Structural Variations of Blood Group A Antigens in Human Normal Colon and Carcinomas

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ABSTRACT

The blood group A determinant is carried by four basic carrier carbohydrate chains. This paper reports the expression of blood group A variants, as determined by immunohistology with highly specific monoclonal anti-A antibodies, in 18 adenocarcinomas of the distal colon, 4 specimens of normal proximal mucosa from group A persons, and 5 specimens of fetal colonic mucosa. Monoclonal antibodies directed to type 1 chain A, type 1 chain ALeb, type 2 chain A, type 2 chain ALeb, and type 3 chain A (repetitive A) were used. In normal mucosa, type 1 chain A and ALeb were expressed in proximal regions. Type 1 chain A was expressed in columnar cells, whereas type 1 chain ALeb was found in goblet cells. Type 2 and type 3 chain A structures were not found in normal adult mucosa. All types of A antigens were detected in adenocarcinomas from the distal colon as well as in normal fetal mucosa. In fetal mucosa, type 1 chain A and ALeb antigens and type 3 chain A antigens were expressed in columnar cells, whereas type 2 chain A and Le' and type 1 chain ALeb antigens were found in goblet cells. The results indicate that blood group A antigens with type 1, 2, and 3 carriers are present in fetal mucosa and adenocarcinomas of distal colon, while epithelial mucosa of normal adult colon is characterized by the exclusive expression of type 1 chain A antigens.

INTRODUCTION

Blood group ABO(H) antigens are the major human alloantigens expressed not only in erythrocytes but also in many other cell types and in tissue and secretions throughout the body, particularly in gastrointestinal, colorectal, urogenital, and bronchopneumonal epithelia and their secretions (1). In the digestive tract, they are present in the cell membranes of oral mucosal and esophageal squamous epithelia, whereas in the mucosa of the stomach, small intestine, and proximal part of the colon, they are mainly present in the goblet cells and the cytoplasm of the columnar epithelial cells (2, 3). During neoplastic transformation, blood group antigen expression changes profoundly (4). Thus, neoplastic cells may “lose” certain blood group antigens that are expressed by their normal counterparts, or they may acquire new antigens that are not normally expressed (4, 5).

Most studies of blood group antigen expression in tissue have been performed with conventional polyclonal blood group test sera or monoclonal antibodies with wide specificity that do not provide detailed structural information of the antigens (4, 6). Increasing evidence, obtained both by chemical and immunochemical means, points to the significance of two major factors influencing the expression of blood group antigens. First, changes in core or carrier chain assembly are associated with development and transformation; branching status (blood group II), and carrier chains of type 1 and 2 are good examples (for structures, see Table 1) (4, 6, 7). Second, modifications through fucosylation and sialylation have been shown to affect expression of blood group antigens and to generate most of the known tumor-associated carbohydrate antigens. Typical examples of tumor-associated antigens in gastrointestinal and colorectal lesions are fucosylated mono- and multimeric Le', Le' (4, 8–12), and sialylated Le' and Le' structures (4, 8–12).

Blood group ABH antigen expression in colonic epithelia and tumors has been extensively investigated (12–15). These studies have shown that the antigens are present only in the proximal part of normal adult colon, whereas they are found throughout fetal colon. Generally, blood group antigen expression is maintained in adenocarcinomas of the colon and, importantly, tumors originating from the distal part have been found to reexpress ABH antigens, thus suggestive of the appearance of oncodevelopmental antigens. As it is unknown whether the ABH antigens of malignant cells are of the same structural variety as those found in fetal and/or normal adult cells, it is of special interest to determine the structure of the ABH antigens reexpressed in distal colon tumors.

With the use of a set of monoclonal antibodies directed to various structural variants of blood group A antigens (Table 1), we were able to study A antigen polymorphism in detail, including carrier chain differences and modifications through fucosylation. In the present paper, we describe an immunohistological study of normal adult colon, fetal colon, and adenocarcinomas of the distal colon using this set of well-defined monoclonal antibodies. The results show that blood group A antigens reexpressed in tumors from the distal colon are structurally similar to those from fetal colon, but distinctly different from those of normal adult proximal colon.

MATERIALS AND METHODS

Patients and Tissue. Tissue from a consecutive series of 18 patients belonging to blood group A (9 men and 9 women; mean age, 71.2 years) who had undergone surgery for adenocarcinoma of the sigmoid colon (9 patients) and rectum (9 patients) at Kommunehospitalet, Copenhagen, during the period 1976–1979, were studied. Lewis and secretor blood group status was not available. In the selection of the filed paraffin blocks, special attention was paid to obtain representation of both normal colonic mucosa (resection line) and adenocarcinoma from each patient. The study also included normal mucosa from the fecal of four blood group A individuals (mean age, 64 years) treated for non-malignant diseases, and fetal colonic tissue from five blood group A fetuses aborted at 8–12 weeks of gestation. The five were selected from 14 after positive staining with anti-A antibodies. In specimens of fetal colon, distinction between proximal and distal colon was not possible, and blood group information was not available. All tissues were placed in 10% (w/v) formaldehyde in 0.1 M sodium phosphate buffer, pH 7.2, immediately after removal and fixed for 24 h, followed by embedding in paraffin by routine histological methods. Fetal tissues were removed at 1–24 h after abortion.

Antibodies and Staining Method. Blood group A antigen was demonstrated in the tissue by an indirect immunofluorescence staining technique. Five monoclonal anti-A antibodies with different specificities were used as primary antibodies: (a) AH21 recognizes only monofucosylated blood group antigen A with type 1 chain structure (16); (b)
### Table 1  Structure of A variants with regard to carrier carbohydrates, and the specific antibodies defining each structure

<table>
<thead>
<tr>
<th>Structure</th>
<th>A variant</th>
<th>Monoclonal antibodies defining the structure</th>
<th>Ref.</th>
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</thead>
</table>
| Type 1 chain  
GalNAc1→3Galβ1→3GlcNAcβ1→3Galβ1→R  
| ALeα | AH21 IgM | 16 |
| 2   |            |                                             |      |
| Fuca1 |            |                                             |      |
| GalNAc1→3Galβ1→3GlcNAcβ1→3Galβ1→R  
| ALeα | HH3 IgG2a | 17 |
| 2   |            |                                             |      |
| 4   |            |                                             |      |
| Fuca1 | Fuca1 |                                             |      |
| Type 2 chain  
GalNAc1→3Galβ1→4[GlcNAcβ1→3Galβ1→R  
| ALeα | HH2 IgG3 | 17 |
| 2   |            |                                             |      |
| Fuca1 | n = 1, 2, or 3 |                                        |      |
| GalNAc1→3Galβ1→4GlcNAcβ1→3Galβ1→R  
| ALeα | HH4 IgG3 | 18 |
| 2   |            |                                             |      |
| 3   |            |                                             |      |
| Fuca1 | Fuca1 |                                             |      |
| GalNAc1→3Galβ1→4GlcNAcβ1  
| ALeα | HH4 IgG3 | 18 |
| 2   |            |                                             |      |
| Fuca1 | n = 1 or 2 |                                        |      |
| Type 3 chain A (A specific)  
GalNAc1→3Galβ1→4[GlcNAcβ1→3Galβ1→R  
| TH1 IgG2a | 19 |
| 2   |            |                                             |      |
| 2   |            |                                             |      |
| Fuca1 | Fuca1 |                                             |      |
| Type 4 chain A  
GalNAc1→3Galβ1→3GlcNAcβ1→3Galβ1→R  
| HH5 IgG3 | 18 |
| 2   |            |                                             |      |

### Table 2  Staining pattern of colorectal tissue and adenocarcinomas of distal colon

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
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<tr>
<td>Fetal columnar cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fetal goblet cells</td>
<td></td>
<td>+</td>
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<tr>
<td>Adult proximal columnar cells</td>
<td>+</td>
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<tr>
<td>Adult proximal goblet cells</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Adult distal (resection line)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adenocarcinoma (distal part)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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**Summary**  
(18/18) (18/18) (4/18) (11/18) (7/18)

### Patient

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<td>Adult proximal columnar cells</td>
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<td>Adult distal (resection line)</td>
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* Symbols in parentheses, weakly and partially positive.

Fig. 1. Normal proximal adult colonic mucosa with diffuse staining of monofucosylated type 1 chain A antigen in columnar absorptive cells. Bar, 50 μm. Stained with AH21 antibody, × 160.
HH3 recognizes difucosyl blood group A antigen with type 1 chain structure (17); (c) HH4 recognizes monofucosylated type 2 chain A, but cross-reacts to some degree with difucosylated type 2 chain A (18); (d) HH2 recognizes difucosyl blood group A antigen with type 2 chain structure (17); and (e) TH1 recognizes type 3 chain A (repetitive A antigen), which is essentially an A antigen extended from a type 2 chain A structure (19). The chemical structures of these antigens are shown in Table 1. Production and characterization of these antibodies have previously been described (16–19).

The secondary antibody was a rabbit anti-mouse immunoglobulin conjugated with fluorescein isothiocyanate (Dako, Copenhagen). The staining procedures have been described elsewhere (20). In brief, rehydrated paraffin sections were incubated with the primary antibody for 20 h at 4°C, washed three times for 5 min in phosphate-buffered saline, pH 7.2, and incubated with fluorescein-conjugated rabbit anti-serum to mouse immunoglobulin (Dako) for 1 h at 20°C. To allow comparison between staining reactions, serial sections of each specimen were stained with different monoclonal antibodies. Control reactions consisted of (a) staining with conjugate alone; (b) substitution of the primary antiserum with culture supernatant from the myeloma cell line (SP/2-0) used for hybridization; (c) substitution with mouse monoclonal antibody of irrelevant specificity (OKT4); and (d) substitution of primary antibody with antibody to blood group B.

RESULTS

The control reactions did not result in any staining. All anti-A antibodies stained enterocytes, and considerable differences in binding patterns were observed. A survey of the staining is shown in Table 2.

Normal Adult Proximal Colon

Type 1 Chain A. AH21, which recognizes monofucosyl type 1 chain A, showed a diffuse cytoplasmic staining of columnar (absorptive) cells, but not of goblet cells (Fig. 1) in normal adult mucosa from the cecum. HH3, which recognizes difucosyl type 1 chain A, stained goblet cells, with only a slight granular staining in the cytoplasm of columnar (absorptive) cells (Fig. 2). Thus, a distinct difference in distribution of mono- and difucosylated type 1 chain A antigens in normal proximal colon was found.

Type 2 and 3 Chain A. The antibodies HH4 (defining monofucosyl type 2 chain) and HH2 (defining difucosyl type 2 chain) did not stain any of these cells, indicating the absence of type 2 chain A antigens in normal adult colon. Likewise, no staining of these cells was found with antibody TH1, which reacts with extended A structure (type 3 chain A).

Normal Adult Distal Colon

Colon mucosa of specimens from sigmoid colon and rectum showed no staining with any of the antibodies.

Adenocarcinomas of Distal Colon

A detailed summary of the staining of adenocarcinomas is shown in Table 2.

Type 1 Chain A. Antibody AH21 (defining monofucosyl type 1 chain) showed diffuse staining of cytoplasm and no staining of mucus in goblet-like cells similar to normal proximal epithelium (Fig. 3). HH3 (defining difucosyl type 1 chain) stained only a few tumor cells in some specimens, but stained groups of cells in other tumors (Figs. 4 and 5). Mucus in tumor cells was also stained.

Type 2 and 3 Chain A. In general, the antibodies HH4 (defining monofucosyl type 2 chain) and HH2 (defining difucosyl type 2 chain) stained cells which were stained with HH3, although HH3 stained more cells (Figs. 5 and 6). In most areas, a fine evenly distributed granular staining of the cytoplasm was seen, but in some areas an intense fluorescence was also observed at the cell membranes. Positive staining with TH1 (which recognizes type 3 chain A) appeared as a granular staining in the cytoplasm (Fig. 7). Adenocarcinomas from distal colon were therefore found to express type 1, 2, and 3 chain A antigens, in contrast to normal adult proximal colon.
BLOOD GROUP A ANTIGENS IN COLORECTAL CANCER

Fig. 4. Difucosylated type 1 chain A antigen at the plasma membranes of cells of an adenocarcinoma of the distal colon. Bar, 50 μm. Stained with HH3 antibody, × 160.

Fig. 6. Difucosylated type 2 chain A antigen in an adenocarcinoma of the distal colon (neighboring section to that shown in Fig. 5). Bar, 50 μm. Stained with HH2 antibody, × 160.

Fig. 5. Monofucosylated type 2 chain A antigen in an adenocarcinoma of the distal colon. Bar, 50 μm. Stained with HH4 antibody, × 160.

Fig. 7. Adenocarcinoma of the distal colon with granular cytoplasmic staining of type 3 chain antigen A. Bar, 50 μm. Stained with TH1 antibody, × 100.

Fetal Colon

Staining of fetal tissue was evident in 5 of the 14 cases, and these were regarded as blood group A individuals. Except for TH1, all antibodies stained only scattered group of cells. The positive cases were stained with all five anti-A antibodies. Proximal and distal fetal colon could not be distinguished, but serial sections through the entire embedded material showed no variation in staining with the different antibodies. This is in agreement with other authors who have found no major regional variation in the distribution of type 1 and type 2 chain structures, namely, Le⁺, Le⁺, Le⁺, and Le⁺ structures (8, 12, 21–23).

Type 1 Chain A. AH21 (defining monofucosyl type 1 chain) produced a fine granular perinuclear staining as well as a distinct cell membrane staining, but did not stain goblet cells (Fig. 8). HH3 (defining difucosyl type 1 chain) produced a focal and discrete cytoplasmic staining of columnar cells and goblet cells (Fig. 9).
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Fig. 8. Fetal colon mucosa with distinct staining of monofucosylated type 1 chain A antigen at the luminal cell membrane. Bar, 50 μm. Stained AH21 antibody, × 160.

Fig. 9. Fetal colon mucosa with focal staining of difucosylated type 1 chain A antigen in goblet cells. Bar, 50 μm. Stained with HH3 antibody, × 160.

Type 2 and 3 Chain A. HH4 (defining monofucosyl type 2 chain) and HH2 (defining difucosyl type 2 chain) stained only goblet cells, which showed weak granular staining (Fig. 10). This staining was, however, more pronounced with HH3 than with HH4 and HH2. TH1 (which defines extended A) stained all columnar cells with comma-shaped fluorescent granules in the cytoplasm and did not stain goblet cells, in contrast to other type 2 chain A structures (Fig. 11). These results clearly indicate the presence of all type 1, 2, and 3 chain A antigens in fetal colon in contrast to normal adult colon but similar to adenocarcinomas.

DISCUSSION

The blood group A determinant is a trisaccharide GalNACα1→3(Fucα1→2)Galβ1→R (24) that is carried by different basic core structures as shown in Table 1. Differences in carrier core structure, but not in the antigen determinant itself, provide the basis for the presence of structurally and immunologically distinct variants of A antigens investigated in the present study. The antibodies were selected to differentiate A determinants carried by different lacto-series structures: (a) type 1 chain, Galβ1→3GlcNAc; (b) type 2 chain, Galβ1→4GlcNAc; and (c) type 3 chain, which is type 2 chain A with a repetitive A epitope (Table 1). These are believed to be the main carriers of ABH antigens in gastrointestinal tissue, although other carrier types exist, such as O-linked type 3 chain (mucin type) and type 4 chain ( globo series) (25, 26).

Tissue-specific expression of carbohydrate carrier chains has been well documented (7). The present study supports previous findings (7, 27) that blood group antigens in normal adult colon mucosa are primarily carried on type 1 chain structures, since neither type 2 or type 3 (repetitive) structures were found.

Several studies have indicated regional variation in expression of blood group antigens in normal adult colon mucosa (2, 12, 13, 23). In the present study, we have shown that there are distinct differences in cellular distribution of monofucosylated and difucosylated type 1 chain A structures. Whereas monofucosylated type 1 chain A antigen was evident in columnar cells but not in goblet cells, difucosylated type 1 chain A (Aleβ) was found primarily in goblet cells. As the internal fucosylation of the Aleβ structure is thought to be catalyzed by the Lewis gene-encoded fucosyltransferase, it is likely that goblet cells and columnar cells differ in the expression or activation of this transferase. Interestingly, tumor tissue appeared to maintain the differentiation-dependent expression of type 1 chain A structures. Another difference between columnar and goblet cells was observed in fetal colon, in which type 2 chain A antigens were found only in goblet cells, in contrast to type 3 chain A (extended type 2 chain A), which was only found in columnar cells.

The distribution of mono- and difucosylated type 2 chain A antigens in the tumor tissue was similar to that of Aleβ, although there were more cells expressing Aleβ. The expression of type 2 chain A antigen in tumor tissue, but not in normal colon, may represent a change in the core structure of the carbohydrates in relationship to tumor development. This correlates with previous findings that type 2 chain structures such as Leα, Leβ, and sialylated Leα are highly accumulated in colonic adenocarcinomas and essentially absent in normal adult epithelium, although recent immunohistochemical studies have indicated detectable type 2 chain Leα in normal proximal colonic epithelium, particularly of the crypt area (4, 8, 12, 23). The absence of type 2 chain A antigens in this epithelium is probably due to lack of type 2 chain H precursor substances, since neither Leα nor Leβ are good substrates for the A enzyme.

Changes in certain cell surface carbohydrate structures during embryonic development have been clearly established (2, 21, 22), and many of these structures, minimally expressed in adult tissue, have been found to be coexpressed in fetal and tumor tissue (21, 22, 28, 29); thus, these antigens have been described as oncodevelopmental antigens as classically defined (30).
Therefore, in the present work, we correlated the expression of blood group A structures in adenocarcinomas of the colon with the expression of the same structures in fetal colonic mucosa. We found the same blood group A variant antigens, defined by monoclonal antibodies AH21, HH3, HH4, HH2, and TH1, in fetal colon as well as in tumors, although the histological distribution of type 1 chain monofucosylated A was limited in adult normal mucosal cells. The basis for this change in expression of carrier chains in normal adult colon and in fetal colon and tumors is not clear. These differences could be in the synthesis of the respective carrier chains; however, carrier chains could also be modified by other glycosyltransferases, especially sialyltransferases (8). In summary, the present study has established the differential expression of blood group A variants in the distal region of normal adult colonic epithelia as compared with that of fetal colonic epithelia and colonic carcinomas, and showed clear structural characteristics of the reexpressed blood group A antigens in carcinomas of the distal colon. The study also provides evidence that this reexpression can be regarded as an oncodevelopmental change.

REFERENCES

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