell serology and hapten inhibition.4 All 5 are mouse IgM antibodies and have the following specificities: (a) anti-A reacts with type 1 and 2 A antigens; (b) anti-B reacts with type 1 and 2 B antigens; (c) anti-H reacts only with type 2 H antigen; (d) anti-Le6 reacts with A1, A2, AB, B, and O donor tissue, and does not react with human Le6 or Le6+ erythrocytes; (e) anti-Le6 reacts with A1, A2, AB, B, and O donor tissue and does not react with human Le6 or Le6+ erythrocytes. By hemagglutination, anti-A does not react with Forssman-positive sheep erythrocytes.

UEA-1 and DBA lectins, as well as anti-UEA-1, anti-DBA, rabbit anti-PAP and goat anti-rabbit antibodies were provided by EY Laboratories (San Mateo, CA). Rabbit anti-mouse IgM + IgG + IgA was provided by Zymed Laboratory (South San Francisco, CA).

Working concentrations were as follows: UEA-1, 50 µg/ml; DBA, 100 µg/ml; rabbit anti-UEA-1, 19 µg/ml; and rabbit anti-DBA, 34 µg/ml.

**Antisera**

The PAP technique of immunohistochemical staining was used as detailed below (23).

1. Deparaffinize, clear, and hydrate sections. Each subsequent step is preceded by 3 washes with PBS (0.1 M phosphate, 1.5 M NaCl, pH 7.4).

2. Incubate sections with methanol-1% hydrogen peroxide 30 min.

3. Incubate with mouse anti-BGS, 20 µg/ml, diluted in 10% normal goat serum, 2–4 h.

4. Incubate with rabbit anti-mouse IgM + IgG + IgA, 20 µg/ml, diluted in 1% normal goat serum, 60 min.

5. Incubate with goat anti-rabbit IgG, 20 µg/ml, 30 min.

6. Incubate with rabbit peroxidase anti-peroxidase complex, 6 units/ml, 30 min.

7. Flood slide for 5 min with 0.05 mg of 3,3'diaminobenzidine/100 ml in 1 ml of PBS with 0.1% hydrogen peroxide added just prior to use.

8. Counterstain with 1% methyl green in methanol.

9. Dehydrate, clear, and mount.

For lectin immunohistochemistry, UEA-1 and DBA were substituted in Step 3, and rabbit anti-UEA-1 or rabbit anti-DBA were used in Step 4 above.

**Controls**

Substitution of PBS for specific antisera or secondary antibodies completely abolished any staining. With anti-A, B, H antibodies, positive staining of erythrocytes and blood vessel endothelial cells served as useful internal controls.

**Semiquantitative Analysis of BGS Expression**

In each case of mucosa obtained from normal colon, fetal colon, and transitional zone of cancers, well-oriented crypts were examined for the presence of BGS, and results were expressed as the percentage of crypts which were positive. In the cancer specimens, all of the low-power (×10 objective) optical fields were examined for BGS expression, and results were expressed as percentage of total fields which were positive.

**Determination of BGS Phenotype and Secretor Status**

Saliva and gastric secretions were not available to confirm the secretor status of these patients. We therefore predicted the patient's blood type on the basis of ABH erythrocyte staining, and assumed the secretor status based on Lewis antigen staining of erythrocytes and ABH staining of colonic epithelium (1). Cases that demonstrated Le6+ erythrocytes and lack of epithelial A, B, or H staining were considered "unknown" secretor status.

**RESULTS**

**Normal Colonic Mucosa**

**BGS Phenotype and Secretor Status**

The blood type of the 18 normal colon specimens was predicted as follows: 8 type A, 4 type B, and 6 type H. Based on Lewis antigen staining, we predicted 11 secretors, 2 nonsecretors, and 5 unknown secretor status (secretor status).

**Regional Distribution**

Two patterns emerged from analysis of BGS in normal colon (Chart 1). BG-A, B, H, and Le6 were expressed in proximal segments with progressively less expression more distally. In Chart 1, although it appears that BG-A is indeed expressed in the rectum, this represents only 2 cases, both of whom demonstrated weak staining with BG-A on fewer than 20% of the crypts. A second pattern was demonstrated for Le6 which was uniformly distributed throughout the colon. In Chart 1, the reason why the percentage of cases appears somewhat low in the proximal segments of normal colon is because several patients were nonsecretors, and would not be expected to express BGS.

**Cellular Distribution**

The expression of BGS in the normal colon is found mainly in the goblet cells. If only the apical membrane of an epithelial cell stained for a particular BGS, this was considered nonspecific and was therefore excluded from analysis, since this may reflect adsorption of luminal mucin. The secretory part of the goblet cells was responsible for most BGS staining. Staining of the goblet cells was confined to the...
BLOOD GROUP ANTIGENS IN HUMAN COLON CANCER

Regional Distribution

Unlike the normal colon which demonstrated a proximal-distal gradient for the expression of 4 BGS (A, B, H, and Le⁰), colorectal cancers expressed all BGS uniformly throughout the colon (Chart 1). It is actually the enhanced distal expression of BGS which accounts for the loss of the proximal-distal gradient in colorectal cancers. This observation confirms previous reports (8, 9, 21).

Cellular (Glandular) Distribution

The cellular distribution of all BGS tended to correlate with the degree of histological differentiation (Fig. 2). In well-differentiated tumors, BGS were expressed mainly in the apical membrane and luminal contents of neoplastic glands, with occasional expression in the supranuclear region of cell cytoplasm (Fig. 2A). Moderately well-differentiated tumors demonstrated staining diffusely in the whole cell membrane or localized to the cell apical cytoplasm (Fig. 2B). Poorly differentiated tumors exhibited diffuse intracytoplasmic staining (Fig. 2C).

Although there was some variability, each individual antigen displayed a particular cellular staining pattern. BG-A and Le⁰ were located primarily on apical membranes of cells and glandular luminal contents. The other 3 antigens were more evenly distributed among the various cell locations, but BG-H was more often present in the cytoplasm and BG-B was rarely expressed on the whole membranes.

By examining serial sections, considerable heterogeneity within a given area was demonstrated. For example, 2 or more BGS might be simultaneously coexpressed in the same field (Fig. 3). Alternatively, a mosaic pattern was noted whereby one BGS but not another was expressed in the same optical field (Fig. 4).

Patterns of Altered BGS Expression in Colorectal Cancer

Compatibility. With few exceptions, when the erythrocytes of a particular specimen expressed an ABO or Lewis antigen, the malignant epithelial cells also expressed the antigen, regardless of tumor location or degree of differentiation. In addition, the tumors of more than one-half of the patients also expressed ABO antigens which were incompatible with the patient’s blood type (Table 1). Of 10 cancers from type A patients, 5 expressed BG-B (3 proximal cancers and 2 distal cancers), and of 13 cancers from type B patients, 7 expressed BG-A (4 proximal and 3 distal). Five of 5 type H patients also expressed BG-A and/or BG-B in their tumors. All 3 Le⁰ patients (nonsecretors) expressed tumor cell Le⁰ (not shown).

Deletion. In 2 of 10 patients with blood type A (one secretor and one unknown secretor status), and 1 of 4 with blood type AB (secretor), tumor cells failed to express BG-A. All 3 of these tumors were in the ascending colon. In 2 of 13 patients with blood type B and 1 with type AB, BG-B expression by cancer cells was absent in the distal colon. However, this latter observation resembles that which has been described in normal distal colon, and therefore may not represent a true “deletion.” Four of 20 patients with Le⁰-positive erythrocytes demonstrated tumor cell deletion of Le⁰, 1 cecal, 2 ascending colon, and 1 rectosig...

Chart 1. Regional distribution of BGS in normal colon (solid bars) and colon cancers (open bars). Abscissa: C, cecum; AC, ascending colon; TC, transverse colon; DC, descending colon; S, sigmoid; R, rectum. Ordinate: percentage of specimens from a region of colon which stained for BGS. Numbers beneath bars, total number of specimens. *, 2 cases with weakly staining BG-A in fewer than 20% of crypts. Rectosigmoid cancers have been combined with rectal cancers.
BLOOD GROUP ANTIGENS IN HUMAN COLON CANCER

Table 1

Incompatible BGS expression in colorectal cancer and transitional mucosa

<table>
<thead>
<tr>
<th>Proximal</th>
<th>Distal</th>
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<tbody>
<tr>
<td>Patient blood type (n)</td>
<td>Incompatible BGS expressed (n)</td>
</tr>
<tr>
<td>A (5)</td>
<td>B (3)</td>
</tr>
<tr>
<td>B (7)</td>
<td>A (4)</td>
</tr>
<tr>
<td>H (1)</td>
<td>AB (1)</td>
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Totals: 13 B (62%) 15 B (60%)

Transitional mucosa

<table>
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<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (4)</td>
<td>B (3)</td>
</tr>
<tr>
<td>B (7)</td>
<td>A (4)</td>
</tr>
<tr>
<td>H (1)</td>
<td>AB (1)</td>
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</tbody>
</table>

Totals: 12 B (57%) 10 A (30%) 3 B (30%)

Table 2

BGS precursor expression in colorectal cancer

<table>
<thead>
<tr>
<th>Tumor phenotype</th>
<th>RBC phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Precursor absent: (n = 3)</td>
<td>H<em>A</em>B* (n = 2)</td>
</tr>
<tr>
<td>H<em>A</em>B' (n = 1)</td>
<td>BG-A</td>
</tr>
<tr>
<td>H*A'B' (n = 1)</td>
<td>BG-AB</td>
</tr>
<tr>
<td>H*A'B'' (n = 2)</td>
<td>BG-A</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Prevalence of BGS Precursor*</th>
<th>Staining Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Precursor present; appropriate BGS absent: (n = 4)</td>
<td>H &gt; A or B</td>
</tr>
<tr>
<td>H = A or B</td>
<td>13/25</td>
</tr>
<tr>
<td>H &lt; A or B</td>
<td>6/25</td>
</tr>
</tbody>
</table>

*For each tumor, the percentage of optical fields which were positive for BG-H were compared to the percentage positive for BG-A or BG-B.

BGS Precursor Accumulation. As mentioned above, substance H is a precursor to both BG-A and BG-B. The latter antigens were expressed only if the appropriate glycosyltransferase was available to complete these "finished" antigens. BG-H was expressed in all but 3 cancers, suggesting that ABO precursor substance was present in most tumors (Table 2). Of the 3 BG-H negative cancers, 1 demonstrated the capacity to express BG-A in approximately 20% of the tumor, but the other 2 cases could not express either BG-A or BG-B in the absence of BG-H. The remaining 29 patients had demonstrable precursor substance. However, in 4 instances, the cancer cells were unable to express the BGS corresponding to the patient's blood type (Table 2); 1 blood type AB patient lacked cancer cell BG-B, and 3 patients (2 blood type A and 1 blood type AB) lacked cancer cell BG-A. The latter 3 patients are the same as those described in the previous paragraph of BGS deletion, and it is therefore probable that the cancer cells of these patients have a deficiency or total absence of the proper glycosyltransferase enzyme.

There were 25 cases in which the cancer cells not only expressed BG-H but also expressed the appropriate BG-A or BG-B relative to the patient's blood type (Table 2). In these specimens, BG-H expression was compared to BG-A or BG-B expression by evaluating both the staining intensity of each antigen and the proportion of each tumor which exhibited the particular antigen. When this was done, 19 specimens demonstrated BG-H staining which was at least as strong as BG-A or BG-B. In addition, BG-H was present in at least as many optical fields as was BG-A or BG-B in 21 of the 25 specimens. These findings suggest that when colorectal cancers express BG-A or BG-B, there is often considerable expression of precursor BG-H as well.

Transitional Mucosa

General Characteristics

Well-oriented mucosa immediately adjacent to cancer was available in only 25 of the 32 cancer specimens. Eight patients were type A, 10 were type B, 3 were type AB, and 4 were type H. There were 15 secretors, 3 nonsecretors, and 7 unknown secretor status.

Regional Distribution

Compared to normal mucosa, all BGS were enhanced in the proximal colon and, with the exception of BG-A, the same was true of the distal colon (Chart 2). Thus, transitional mucosa mimicked colorectal cancer by demonstrating a general loss of the proximal-distal gradient for BGS.

Cellular Distribution

Unlike normal mucosa which demonstrated goblet cell cytoplasm staining only with BG-H, transitional mucosa was remarkable for cytoplasmic expression of all BGS in both proximal and distal colon (Fig. 5).

Proximal Distal

Chart 2. Summary of BGS expression in human colonic tissue. N, normal adult colon; C, colon cancer; TM, transitional mucosa; F, fetal colon. *, 2 cases of normal rectum with <20% of crypts positive, and 4 cases of normal descending colon with <45% of crypts positive. Proximal, cecum, ascending colon, and transverse colon. Distal, descending colon, sigmoid, and rectum. In the case of normal colon, a case was scored positive if any 1 of the 3 proximal or distal segments stained for a particular BGS.
BLOOD GROUP ANTIGENS IN HUMAN COLON CANCER

Secretor Status

In normal mucosa, patients who were nonsecretors did not express BGS in the colonic epithelium, with one exception: Le" was present in epithelium. However, in the case of transitional mucosa, 2 of 3 nonsecretors were able to express several BGS in the epithelium.

Patterns of Altered BGS Expression in Transitional Mucosa

Compatibility. In normal mucosa, no incompatible BGS expression was encountered. However, transitional mucosa did exhibit incompatible BGS, especially in the proximal colon (Table 1). Although not shown, if transitional mucosa expressed incompatible BGS, then the cancer from that same patient expressed incompatible BGS in the same way, with only 3 exceptions: one type A patient had BG-B expressed in transitional mucosa but not cancer; another type A patient had BG-B expressed in cancer but not transitional mucosa; and one type H patient expressed BG-B in transitional mucosa but both BG-A and BG-B in cancer.

Deletion. Transitional mucosa was similar to normal mucosa in that no examples of BGS deletion were detected.

BGs Precursor Accumulation. In normal mucosa, BG-H expression was found only in the proximal colon (9 of 18 patients). However, in transitional mucosa, precursor expression was noted in 12 of 13 proximal colonic and 8 of 12 distal colon specimens. This resembles the pattern for colon cancer in which 13 of 14 proximal and 16 of 18 distal cancers demonstrated precursor substance.

Fetal Colon

General Characteristics

From 12 fetuses, 9 proximal colon and 9 distal colon specimens were available. Six fetuses were blood type A, 3 were type B, and 3 were type H. All were secretors.

Regional Distribution

No proximal-distal gradient was noted for any of the BGS (Chart 2). This confirms earlier immunofluorescence studies which documented distal colonic expression of BGS in the fetus (5, 24).

Cellular Location

Fetal colon demonstrated cytoplasmic and whole membrane expression of BG-H and Le" more so than in normal mucosa.

Patterns of BGS Expression in Fetal Colon

Compatibility. No case of incompatible BGS expression was found.

Deletion. In blood type A fetuses, BG-A was deleted in 2 of 5 proximal but in 0 of 5 distal colons. In blood type B fetuses, 1 of 2 proximal and 1 of 2 distal colons had BG-B deletion from the epithelium.

BGs Precursor. BG-H was present in 8 of 9 proximal and in 7 of 9 distal fetal colons.

Summary of Results with ABH Monoclonal Antibodies

Table 3 summarizes the results of ABH expression in the present study.

Comparison of Lectins with Monoclonal Antibodies

Because lectins, which recognize certain terminal saccharides on glycoconjugates, have been used to investigate antigenic alterations in cancer tissues, we decided to compare staining patterns between lectins of blood group H- and A-like specificity and our anti-H and anti-A monoclonal antibodies (Table 4). When UEA-1 and BG-H were compared, an almost identical staining pattern emerged, both in terms of the number of positive cases as well as the proximal-distal distribution. However, DBA and BG-A gave discordant results for these 2 parameters and considerable heterogeneity within cancers was demonstrated, such that only 10 of 16 cancers stained with both compounds.

DISCUSSION

Blood group substances are a group of carbohydrate antigen structures which can be detected on erythrocytes, endothelium, epithelium, and body secretions. These structures are formed by the sequential addition of saccharides to the carbohydrate side chains of glycolipids and glycoproteins. Although 15 independent blood group systems have been defined in man, the biochemistry and genetics which control antigenic expression have been elucidated for only a few groups. Analysis of these well-characterized carbohydrate antigens, as well as other tumor-associated

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Table 3

<table>
<thead>
<tr>
<th>Summary of ABH expression in human colon</th>
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<tbody>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Regional distribution</td>
</tr>
<tr>
<td>Proximal</td>
</tr>
<tr>
<td>Distal</td>
</tr>
<tr>
<td>Cellular location</td>
</tr>
<tr>
<td>Supranuclear cytoplasm</td>
</tr>
<tr>
<td>Whole membrane</td>
</tr>
<tr>
<td>Goblet cell</td>
</tr>
<tr>
<td>Incompatible BGS</td>
</tr>
<tr>
<td>Deletion of BGS</td>
</tr>
<tr>
<td>Precursor present</td>
</tr>
</tbody>
</table>

* NA, not applicable.

Table 4

<table>
<thead>
<tr>
<th>Comparison of lectins and monoclonal antibodies in normal and malignant colorectal tissues</th>
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</thead>
<tbody>
<tr>
<td>Normal (n = 9)</td>
</tr>
<tr>
<td>BG-H</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Proximal:distal</td>
</tr>
<tr>
<td>Cancer</td>
</tr>
<tr>
<td>Proximal:distal</td>
</tr>
</tbody>
</table>

* Specificity for α-L-fucose.
* Specificity for N-acetylgalactosamine.
* One case positive in descending colon only; 1 case positive in sigmoid and rectum.

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carbohydrate antigens, has provided tremendous insight into the alterations of cell surface carbohydrates that may occur with neoplastic transformation (25, 26).

The colon is well suited for the study of BGS expression by normal and malignant cells, not only because it contains a secretory epithelium, but also because it is a relatively accessible organ (surgically and endoscopically) that has an increased tendency toward neoplastic transformation, as evidenced by the high prevalence of colorectal adenocarcinoma in Western society. The colorectum undergoes developmental changes in ABO and Lewis antigen expression. In the fetus, ABO determinants are expressed throughout the colon (5), but at parturition, these substances disappear from the rectum and sigmoid and this pattern persists in the adult (6, 7). Expression of Le\textsuperscript{a} antigen often parallels that of the ABO antigens (20, 24) but Le\textsuperscript{b}, which lacks an $\alpha 1 \rightarrow 2$ linked fucosyl moiety in its oligosaccharide determinant, appears to be more uniformly distributed throughout the proximal and distal adult colon (20). In the case of colorectal cancer, lesions of the distal colon often have the capacity to reexpress BGS, thereby abolishing the "normal" proximal-distal gradient and recapitulating the pattern found in fetal colon (8, 9, 18, 21). Thus, at least for BG-A, B, H and Le\textsuperscript{a}, BGS may be considered oncodevelopmental antigens in the colon.

Most of our knowledge about BGS expression in normal and neoplastic colonic tissue comes from studies using specific erythrocyte adherence, mixed-cell agglutination reaction, or immunofluorescence with polyclonal antibodies and, in the case of BG-H, lectins. Only recently have monoclonal antibodies been available for the study of BGS expression in tissues, and some studies have demonstrated that mouse monoclonal anti-A and anti-B antibodies perform as well if not better than routine human polyclonal antibodies for serological blood typing (27). The present study extends previous observations by using a very sensitive immunoperoxidase technique (PAP method) with specific monoclonal antibodies and by simultaneously assessing the expression of 5 BGS in serial sections. Moreover, the use of autopsy-derived colonic tissue has provided the opportunity to systematically study regional variation of BGS expression in the same patient, not possible in most previous studies of normal tissues from surgically removed cancer specimens of only one segment of colon.

Using these methodological refinements, we obtained results in normal colon similar to those which have been previously reported; namely, that BG-A, B, H, Le\textsuperscript{a} expression progressively diminishes in a caudal direction, whereas Le\textsuperscript{b} is more uniformly expressed (6, 7, 20). To allow for more direct comparison with previous literature, and to more readily compare normal tissue to cancer tissue, our data have been expressed according to what percentage of each colonic segment expressed a particular BGS (Table 1). However, if one were to express the data to ask what is the segmental distribution of BGS in a patient with a particular blood type, a similar proximal-distal gradient would be obtained (data not shown).

The present study also extends previous observations concerning patterns of altered BGS expression in colon cancer. In general, 3 mechanisms of altered BGS expression in carcinoma cells have been described (26). First, tumor cells may exhibit a deletion of the appropriate BGS found in normal tissue. Second, precursor substances of BGS may accumulate in cancers. And third, cancer cells may demonstrate incompatible BGS expression with respect to the patient's erythrocyte phenotype.

In colon cancer tissues, each of these mechanisms has been demonstrated. Deletion or marked diminution of ABO substances has been detected by mixed-cell agglutination reaction (8, 10), immunofluorescence (18, 28), immunoperoxidase (21), and biochemical (29, 30) methods. We encountered 3 examples of BG-A deletion and 4 cases of Le\textsuperscript{b} deletion in colon cancer. Ernst et al. also found 3 Le\textsuperscript{a} negative colon cancers in 9 patients of salivary phenotype Le\textsuperscript{a+} (20), and 2 cases of proximal BG-A deletion (21). The probable explanation for BGS deletion in colon cancer is a lack or deficiency of glycosyltransferase activities responsible for completing the immunodeterminant oligosaccharides (30, 31), although "masking" of the antigen by addition of other sugars or degradation of antigen by luminal bacteria cannot be completely excluded.

A deficiency of glycosyltransferase activity may likewise result in the accumulation of precursor substances within cells, and this has been demonstrated in colon cancer and even in premalignant polyps (17-19, 32, 33), as well as in gastric cancer (34). Others, however, using a variety of biochemical and histochemical techniques, have either failed to find precursor accumulation in colon (30, 35) or gastric (36) cancers, or have actually noted a loss of precursor substance in these tissues (18, 28, 37).

In the present study, we found 21 cancers in which precursor BG-H was not only present, but was found in a greater percentage of each specimen than was either BG-A or B (Table 2). In only 3 cases was BG-H completely absent. A recent report by Brown et al. (19) used the same monoclonal antibody as we did to detect BG-H type 2 chains in colon cancers and found that all patients with blood type O expressed BG-H in tumors, but 4 of 12 blood A patients demonstrated no BG-H in their cancers. They speculated that BG-H expression was absent in these type A patients because their tumor cells converted BG-H to A or A-like substances. This could not be confirmed because the tissues were not examined for BG-A activity. In our study, there were 3 BG-H-negative cancers: one expressed the "complete" BG-A and was from a type A patient, but the other 2 did not express any ABO antigens, even though both patients were blood type B (Table 2).

One of our most striking observations was the relatively high incidence of incompatible BGS expression in cancer tissue. A recent report noted no incompatible BG-A or B expression in colon cancers, even with the use of monoclonal antibodies and immunoperoxidase methodology (21). In addition, none of the blood type H patients in that study expressed BG-A or BG-B in colon cancer tissue. We found that the tumors of over one-half of our patients expressed incompatible BGS (Table 1). Moreover, all patients who were blood type H had cancers which were able to express BG-A and/or BG-B. We speculate that the discrepancy between our results and those of Ernst et al. could be related to differences in the degree of fucosylation or the oligosaccharide chain length of the antigen, as Ernst et al. (21) and others\textsuperscript{a} suggest. Differences in specificity or subclass of the antibodies may also be a contributing factor.

In our study, 3 nonssecretors (i.e., erythrocyte Le\textsuperscript{a}) were capable of expressing not only the compatible Le\textsuperscript{a} in their tumors, which has been observed by others (20), but also expressed Le\textsuperscript{b}

\textsuperscript{a}H. T. Chen and E. A. Kabat, personal communication.
and A, B, O determinants, a phenomenon which has not, to our knowledge, been reported. Although saliva and gastric secretions were unavailable to directly assess secretor status, the method of predicting secretor status by erythrocyte Lewis phenotype is well established (1). The transitional mucosa shared the ability to express incompatible BGS in both secretors and nonsecretors, but to a lesser degree than did cancers. No examples of incompatible BGS expression were noted in our normal adult or fetal colonic specimens.

Incompatible BGS expression was originally described in gastric carcinoma (38) and was confirmed in other cases of gastric as well as pancreatic and hepatocellular carcinoma (21, 34, 36, 39). Only a handful of cases have been reported which exhibit ABO incompatibility in colon cancer (12, 15), a colonic polyp (12), and an appendiceal cancer (40). The mechanism for “unexpected” BGS expression is not known. Denk et al. (36) noted that BG-A and B were expressed independently in different regions of a gastric cancer from a patient of type AB blood. Hakomori et al. (41) suggested that Forssman antigen, which has a terminal N-acetylgalactosamine and cross-reacts with BG-A, might actually be accounting for “neo-A” incompatibility. In vitro experiments suggest that under certain conditions, the BG-A glycosyltransferase can synthesize B determinant structures (42). Somatic mutation has also been implicated as an explanation for incompatible BGS expression (38). The use of highly specific monoclonal antibodies in our study, which react only to the appropriate BGS and not with Forssman antigen, makes it unlikely that cross-reacting antigens are accounting for the incompatible BGS. Indeed, the absence of this phenomenon in normal and fetal colon suggests that this is a cancer-associated process which, after further research, may help to identify biochemical or genetic regulatory mechanisms intrinsic to antigen expression in colon cancer cells.

As mentioned above, the source of normal mucosa in most previous studies of BGS expression in the colon has been that which is adjacent to or several cm from a surgically removed cancer. There is evidence, however, that these regions may not be morphologically or histochemically normal (22). While we realize that in our study, the normal and cancer tissues were obtained from 2 different sets of patients, we find it intriguing that BGS expression in our normal colonic specimens was different from that of transitional mucosa, which behaved more like the adjacent cancer tissue (Table 3). In fact, the only feature not common to both TM and cancer was the absence of the deletion phenomenon in TM. Moreover, the pattern of incompatible BGS expression in TM was practically identical to that of cancer tissue in the same patient. Although others have also noted a similarity in BGS expression between TM and colon cancer (or polyps) (13), several authors have concluded that for BGS expression, adjacent mucosa behaves more like normal tissue than like cancer (18, 21, 34). Controversy persists as to the neoplastic potential of transitional mucosa (43), but the present study supports the view that antigen expression in TM resembles cancer more than normal tissue.

A systematic study of fetal, adult, transitional mucosa, and malignant colonic tissues disclosed some interesting cytological patterns of BGS expression. In the adult, the goblet cell vacuole accounts for most of the staining, although, as noted by others (7), supranuclear staining may also be present. Fetal colon demonstrates cytoplasmic and whole-membrane staining. Transisional mucosa and cancer resemble fetal cells in that cytoplasmic localization is enhanced in TM and poorly differentiated cancers. With well-differentiated tumors, expression on apical membranes and luminal contents predominates, suggesting perhaps that in these more polarized cells, BGS can be properly sorted and secreted. In the present study, although cellular localization correlated with tumor differentiation, there was no correlation between patterns of altered BGS expression and tumor differentiation.

Many of the earlier studies had technical difficulties in identifying BG-H in tissue specimens, and therefore had to either exclude this antigen from analysis (11, 12), or use the Ulex europeas lectin to identify BG-H-like activity (17, 18). We are encouraged that in the present series, the monoclonal BG-H antibody was generally similar to that of UEA-1 (Table 4). In 2 cases of normal distal colon, we noted UEA-1 reactivity without evidence of BG-H activity. The literature to date suggests that UEA-1 reactivity is absent in normal distal adult colon, so we cannot easily explain our lectin results. Our results with monoclonal anti-H antibody are in accord with those of Brown et al. (19), who used the same monoclonal anti-H antibody specific for type-2 but not for type-1 blood group chains, and found no BG-H expression in normal distal colon. Perhaps the staining differences between lectins and antibodies are, in part, related to differences in blood group chain backbones (e.g., UEA-1 versus monoclonal anti-H), or differences in the subgroup of BG-A (e.g., DBA versus monoclonal anti-A).

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REFERENCES

BLOOD GROUP ANTIGENS IN HUMAN COLON CANCER


Fig. 1. Normal prostatic colonic mucosa. A, staining with anti-BG-A antibody. Secretory vesicle of goblet cells and apical membrane of epithelial cells are stained. Supranuclear region of cytoplasm is positive (arrows). × 250. B, staining with anti-BG-1 antibody. Note positive staining of blood vessel endothelium (bottom) × 650.
Fig. 2. Colon carcinoma stained with anti-BG-H antibody. A, well-differentiated; strong apical membrane and luminal contents staining; weakly stained supranuclear region. × 250. B, moderately differentiated; whole membrane and apical cytoplasm staining. × 650. C, poorly differentiated; whole membrane and diffuse cytoplasmic staining. × 650.
Fig. 3. Colon carcinoma showing coexpression of BG-A, B and H. A, anti-BG-A; B, anti-BG-B; C, anti-BG-H. Note intense staining of apical membrane and luminal contents with all 3 BGS. × 250.
Fig. 4. Colon carcinoma showing heterogeneous expression of BGS. A, anti-BG-A; B, anti-BG-H. In A, area on right is positive on apical membrane and cytoplasm (solid arrow), but area on left is negative (open arrow). In B, area on right is negative (solid arrow) but area on left is positive on apical, lateral, and basal membranes (open arrows). × 650.
Fig. 5. Transitional mucosa stained with anti-Lea antibody. Note supranuclear staining (arrow). × 650.
Distribution of Blood Group Antigens A, B, H, Lewis\(^a\), and Lewis\(^b\) in Human Normal, Fetal, and Malignant Colonic Tissue

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