ABH and Lewis Blood Group Expression in Colorectal Carcinoma

Robert Schoentag, F. James Primus, and William Kuhns

ABSTRACT

The expression of ABO(H) and Lewis blood group antigens on 68 colorectal carcinomas from 63 patients was studied by immunohistochemical staining of tissue specimens. The pattern of antigen expression was as follows: (a) ABH was expressed in normal tissue only in secretors and was expressed in the proximal but not distal colon. In tumors, there was net loss of ABH expression in the proximal and net gain in the distal colon. Some nonsecretor tumor tissue expressed ABH. (b) Lewis expression was similar to but less strong than ABH. Its expression occurred only in secretors and in normal epithelium only in the proximal colon. In tumors, there was net loss of antigen expression proximally and net gain in the distal colon. There was no expression of Lewis in tumors of nonsecretors. (c) Lewis antigen was expressed throughout the normal colon in secretors and nonsecretors with no discernible difference between proximal and distal colon. In tumors, net loss of expression of Lewis occurred throughout the colon. (d) No inappropriate blood group expression was observed in this study. (e) With few exceptions, H expression paralleled expression of A and B in non-0 patients. (f) Metastatic tumor antigen expression was similar to that of the primary in most cases. (g) Alterations in antigen expression were not clinically or histologically distinctive.

INTRODUCTION

Changes of blood group antigens have been described in several types of cancer (1, 2). Normally expressed antigens may be deleted or antigens not normally expressed may emerge. The explanation for these alterations in expression has not been resolved. On occasion, terminal sialic acid may mask blood group determinants (3). Tumor markers have been described whose acceptors for non-blood-group sugar termini possess blood group specificities (4). Incomplete biosynthesis of oligosaccharide chains associated with cancer may result in core sugar specificities (5).

The biosynthesis of such carbohydrate determinants occurs by the sequential addition of single sugars to growing precursor chains, each addition being made from a nucleotide and catalyzed by a specific glycosyltransferase (6). These synthetic mediators may be altered in malignancy (7), or normally constituted antigens may be cryptic (4). In the gastrointestinal tract, blood group expression may be abrogated by specific glycosidases produced either by the normal bacterial flora (8, 9), or possibly by increased glycosidases produced by carcinoma cells (10). Ectopic sucrase production in colon cancer has been suggested as a possible cause of blood group gain (11, 12); this enzyme is known to possess blood group specificities.

Normal gastrointestinal mucosa contains large quantities of blood group substances (1). The pattern of expression in normal colonic mucosa was delineated by Szulman (13, 14) who found ABH antigens expressed only in secretors in the proximal half of the colon. Subsequently much work has been done on ABH alterations in gastrointestinal carcinoma (15–18) and recent publications have placed special emphasis on Lewis blood group antigen expression in carcinoma of this area (19–23). From these studies, general patterns of blood group expression in normal colon and colonic tumor have emerged. ABH and Le3 are expressed in normal colon and only in the proximal portion. Lewis is independent of secretor status and is expressed throughout the colon. In tumors, reexpression of ABH and Le3 antigens in distal colon carcinomas has been a uniform finding. Some studies have demonstrated incompatible blood substances in colonic tumors (21, 22), at variance with the individual’s erythrocyte blood group, whereas other studies have not shown such differences (20). In nonsecretors, expression of ABH (21) and Le3 (23) has been reported in tumor-involved colonic epithelium.

We now report our findings on ABO(H) and Lewis alterations in 68 tumors of the colon from 63 patients using immunohistochemical techniques. In the cases to be described, the findings on primary tumor and tumor metastases were compared with those observed on normal adjacent tissue and with the patient’s phenotype as determined by red cell agglutination or by neutralization inhibition studies of saliva. In some cases, secretory status was deduced by the blood group reactivity pattern of normal small bowel. Part of this work has appeared in preliminary form elsewhere (19).

MATERIALS AND METHODS

Tissue Specimens. Tissue specimens were obtained from the departments of surgical pathology of the North Carolina Memorial Hospital, the University of Kentucky Medical Center, and the New York Veterans Administration Medical Center over a several-year period. The tissues were regularly fixed in 10% buffered formalin and embedded in paraffin. The tumors were classified according to the criteria proposed by the Joint Committee on Cancer (25). Stage I indicates tumors confined to the bowel wall, Stage II involves all layers of the wall but no lymph node involvement, Stage III indicates regional lymph node involvement regardless of penetration of bowel wall. Stage IV tumors have distant metastases.

Antiserum and Lectins. Ulex europaeus I lectin and rabbit anti-Ulex europaeus I were purchased from Vector Laboratories, Burlingame, CA. Anti-A, anti-Le3, and anti-Le3 were kindly supplied to us by Dr. Don Baker, Chembio Med, Ltd., Edmonton, Alberta, Canada. These were all polyclonal rabbit antiserum raised to synthetic antigens and rendered specific by cross-absorption with solid-phase immunoabsorbents (26). Human sera having hemagglutinating anti-A or anti-B titers of 512 or greater were pooled. No minor blood group antibody activity was revealed in these pools following screening with the Spec-
trogen Duo System (Spectra Biologicals, Oxnard, CA).

Erythrocyte Blood Groups. Standard hemagglutination reagents and techniques were used to test patients for ABO(H) and Lewis blood groups (27).

Secretor Status. Secretor status was determined either directly by standard hemagglutination inhibition techniques (27), inferred by Lewis phenotype on patient's red cells or by blood group antigen testing of normal ileum.

Immunohistochemical Procedures. An unlabeled antibody method using GAG complexes was carried out for all blood group antigen detection procedures except for the demonstration of B (28). For H antigen, hydrated sections (5 μm) were first incubated with Ulex lectin (25 μg/ml), and then test sections were exposed to rabbit anti-Ulex and control sections to normal rabbit serum, both diluted 1/300. Following incubation with donkey anti-rabbit IgG (1/50), the sections were treated with rabbit GAG at 1/100 dilution (Jackson ImmunoResearch, Avondale, PA). The enzymatic disclosing reaction consisted of the following: 6.7 mg/ml β-D-glucose (Calibiomed-Behring Corp., LaJolla, CA); 0.67 mg/ml nitroblue tetrazolium (Sigma Chemical Co., St. Louis, MO) and 0.0167 mg/ml phenazine methosulfate (Sigma) in 0.05 m Tris, pH 8.3. After further incubation for 45 min at 37°C, the slides were washed and counterstained with Nuclear Fast Red. The demonstration of A and Lewis antigens followed similar procedures except that rabbit antisera (1/25) against these antigens were substituted for Ulex lectin and rabbit anti-Ulex antiserum. All immunological reaction steps were done for 20 min at 37°C in a humidified atmosphere. These reaction conditions were established in previous studies (28) as being optimal by staining of conventionally processed tissues known to be antigen positive. An immunoperoxidase procedure was used to localize B antigen by first incubating test sections for 1 h at room temperature with human anti-B serum, or parallel negative control sections with human anti-A serum. These were then incubated with peroxidase-conjugated, rabbit anti-human IgM antibody (Jackson ImmunoResearch) diluted 1/50 for 30 min at room temperature. The enzyme site was disclosed using 3'-3'-diaminobenzidine (Sigma) as the chromagen. Sections were then lightly counterstained with Harris' hematoxylin before mounting.

Positive control sections were included in all staining reactions for any of the blood group antigens, and, in addition, staining of blood vessels was used as an internal positive control of A, B, or H reactivity in specimens from individual cases. Inappropriate expression of A, B, or H was not observed in blood vessel endothelial or erythrocytes. Gastrointestinal tissue from 3 Le°(−−) patients served as a negative control for Lewis antigens.

The staining intensity of the specimens was evaluated at ×125 magnification and was graded as − or +. All tumor specimens were considered positive when greater than 10% of the tissue was stained.

RESULTS

The red cell phenotype of patients with these 68 tumors was as follows: group 0, 35; group A, 17; group B, 14; group AB, 2. Four patients had multiple tumors (three had two and one had three).

There were 50 tumors from secretors and 18 from nonsecretors. The tumors fell into the following American Joint Committee on Cancer stages: stage I, 6; stage II, 19; stage III, 33; stage IV, 10.

The histological grades were as follows: grade 1, 14; grade 2, 44; grade 3, 4; mucinous, 6.

The results of blood group antigen expression are summarized in Fig. 1. ABH and Le° had generally similar patterns of expression. In secretors, normal mucosa in the proximal colon expressed ABH in a high percentage of cases and Le° in almost half, whereas these antigens were not expressed in normal mucosa of the distal colon. In nonsecretors, there was no expression of these antigens anywhere in the normal colon. In tumors of the proximal colon, obtained from secretors, ABH and Le° antigens were often expressed, but to a lesser extent than normal tissue; the effect being net loss of the antigens in carcinoma. In approximately two-thirds of the specimens from the proximal colon in secretors, tumor expression was similar to that found in normal epithelium for both ABH and Le° although these blood group antigens were usually independently expressed. In the distal colon, most tumors expressed ABH and several expressed Le°, the effect being net gain of these antigens in the distal colon. Figs. 2 and 3 demonstrate strong staining of blood group A in a moderately differentiated rectal tumor, while the overlying normal epithelium remains negative. There were three instances of ABH expression (two H and one B) in tumors from nonsecretors in the proximal half of the colon, two of which were of the mucinous histological type. One metastatic lesion from a primary rectosigmoid tumor in a group O nonsecretor also expressed H. Lewis° expression was independent of secretor status. Normal tissue expressed this antigen more commonly than tumor in both proximal and distal colon, there being a net loss of Le° in tumors throughout the colon. Fig. 4 shows loss of Le° expression in a sigmoid tumor, although expression of this antigen was commonly retained in tumors throughout the colon.

Certain generalizations about localization of these blood group antigens could be made from this study. In normal tissue the antigens were located primarily in cytoplasmic vacuoles or goblet cells with lesser degrees of glycocalyx, cytoplasmic, and intraluminal distribution. Lewis° had somewhat more tendency to localize in glycocalyx or intraluminal regions in normal mucosa. In tumors, localization of antigens was more common in the glycocalyx and intraluminal areas for both ABH and Lewis blood groups. Cytoplasmic expression was observed in a definite but small percentage of cases and did not appear to be related to histological grade (Fig. 3).

H antigen was studied in 19 A, B, and AB tumors and was
found to be similar to the expression of A and/or B. In most cases tested, the expression of H was identical to A and B except for minor variations in relative strength or degree of positivity. In two specimens from the cecum in blood group B patients, nontumor epithelium expressed H but not B antigens.

We failed to observe any instance of incompatible blood group expression in carcinoma. This includes A expression on B cases, B on A, and A and B on O tumors. In none of these cases was less than 10% reactivity found.

There was no discernible relationship between the pattern of blood antigen expression and histological grade or pathological stage.

In 11 patients, metastases in lymph nodes (Fig. 5) and/or liver were studied for antigenic comparison with the primary tumor (Table 1). With few exceptions, antigenic expression was identical in primary tumor and metastases. Sampling variations could account in part, for these discrepancies. Antigenic expression (H) in a metastatic liver lesion from one nonsecretor patient in which the primary was negative has already been noted.

**DISCUSSION**

The data presented here concerning ABH and Lewis blood group antigen expression in colorectal carcinoma are in general agreement with several recent reports (15–23). ABH and Le\textsuperscript{b} have similar distribution patterns both in normal colonic epithelium and in tumors, the overall expression of which is related to secretor status. Our findings indicate that these antigens have decreased expression in tumors relative to normal in the proximal colon, but the opposite relationship of increased expression appears in the distal colon. Lewis\textsuperscript{*} expression is independ-
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Fig. 4. Sigmoid tumor (below) in group O nonsecretor reacted with anti-Le* and GAG. Cytoplasmic staining of normal mucosa (above) is present. Tumor is negative. × 200.

Fig. 5. Lymph node metastasis from a right colon tumor in a secretor patient of blood group B reacted with anti-Le* and GAG. Positivity is predominantly intraluminal and glycocolyceal. × 200.

ent of secretor status, and in contrast to LeA, its expression in proximal and distal colon is similar. Overall, there is decreased expression of LeA antigen in tumors as compared to normal tissue. A goblet cell localization of normal blood group antigens, contrasted to their cell surface or luminal location in cancer support the findings of other investigators (21).

Changes in blood group antigen expression which can accompany malignant transformation in the colon could result from several disease mechanisms. Since the ABH and Lewis antigens represent indirect gene products, changes of the enzyme mediators, i.e., transferases, the acceptors, or the phenotypes themselves may occur (29). Sialic acid is frequently produced in excess and may mask blood group linkages, thus converting blood group antigen expression to a cryptic state (3). Tumor-associated antigens may also compete with normal carrier molecules for limited glycosyltransferases and nucleotide sugars (4, 30, 31). Intestinal microflora possess a subpopulation of anaerobes which produce blood group-splitting enzymes (29). Glycosidases in addition to proteases may also be produced in excessive quantities by cancer cells (10). Ectopic enzymes in cancer may possess blood group specificities (11, 12).

Normal and tumor cell membrane glycoconjugates display extensive microheterogeneity (32–34). In colonic tumors, mixed evidence favors simplification of carbohydrate structures with accumulation of precursors on the one hand (35), but increased complexity of certain tumor-associated molecules on the other (36). The prevailing membrane blood group acceptor linkage consists of 1–3 type 1 chains (37) (in contrast to 1–4 type 2 chains prevalent on erythrocytes), and this may explain the normal colon-wide distribution of Lewis antigen on cell membranes (20). How this can be reconciled with normal ABH variation is less clear. It is possible that water-soluble ABH, particularly prevalent on brush borders and in the lumen may become progressively vulnerable to the action of bacterial glycosidases (9) throughout the length of the colon. The integral nature of LeA-associated membrane molecules, presumably less...
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Table 1  Blood group expression of metastases in 11 patients with colorectal carcinoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Secretor status</th>
<th>Location of primary</th>
<th>Specimen</th>
<th>ABH</th>
<th>Le&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Le&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>1</td>
<td>S</td>
<td>Right</td>
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<td>2</td>
<td>S</td>
<td>Left</td>
<td>Primary</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>S</td>
<td>Left</td>
<td>Primary</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>S</td>
<td>Rectum</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>NS</td>
<td>Cecum</td>
<td>Primary</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>NS</td>
<td>Right</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>NS</td>
<td>Transverse</td>
<td>Primary</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>NS</td>
<td>Sigmoid</td>
<td>Primary</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>NS</td>
<td>Sigmoid</td>
<td>Primary</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>NS</td>
<td>Rectosigmoid</td>
<td>Primary</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>NS</td>
<td>Rectum</td>
<td>Primary</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> S, secretor; NS, Nonsecretor.

accessible to contents of the lumen, together with the subter-
minal location of this determinant on the complex molecule,
may provide some resistance against enzyme degradation.

The absence of detailed comparative structural studies in
human proximal and distal colon makes it difficult to resolve
the apparent paradox of proximal blood group loss on the one
hand, and distal blood group gain on the other. Accumulation
of glycolipid precursors (35) may account for this effect. How-
ever, a significant number of tumors demonstrated no apprecia-
table change from normal in their blood group expression. These
cases cannot be distinguished from the remainder on any clear-
cut clinical or histological basis, as shown in our study. A
definitive future role for blood groups as histological aids in
colon cancer diagnosis and prognosis will depend upon further
regional chemical dissections of their carrier molecules, their
possible alterations following tumor transformation, and their
specific interrelationships with known tumor-associated mole-
cules. Tumor structure-specific reagents could then possibly
be made available for precise immunohistochemical diagnosis.

Inappropriate expression of blood group antigens in gastroin-
testinal cancer has been described in some series of cases (21,
22) but not in others (20). Most instances of this phenomenon
have occurred in gastric and colonic malignancies of individuals
of erythrocyte group O or B in whose tumors A or A-like
antigen was expressed (38–40). Occasionally, apparent nonge-
netic B and human lymphocyte antigen expression have been
reported (22). Explanations for this behavior could relate to
cancer-altered genes, changes in specificity of blood group
enzymes, or competition for limiting substrates between the
latter and abnormally expressed enzymes. On the other hand,
tumor-transformed structures may at times yield A-like reac-
tions in the presence of certain anti-A reagents (1). Recent
studies using panels of monoclonal anti-A antibodies may clar-
ify this apparent paradox. Thus, Gooi et al. (41) found several
patterns of reactions using this approach, for example, (a) broad
specificities against mono- and difucosyl A on type 1 and 2
chains, (b) restricted specificities against combinations of these
structures, (c) reactions with oligosaccharides containing ter-

netal GalNAc in an afucosyl sequence producing A-like pat-
tterns unrelated to blood group A-gene specified NGalNAc
transferase. A monoclonal anti-A-like reagent has been de-
scribed which produces cross-reactions with Tn blood group
antigens (42) whose terminal GalNAc would be synthesized by
an enzyme unrelated to A transferase (43). Although incom-
patible "real A antigen" has been claimed based upon demon-
stration of an A-transferase in a tumor expressing A antigen,
additional studies using defined fucosyl and afucosyl acceptors
would be necessary to substantiate this finding (44).

In contrast to other investigators (21), we did not find inap-
propriate expression of Le<sup>b</sup> in nonsecretors. However, there
were three instances of compatible ABH expression in tumors
of the proximal colon in nonsecretor patients. The expression
of Le<sup>b</sup> in nonsecretors as found by others or the expression of
ABH in nonsecretors as observed in the present study, has been
suggested to occur as the result of the activation during tumor
transformation of a quiescent α-fucosyl transferase gene (23).

The differences between our results and those of other inves-
tigators may derive from several factors, including variations in
tissue processing and fixation, use of monoclonal versus poly-
clonal antibodies, reagent incubation times and temperatures,
labeling systems, and thresholds for categorizing tissues as
positive or negative for antigen. From some of the studies cited
above it would seem that inappropriate blood group expression
in particular, may be more susceptible to definition by mono-
clonal reagents than by polyclonal reagents or lectins. This
could be true in some circumstances because hybridoma-defined
reagents can define subtle molecular changes (45) more readily
than polyclonals which possess multiple specificities and affin-
ities. However, specificity problems can occur with monoclonal
antibodies as well, and they have been shown to participate in
unexpected cross-reactions involving dissimilar antigens (46).
Inappropriate blood group expression in gastrointestinal car-
cinoma has been reported using both polyclonal (38, 47) and
monoclonal antibodies (21, 22) but lack of such expression has
likewise been reported using both types of reagents (20, 48).
The reasons for such discrepancies are unclear, and may relate
to factors as previously described rather than to reagent selec-
tion. The increasing availability of hybridoma-derived antibo-
dies invites their future comparisons with standard reagents.

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