Monoclonal Antibody Localization of A and B Isoantigens in Normal and Malignant Fixed Human Tissues

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The expression of human blood group A and B isoantigens in normal and malignant tissues from stomach, colon, and pancreas was analyzed in an immunoperoxidase assay using monoclonal antibodies specific for these isoantigens. Appropriate isoantigen expression was demonstrated in the normal epithelium from the stomach, pancreas, and proximal but not distal colon of blood group A, AB, or B patients. Half of all gastric carcinomas and of proximal colon carcinomas showed complete loss of isoantigen, whereas the adjacent mucosa in these cases continued to express appropriate isoantigen. Isoantigen expression was completely lost in only 13% of pancreatic carcinomas tested. Neither A nor B isoantigen was detected in normal epithelium from the distal colon. By contrast, 85% of carcinomas derived from this site showed reexpression of isoantigen. Inappropriate expression of A isoantigen was detected in pancreatic carcinomas (2/5) but not in gastric or colon carcinomas (0/21). Inappropriate expression of B substance was not detected in any tissue (0/38). Interestingly, differential binding of antibodies to Type 1 versus Type 2 and/or difucosyl versus monofucosyl blood group B substances was manifested by differences in intensity of staining for endothelium and red blood cells. (Am J Pathol 1984, 117:451–461)

BLOOD GROUP ABH antigens comprise a group of carbohydrate cell surface markers found not only on erythrocytes but in a wide variety of epithelial cells. There has been increasing interest in the changes in blood group ABH antigen expression in various epithelial malignancies. These changes include deletion of A,B determinants,[1,2] accumulation of precursor substances,[3] increased or neosynthesis of certain glycolipids with Lewis or A,B determinants,[4–6] synthesis of incompatible blood group antigens[7–12] and synthesis of sialylated substances bearing blood group carbohydrate chains.[13–15]

Immunohistologic characterization of blood group antigens in tissue has relied upon the use of conventional polyclonal antisera in the specific red cell adherence test,[1,2,4,5,16,17] immunofluorescence assays[18–20] or immunoperoxidase (IP) assays.[21] The introduction of monoclonal antibodies (MAbs) that specifically detect blood group antigens has greatly facilitated standardization of immunohistochemical methods and enables valid comparisons with the numerous other tumor markers defined by MAbs. The MAbs used in this study have been well characterized.[22,23] The aim of this work was to describe their immunoreactivity in tissue with the use of an immunoperoxidase (IP) assay.

Materials and Methods

Tissues

Paraffin-embedded Bouin's fixed tissue from the surgical pathology file of the Hospital of the University of Pennsylvania was used. Representative sections from

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gastrectomy specimens for ulcer disease or endoscopic biopsies from patients with no clinical or pathologic diagnosis of malignancy, colectomy specimens for diverticulitis, and open pancreatic biopsies for chronic pancreatitis were used as "normal" tissues. From specimens resected for malignant disease, sections of both the tumor and adjacent mucosa were obtained when possible. Histologically normal tissues from these specimens are referred to as adjacent normals. Blocks were stored from 6 months to 5 years; no correlation between storage time and binding of MAb was observed.

Glycolipid Fractions

The total nonacid glycolipid fractions from human blood group A erythrocytes were prepared with the use of the toluene-flotation procedure[24] and as previously described.[25] Preparation of the total neutral glycolipid fraction from meconium was made as described earlier.[26] The Forssman glycolipid and A7-2 glycolipid (Table 1) were prepared in pure form from dog intestine[30] and were characterized by mass spectrometry and NMR[32,33] as a permethylated derivative. The total neutral glycolipid fraction from human erythrocyte from a blood group B individual was kindly supplied by Dr. B. E. Samuelsson, University of Gothenburg, Gothenburg, Sweden.

MAbs

Antibodies PA15-2 (IgG3), PA23-48 (IgG1), PA66-18 (IgG1), and PA83-52 (IgA) were derived from mice immunized with pancreatic carcinoma cell line Capan-2; binding characteristics to various glycolipids and determination of blood group specificities have been described elsewhere in detail.[22] These antibodies were previously named $E_1$15-2, $E_1$23-48, $E_1$66-18, and $E_2$83-52, respectively. In live-cell radioimmunoassay (RIA) these antibodies bind to red cells of blood group B but not A or O individuals. In solid-phase RIA, antibody PA15-2 binds approximately 100 times more strongly to Type 2 B glycolipids (B6-2, B7-2) than Type 1 B glycolipids (B6-1, B7-1) (for explanation of abbreviations and trivial names of glycolipids, see Table 1). MAb PA83-52 binds with about equal strength to both Type 1 and Type 2 B glycolipids. Antibodies PA23-48 and PA66-18 bind to both chains but with approximately 10 times greater strength for Type 2 chains. The data for all antibodies regarding the reactivity to Type 1 chains also show a preference for difucosyl structure over monofucosyl structure, especially so because they all react weakly with the Le^b glycolipid.[22]

Antibody 33-25-17 (IgM) was derived from mice immunized with red blood cells from a blood group A patient; in live-cell RIA, the antibody binds to red cells of blood group A but not B or O individuals.[23] A characterization of the carbohydrate binding specificity of antibody 33-25-17 was carried out (see Results and Table 1).

Immunoperoxidase Procedure

The IP assay used was a modification described previously[6,34] of the preformed avidin-biotin-peroxidase complex technique developed by Hsu et al.[35] Five- to six-micron sections of paraffin-embedded Bouin's fixed tissue were deparaffinized and hydrated through xylene and graded alcohols. Sections were incubated for 15 minutes in 0.3% H_2O_2 in methanol to block endogenous peroxidase. Slides were rinsed in phosphate-buffered saline (PBS), incubated with normal horse serum for 20 minutes, and sequentially incubated for 30 minutes at room temperature with undiluted tissue culture medium containing mouse MAb (PA83-52, PA15-2, PA23-48, PA66-18) or ascitic fluid diluted 1:400 (33-25-17) in PBS, biotinylated anti-mouse immunoglobulin (1:200) (Vector Laboratories, Calif), and avidin-biotin-peroxidase ("ABC" complex) (Vector Laboratories) (10 μl/ml avidin, 2.5 μg/ml biotinylated peroxidase). PBS washes followed each incubation. Finally, sections were incubated for 5 minutes with 0.05% diaminobenzidine (Sigma, St. Louis, Mo) containing 0.01% H_2O_2

Table 1—Some Glycolipids Discussed in the Present Work

Abbreviation for glycolipid*	Trivial names*	Tissue origin	Reference for structure
GalNAcα1→3Gal(2←1αFuc)β1→3GlcNAcβ1→3LacCer	A6-1	Human meconcium	26,27
GalNAcα1→3Gal(2←1αFuc)β1→4GlcNAcβ1→3LacCer	A6-2	Human erythrocyte	28
GalNAcα1→3Gal(2←1αFuc)β1→3GlcNAc(4←1αFuc)β1→3LacCer	A7-1	Human meconium	26,27
GalNAcα1→3Gal(2←1αFuc)β1→4GlcNAc(3←1αFuc)β1→3LacCer	A7-2	Dog intestine	29
GalNAcα1→3Gal(2←1αFuc)β1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3LacCer	A8-2	Human erythrocyte	28
GalNAcα1→3GalNAcβ1→3Galα1→4LacCer	Forssman glycolipid	Dog intestine	30

* The abbreviations for glycolipids follow the IUPAC-IUB recommendations.[31] The trivial names for glycolipid antigens indicate blood group (letter), number of sugars (Arabic numeral), and type of carbohydrate chain (Arabic number).

and counterstained with hematoxylin. Mouse myeloma-P3x63Ag8 supernatant or ascitic fluid was used as a negative control.

Neuraminidase Treatment

Pretreatment of a 5-μ paraffin-embedded tissue sections with neuraminidase has been described elsewhere.[34] Briefly, slides were incubated with 0.5 units type 6 clostridial neuraminidase (Sigma, St. Louis, Mo), diluted in 0.2 ml PBS, pH 6.0, and then incubated with MAb. As controls, slides were incubated in PBS, pH 6.0, without neuraminidase, then stained in the usual IP procedure.

Ablation of binding of 19-9 MAb to SW1116 colorectal cells was used as a positive control.[34]

Blood Grouping

The blood groups of patients were obtained from the files of the blood bank as determined by standard hemagglutination methods using human antisera and *Ulex europaeus* lectin.[36]

Glycolipid Binding Assays

Binding of MAb to glycolipid fractions in solid-phase RIA and chromatogram binding assay was performed as described in detail elsewhere.[22]

Results

Characterization of MAb 33-25-17

Antibody reactivity to blood group A active glycolipids with Type 2 chain was tested in a chromatogram binding assay[22] with the use of a total nonacid glycolipid fraction from human erythrocytes from a blood group A individual. Human erythrocytes are known to contain blood group active glycolipids with Type 2 chain only.[37] In Fraction 1 (Figure 1B), two glycolipid species react strongly with the antibody. Their R_F values correspond to the bands in Fraction 1 (Figure 1A), which are known to contain the A6-2 and A8-2 glycolipids.[28] An equal fraction prepared from a blood group B individual was negative for staining with the antibody in the chromatogram binding assay (data not shown).

Antibody reactivity to blood group A active glycolipids with Type 1 chain was tested on a total glycolipid fraction from a meconium of a blood group A individual Fraction 2 (Figure 1B), because meconium is known to contain mainly Type 1 chains.[29] Strong reactivity was found at an R_F value that corresponded

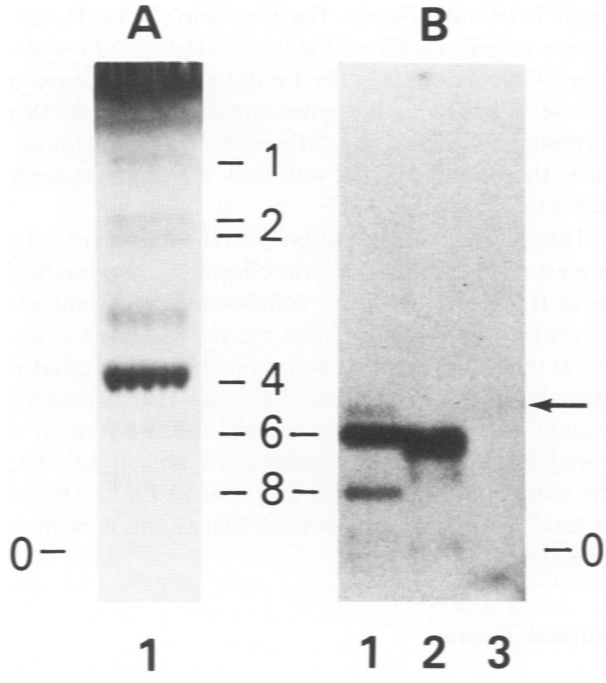

Figure 1 – Thin-layer chromatogram (**A**) and autoradiogram (**B**) of chromatogram binding assay obtained by overlapping the chromatogram with ascites of antibody 33-25-17 diluted 400 times and with goat ^{125}I-labeled anti-mouse F(ab')$_2$. The chemical stain used in chromatogram A is the anisaldehyde reagent. The fractions assayed are 400 μg of total nonacid glycolipid fraction from human blood group A erythrocytes (Fraction 1), 40 μg of total nonacid glycolipid fraction from a meconium of a blood group A active individual (Fraction 2), and 200 ng of pure Forssman glycolipid (Fraction 3). The *arrow* indicates the R_F value for Forssman antigen. Origin of migration is marked with a O at both sides of photographs. The numbers between photographs indicate approximate numbers of sugars in glycolipids. Autoradiogram was developed for 11 hours. Other conditions were essentially as in Hansson et al.[22]

approximately with the A6-2 band in Fraction 1. This strong reactivity, with a slightly lower R_F value, is due to the presence of an A6-1 glycolipid with a more polar ceramide portion than that of the A6-2 in human erythrocytes.[26,27] Meconium is not known to contain any Type 1 glycolipid equivalent to the A8-2 in erythrocytes (ie, no A8-1 present in meconium); but in this particular meconium fraction, which is Le^a- and Le^b-positive, the glycolipid A7-1 (Table 1) should be present.[27] The faint staining seen with an R_F value slightly higher than the A8-2 in erythrocytes Fraction 1, could be this A7-1 glycolipid. These facts agree well with a complete lack of reactivity of the antibody with up to 500 ng of pure A7-2 glycolipid in the solid-phase RIA[22] (data not shown), indicating that the fucose linked to glucosamine might restrict antibody binding to A7-2 and perhaps also to A7-1 glycolipids. Also, faint reactivity is seen in the chromatographic interval of blood group A active glycolipids with more than 10 sugars containing branched carbohydrate chains,[28] which indicates that weaker reactivity to these larger glycolipids

ought to be insignificant. The conclusion is that the antibody seems to react well with the blood group A terminal GalNAcα1→3Gal(2←1αFuc), but when an extra fucose is added to the glucosamine internal to this terminal, or branching of the carbohydrate chain occurs, the binding of the antibody might be severely restricted.

The reactivity of the antibody to the Forssman antigen was also tested in both the chromatogram binding assay (Figure 1) and in the solid-phase RIA (data not shown). In both assays, weak reactivity started to occur at the 100-ng level, as exemplified in the chromatogram binding data fraction 3 (Figure 1B). Normally, a good antibody will start to bind to 0.1–1.0 ng of a pure glycolipid in these two assays.[22] This means that the binding of antibody 33-25-17 to A6-1 and A6-2 is at least 100 times stronger than that to the Forssman glycolipid.

Normal Tissues

To evaluate the specificity of antibodies and localization of isoantigens in normal gastrointestinal tissue, representative sections from stomach (12), pancreas (3), and colon (8) were tested. Neither A nor B isoantigen was detected on the mucosal or ductal epithelia of the six specimens from blood group 0 patients (4 stomach, 1 pancreas, 1 distal colon). Of the remaining 17 specimens comprising blood groups A (2 stomach, 1 pancreas, 2 colon), B (4 stomach, 5 colon), and AB (2 stomach, 1 pancreas) patients, the expected isoantigen was detected on the gastric epithelium, pancreatic ductal and acinar epithelium, and proximal colonic epithelium. Both isoantigens were detected in pancreatic and gastric epithelium from blood group AB patients (Table 2). Thus, with the exception of tissue derived from the distal colon, which expressed neither isoantigen, tissue phenotype as determined by reactivity of MAb corresponded to blood group phenotype determined by hemagglutination assay (Table 2).

Table 2—Reactivity of MAbs Detecting Blood Group Isoantigens to Normal Gastrointestinal Tissue in IP Assay—Correlation With Red Blood Cell Phenotype

Red blood cell phenotype of patients from whom tissue was tested*	A Isoantigen as detected by 33-25-17 (number positive/number tested)	B Isoantigen as detected by PA83-52, PA15-2, PA23-48 (number positive/number tested)
A	4/5†	0/5
AB	3/3	3/3
B	0/9	6/9†
O	0/6	0/6

* As determined by standard hemagglutination assay.
† Tissues not expressing isoantigens were from the distal colon.

Differential Staining of Endothelium and Erythrocytes

The differential reactivity among the four antibodies for Type 1 (Galβ1-3GlcNAc) or Type 2 (Galβ1-4GlcNAc) linkage, and for the difucosyl versus monofucosyl type of the B determinant (MAb PA83-52, PA15-2, PA23-48, PA66-18), was reflected in differences in MAb staining intensity of endothelium and erythrocytes in tissues of B or AB patients. MAb PA83-52 showed strong staining of endothelia and of erythrocytes in all cases, whereas binding of the other antibodies was variable. Early in the study, it was apparent that MAb PA83-52 showed prominent staining of endothelium independent of vessel caliber, and in all B or AB patients, whereas MAbs PA15-2, PA23-48, and PA66-18 in many patients showed either no staining or weak staining of capillaries or small arteries only. In general, staining of endothelium and red cells were parallel, ie, when endothelium stained positively, red cells were also positive (if red cells were present in the specimen). In patients in whom no staining of endothelium was seen with MAb PA23-48, PA66-18, or PA15-2, red cells were also negative.

To further characterize the differential binding to endothelium, we studied tissues from 31 patients who were blood group B (23) or AB (8). There was no appreciable difference in staining seen with the use of MAb PA66-18 or PA23-48; therefore, for further study, only PA23-48 was used. Serial sections of each tissue were incubated with MAbs PA83-52, PA15-2, and PA23-48; and binding to endothelium, red cells, and epithelium was evaluated. Several patterns of reactivity emerged (Table 3). In 14 of 31 patients, the difference in staining intensity was distinct, with ++ staining using MAb PA83-52 and no staining with MAb PA15-2 or PA23-48 (Figure 2). In 5 other patients, there appeared to be

Figure 2—Differential IP staining of endothelium (**A** and **B**) and erythrocytes (**C** and **D**) of a blood group B patient using different MAbs directed against B substances. A section of normal ileum incubated with MAb PA83-52 (**A**), which binds to both type 1 and Type 2 B substances, shows prominent staining of endothelium, whereas a section of ileum from the same patient incubated with MAb PA15-2 (**B**), which binds preferentially to Type 2 B-substances, shows no staining of endothelium in this patient. (×250) A section of bone marrow incubated with MAb PA83-52 (**C**) shows dark staining of mature erythrocytes, whereas no staining of these cells is observed in this particular patient when the section is incubated with MAb PA15-2 (**D**). (×400)

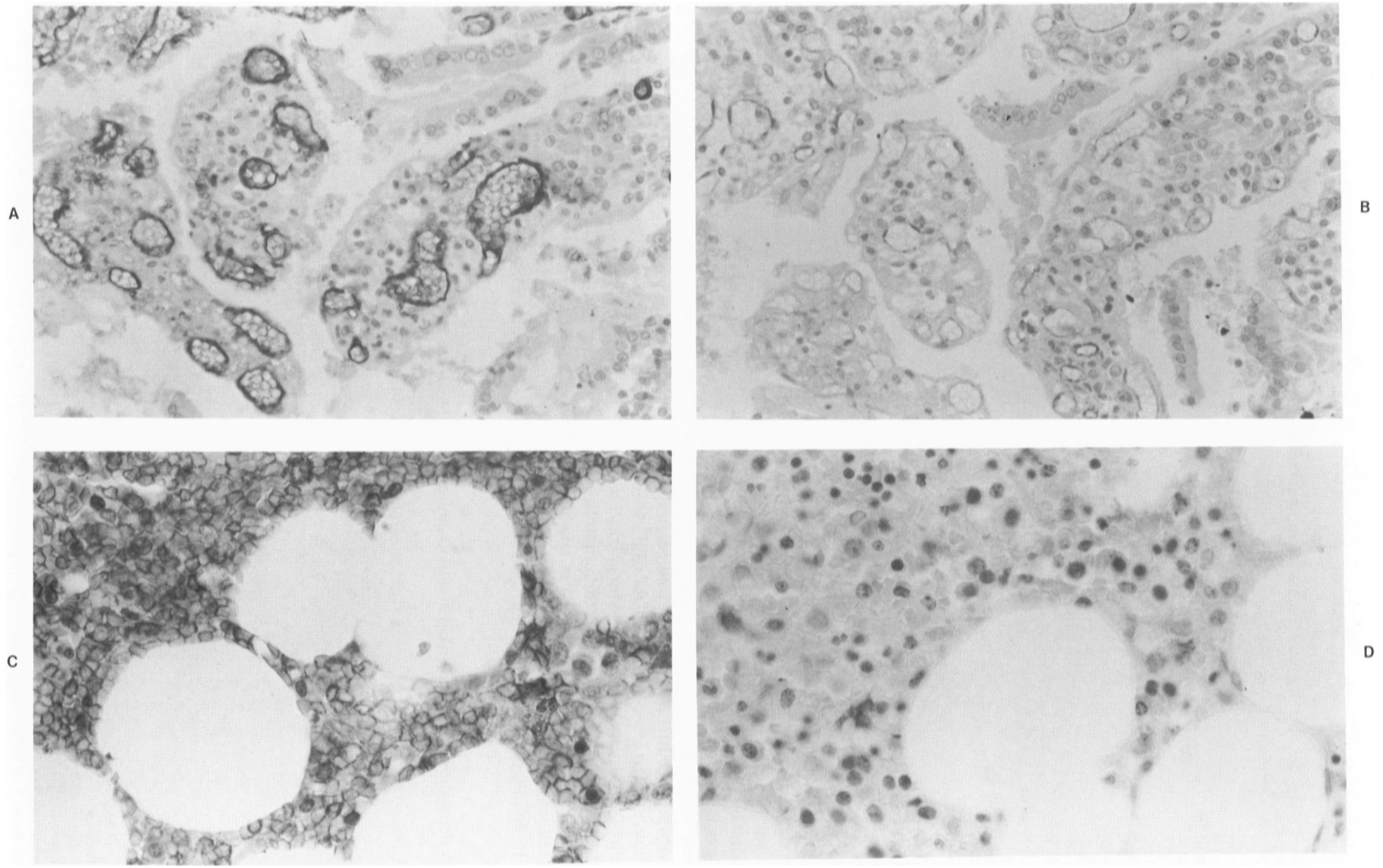
A
B
C
D

Table 3—Differential Staining for B Substance in Endothelium of Blood Group B or AB Patients With the Use of Different MAbs

Pattern of IP reactivity	Overall intensity of IP using MAb			Number of blood group B or AB patients showing pattern (total = 31) (%)	Number of malignant diagnoses/number of tissues showing pattern
	PA83-52	PA15-2	PA23-48		
Distinct difference	+ +	0	0	14 (45)	7/14
Quantitative	+ +	f + *	rare + †	5 (16)	1/5
Regional	+ +	+ + cap‡	+ + cap	1 (3)	1/1
No difference	+ +	+ +	+ +	11 (36)	9/11

* Focal (5–25%).
† Rare (1%).
‡ Capillary only.

some staining with PA15-2 and PA23-48, but staining intensity and percentage of endothelial cells stained were reduced. In 1 patient the distinction was clearly related to vessel caliber; ie, capillaries were strongly stained with each of the three antibodies, but large vessels were stained only with PA83-52 (Table 3). In 11 patients (36%), no difference in reactivity was noted. The proportion of malignant diagnoses was greatest (9/11) in the group showing no distinct differences in binding. These sections studied usually contained representative portions of both tumor and the immediately adjacent mucosa. There was no appreciable difference in reactivity of endothelium of vessels associated with tumor versus those associated with the adjacent mucosa. However, no tissue from other body sites taken from the patient was studied. The differential reactivity appeared to be independent of whether the patient was blood group B or AB. Antibody 33-25-17, which detects A substance, showed only very weak and rare staining of endothelium (and red cells) of all A or AB patients (0/34). The correlation of histochemical binding of endothelium to biochemical characteristics of the various MAbs is shown in Table 4.

Carcinomas

Gastric

A total of 15 gastric carcinomas were tested for isoantigen expression in immunoperoxidase (IP) assay with the different MAbs. No staining of the malignant or adjacent mucosal tissue for A or B antigen was observed in 7 blood group 0 patients, although there was equivocal staining of the carcinoma for A isoantigen and no

Table 4—Correlation of Histochemical and Biochemical Binding by MAbs Detecting A or B Glycolipids

MAb	Isotype	Binding in chromatogram or RIA assays: Preferential substances*	Binding in chromatogram or RIA assays: Preferential form of Type 1 chain	Binding in IP assay (positive for endothelium tested/number tested)
PA83-52†	IgA	B6-2 B7-2 B7-1	Difucosyl	31/31**
PA15-2†	IgG3	B6-2‖ B7-2	Difucosyl	17/31**
PA23-48†‡	IgG1	B6-2¶ B7-2	Difucosyl	17/31**
33-25-17§	IgM	A6-1 A6-2	Monofucosyl	0/34††

* For Table 1.
† Hannson et al.[22]
‡ Same as PA66-18.
§ See Results.
‖ Does bind to B7-1, but 100-fold less than B7-2 or B6-2.[22]
¶ Does bind to B7-1, but 10-fold less than B7-2 or B6-2.[22]
** Blood group B or AB patients.
†† Blood group A or AB patients.

Table 5—Expression of A or B Isoantigen in Gastrointestinal Malignancies as Determined by MAbs in IP Assay

Site (total)	Isoantigen	Blood group of patient*							
		A		AB		B		O	
		A†	B‡	A	B	A	B	A	B
Stomach (15)		1/2§	0/2	0/2	0/2	0/4	3/4	0/7‖	0/7
Colon (24)									
Proximal (7)		1/3	0/3	1/1	1/1	NT¶	NT	0/3	0/3
Distal (17)		7/8	0/8	2/2	1/2	0/3	2/3	0/4	0/4
Pancreas (17)		8/9	0/9	2/3	2/3	2/3	3/3	0/2	0/2

* As determined by standard hemagglutination assay.
† As detected by MAb 33-25-17.
‡ As detected by MAb PA83-52.
§ Number of malignant tissues staining with MAb/total number examined.
‖ One showed equivocal staining. See text.
¶ Not tested.

staining of the adjacent mucosa in 1 case (Table 5). Of the tissue from the remaining 8 patients with gastric carcinoma (blood groups A [2], AB [2], B [4]), specimens from 4 patients showed complete loss of blood group antigens (Table 5). In the tissue from blood group AB patients, neither isoantigen was detected (Table 5). Adjacent normal mucosa in all patients showed appropriate expression of isoantigens in epithelium, despite a loss of staining in malignant tissue. Three cases had metastatic lesions; all 3 lost expression of isoantigen in both the primary and the metastatic lesions.

Colon

A total of 24 colon carcinomas were evaluated: 17 arose in the distal colon, and the others arose in the proximal or transverse colon. Of the 7 patients who were blood group 0, no abnormal expression of A or B isoantigen was detected (Table 5). From the remaining 17 patients, 13 specimens were from the distal colon (blood group A [8], AB [2], B[3]), and 4 were from the proximal colon [blood group A [3], AB [1]). Complete loss of the expected isoantigen was seen in 2 of 4 carcinomas derived from the proximal colon; adjacent mucosa in both cases was positive for the appropriate antigen (Table 6). Figure 3 shows one of the proximal colon tissues that continued to express B antigen as detected in IP assay with MAb PA23-48.

Of the 13 carcinomas derived from the distal colon, 11 showed "reexpression" of the expected isoantigens (Table 6): 7 of 8 group A patients; 2 of 3 group B patients; and 2 of 2 group AB patients, one of which expressed both isoantigens, whereas the other expressed only A antigen (Table 5). As expected, blood group isoantigen was expressed in the tumor tissue and not in the adjacent mucosa derived from the distal colon (Figure 4) (Table 6). Expression in the adjacent normal

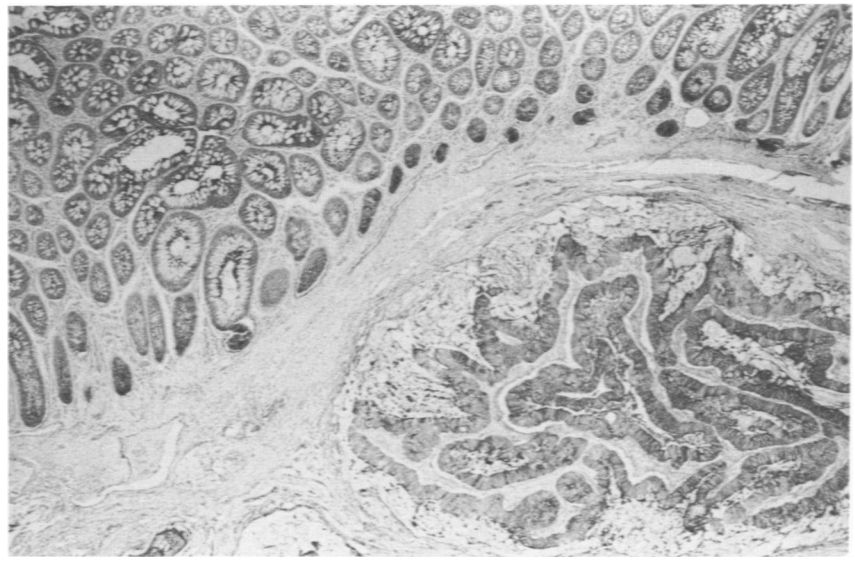

Figure 3—Carcinoma of cecum (proximal) (patient blood group AB). Strong IP staining of both carcinoma and adjacent "normal" mucosa with MAb PA23-48 demonstrates normal expression of B substance in proximal colon and retained expression in tumor. (×100)

Table 6—Expression of A and B Isoantigens in Normal and Malignant Tissue from Colon—Reactivity of MAb in IP Assay

Side from which tissue is derived	Blood group of patient*	Adjacent normal mucosa (Number showing positive staining for A or B isoantigen/total)	Tumor (Number showing positive staining for A or B isoantigen/total)
Proximal	A, AB, or B	4/4	2/4
	O	0/3	0/3
Distal	A, AB, or B	1‡/13	11§/13
	O	0/4	0/4

* As determined by standard hemagglutination assay.
† MAb 33-25-17 and MAb PA83-52 were used to detect A and B substances, respectively.
‡ Positive adjacent normal tissue from rectum.
§ In 1 AB patient, only A isoantigen was expressed in the tumor.

epithelium was detected in one case of carcinoma derived from the rectum.

Pancreas

Seventeen pancreatic carcinomas were evaluated. Neither of the tissues from blood group 0 patients showed staining of adjacent tissue or pancreatic carcinoma. In the remaining 15 patients (blood group A [9], AB [3], B [3]), blood group isoantigen was not expressed in only 2 cases (13%)—1 blood group A patient and 1 AB patient (Table 5). In all patients, adjacent normal tissue showed expression of appropriate isoantigens. Irregular expression of A substance was detected in the tumors of two blood group B patients (Table 5), as indicated by diffuse staining of the malignant cells with the anti-A MAb 33-25-17 (Figure 5).

Effect of Neuraminidase on Expression of Isoantigen

The effect of neuraminidase pretreatment on the expression of isoantigen was tested in 3 cases of gastric carcinoma in which the expected isoantigen was not expressed in the carcinoma but was expressed in the adjacent mucosa. The neuraminidase-treated tissue from these cases did not differ from controls in the amount of staining, which indicates that this enzymatic pretreatment does not unmask antigen.

Discussion

Blood group ABH determinants based on either Type 1 (Galβ1→3GlcNAc) or Type 2 (Galβ1→4GlcNAc) chain have been characterized from a number of gastrointestinal tissues,[38-42] whereas the glycolipids isolated from erythrocyte membranes have been found to have only Type 2 chains.[24,37,43,44]

The differential immunohistochemical staining of endothelia by the MAbs used in this study is an interesting correlate of the differential reactivity for Type 1 and/or 2 linkage and for different reactivity for monofucosyl and difucosyl Type 1 chains of these MAbs in solid-phase RIA and chromatogram binding assay. It is possible that the differences in reactivity seen in endothelia of tissue section are not related to the differences in specificity detected by the other assays. The differences detected immunohistochemically may rest on factors such as degree of branching of antigenic determinant, chain length, relative amount of carbohydrate antigen on glycolipid versus glycoprotein carrier, or differences in MAb affinity or isotype.[45] However, an interesting speculation is that these differences are due to the presence of difucosyl Type 1 chain carbohy-

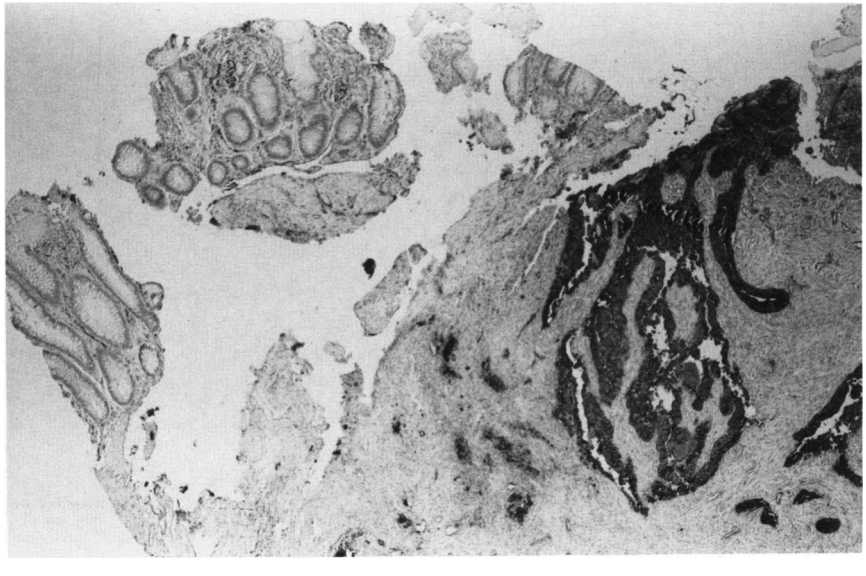

Figure 4—Carcinoma of sigmoid (distal) (patient blood group B). There is strong staining of the carcinoma but no staining of adjacent "normal" mucosa with MAb PA15-2, which detects B isoantigen. (×100)

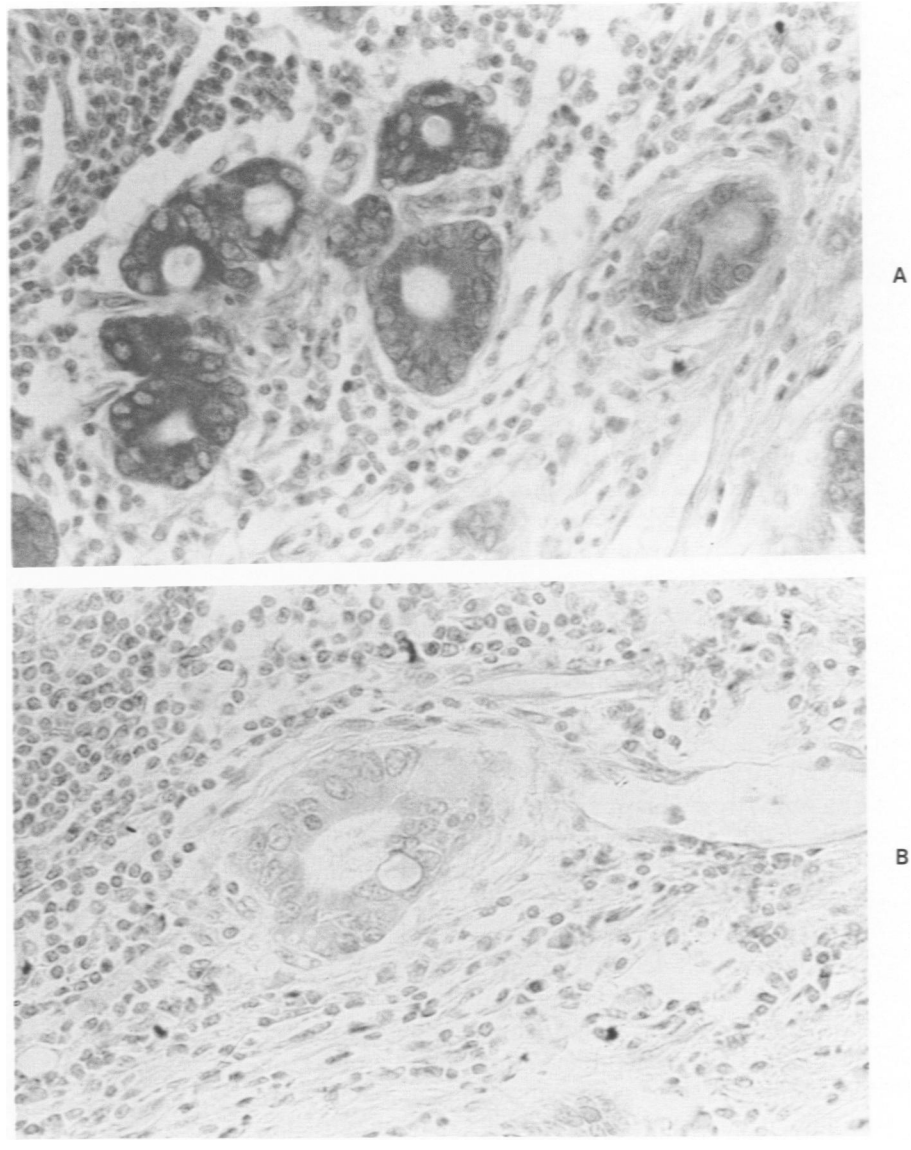

Figure 5 — Pancreatic carcinoma section (patient blood group B) incubated with MAb 33-25-17 (**A**), which binds to A substances, demonstrates irregular or abnormal expression of A substances. **B** — P3 × 63 Ag8 supernatant-negative control. (IP, ×250)

drate detected by PA83-52 and not the other MAb.[22] MAb PA83-52, which shows a high reactivity for difucosyl Type 1 chain,[22] gives the strongest and most consistent IP staining of endothelia of all sized vessels in all B or AB blood group patients. The lack of reactivity of endothelia with PA15-2 or PA23-48 detected in a subgroup of patients (14/31), therefore, may be related to the complete or relative lack of difucosyl Type 1 chain in these endothelia. The nonreactivity to endothelium and difucosyl chains of the blood group A binding antibody 33-25-17 is in line with this data. However, since the binding was also similar for endothelia and red cells and it is known that erythrocytes contain only Type 2 structures,[37] this is less likely. A better explanation might be that these differences reflect quantitative and qualitative differences in Type 2 chain or MAb affinity (Figure 2). Although it may not be possible to arrive at a precise biochemical explanation for the differences in endothelial reactivity detected immunohistochemically, these data allude to the complexity of the ABH system and, in particular, its expression in the mesodermally derived endothelium. Recent evidence suggests that additional factors are involved in control of ABH expression in endothelium.[46]

Alterations involving cell surface glycoproteins and glycolipids with ABH determinants have been documented in a variety of malignancies (see Hakomori and Kannagi[45] for review). The absence or loss of ABH antigens has been reported in several gastrointestinal malignancies detected by immunohistologic[1,2] and biochemical[47] methods. In bladder carcinomas, loss of isoantigen has been correlated with worsened progno-

sis.[17] Variable results regarding loss of antigen, particularly in gastric carcinomas,[18] have been reported. Differences in technique and antisera used might account for some of this variability.[16,48]

Complete loss of antigen was seen in 50% of the gastric carcinomas tested, and antigen expression in metastatic lesions matched that of the primary. The frequency of loss of isoantigen was similar in colon carcinomas from the proximal side (50%) and less in pancreatic carcinomas (13%). It has been proposed that excessive sialylation, which would mask or block antigenic activity,[49] causes the loss of ABH antigenic activity in tumors.[50,51] However, in three gastric carcinomas in which complete loss of isoantigen was demonstrated, neuraminidase pretreatment did not result in an "unmasking" of blocked antigenic sites.

Inappropriate expression of blood group substances, especially of A-like substances in tissue of blood group 0 or B patients, has been reported in a number of malignancies as detected immunohistochemically[5,8,18] or biochemically.[7,11,12] One speculation on the formation of inappropriate A determinants is the production by tumors of an unusual α-GalNAc transferase, so that N-acetylgalactosamine (GalNAc) residues are added to various structures.[45] Immunologic distinction of A isoantigen from Forssman antigen may be difficult because of their similar terminal GalNAc residues; in fact, "A-like" substances detected immunohistochemically by some polyclonal sera may be related to Forssman antigen. One of the major advantages of using MAbs, then, is their well-defined specificity. The MAb used in the present series shows insignificant binding to Forssman antigen. In this series, inappropriate expression of A substance in carcinomas from blood group B or O patients was seen only in pancreatic carcinoma (2/5) and not in gastric (0/11) or colonic carcinomas (0/10).

Regional differences in blood group antigens in normal adult colon are apparent from immunohistologic studies: these antigens can be detected in adult proximal colon but not in the distal colon.[20,52] They are, however, found throughout the colon during fetal development.[19] "Reexpression" of A and B isoantigens in carcinomas derived from the distal colon has been documented in several immunohistochemical studies.[4,5,21] Similar regional differences in Le antigens and reexpression in distal colon carcinomas has been documented using MAb in an IP assay.[6] In the present series, 11/13 carcinomas derived from the distal colon demonstrated such a "fetal" expression of A,B isoantigen (Table 6).

Blood group antigens are closely related to many tumor-associated glycolipids and gangliosides[13-15,45] most of which have been recognized and defined by MAb. The results of the present study lay the groundwork for developing a comprehensive panel of MAb to facilitate comparison of these closely related antigens with other MAb-defined tumor markers.

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