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Immunohistochemical Study of Blood Group Activities in the Alimentary Canal in Normal and Pathologic Conditions with Reference to the Nature of Epithelial Mucopolysaccharides*

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Summary. ABO(H)- and Lewis-activities in the human alimentary canal were investigated by the avidin-biotin-peroxidase complex method using commercial mouse monoclonal antibodies in order to clarify (1) the changes apparent in these activities in carcinoma of the digestive tract or premalignant intestinal metaplasia of the stomach and (2) the relationship of the activities to the histochemical properties of epithelial mucins examined by Alcian blue (pH 2.5)/periodic acid-Schiff (PAS) double staining. In intestinal metaplasia, ectopic goblet cells showed various degrees of ABO(H)-activities according to the PAS stainability of mucins and revealed Le^a-activity even in a Le(a−b+) group. In carcinoma of the alimentary canal, ABO(H)- and Lewis-activities compatible with the donors' blood groups were located mainly at the PAS-positive cell surface, and in the Golgi bodies and secretions. It was thus concluded that quantitative and/or qualitative changes in blood group activities were closely associated with those of epithelial mucopolysaccharides.

Key words: Human alimentary canal, Blood group activity – Blood group activity, human alimentary canal – Epithelial mucopolysaccharides – Immunohistochemistry, blood group activity in the alimentary canal

Zusammenfassung. ABO(H)- und Lewis-Aktivitäten des menschlichen Verdauungskanals wurden durch die Avidin-Biotin-Peroxidase-Komplex-Methode mittels käuflich erworbener monoklonaler Antikörpern (von der Maus) untersucht um (1) die Veränderungen der Aktivitäten in den Karzinomen und in der wie die Krypten der Jejunumschleimhaut ähnlichen Meta-

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plasie von Magenschleimhäuten (intestinale Metaplasie) darzustellen und (2) die Beziehungen der Aktivitäten mit histochemischen Eigenschaften von Mukopolysacchariden in Epithelzellen, die mittels der Alcianblau (pH 2.5)/ Perjodsäure-Schiff (PJS) gefärbt wurden, zu erklären. Bei der intestinalen Metaplasie von Magenschleimhäuten zeigten die fremdörtlichen Becherzellen sogar in Le(a−b+) Gruppen verschiedene ABO(H)-Aktivitäten, die im Verhältnis zur Färbung von PJS stand, und die Le^a-Aktivität. Bei Karzinomen des Verdauungskanals wurden die ABO(H)- und Lewis-Aktivitäten, die mit den Blutgruppen der Gewebespender übereinstimmen, meistens auf den PJS-positiven Zellflächen, im Golgi-Apparatus und in den Karzinomzellsekreten beobachtet. Diese Befunde zeigten, daß die quantitativen bzw. qualitativen Veränderungen von Blutgruppenaktivitäten in enger Beziehung zur Veränderung von Epithelmukopolysacchariden standen.

Schlüsselwörter: Verdauungskanal, Blutgruppenaktivitäten – Blutgruppenaktivitäten, Verdauungskanal – Epithelmukopolysaccharide – Immunhistochemie, Blutgruppenaktivitäten im Verdauungskanal

Introduction

Blood group activities (BGAs) are widely distributed not only on the plasma membranes of red blood cells (RBCs), but also in many kinds of human tissues as well as saliva, gastric juice, semen, and so on [1–3]. They exist as glycoproteins or glycolipids, which have been reported to be closely associated with cell growth, differentiation, and carcinogenesis [4–7]. In consideration of such characteristics of BGAs, several immunohistochemical studies have been conducted on their localization in human pathologic or fetal tissues in addition to normal ones [8–15].

The present study was designed to investigate the changes occurring in ABO(H)- and Lewis (Le^a, Le^b)-activities in the human alimentary canal with such pathologic changes as carcinoma, intestinal metaplasia of the stomach, and tubular adenoma of the colon in comparison with the activities present in the normal state. The relationship between BGAs and the histochemical properties of epithelial mucopolysaccharides was also studied hoping to obtain some clue to the biologic roles of BGAs.

Materials and Methods

Tissue Specimens (Tables 1–3)

Tissue specimens of the human alimentary canal were obtained by surgical resection. They consisted of normal mucosae or pathologic regions, such as carcinoma or intestinal metaplasia [16]. Every stomach specimen had regions of intestinal metaplasia, which were used for this study. The ABO and Lewis phenotypes of tissue donors were examined by usual agglutination tests using fresh peripheral venous blood. The Lewis phenotype, however, could be examined only in some of the tissue donors. The specimens were fixed in 10% formalin solution, embedded in paraffin, and sections 4–5 μm thick were cut.

Immunohistochemical Detection of Blood Group Activities [17]

Deparaffinized tissue sections were rinsed with 0.02 *M* phosphate-buffered saline (PBS, pH 7.3) and immersed in 0.3% H_2O_2/methanol solution containing 0.074% HCl for 30 min to block the endogenous peroxidase activity [18]. Following this, the sections were first incubated with 2% normal goat serum for 30 min and then incubated with each of the following mouse monoclonal IgM antibodies: anti-A (Biotest, Frankfurt/Main, FRG, 1:50 dilution), anti-B (Biotest, 1:40), anti-H (Chembiomed, Edmonton, Canada, 1:40) and anti-Le^a and -Le^b (Biotest, 1:40) for 2 h (if necessary, at 4°C overnight in addition). The specimens were then incubated with affinity-purified biotinylated goat anti-mouse IgM (Tago, Burlingame, Cal., 1:100) for 1 h. Finally, BGAs were immunostained using an avidin-biotin-peroxidase kit (Vector, Burlingame, Cal.) with 3,3′-diaminobenzidine as a substrate for peroxidase. These reactions were performed in a humidified chamber at 20°C, and the sections were rinsed well with PBS after each incubation. Counterstaining was carried out with Mayer's hematoxylin or veronal acetate-buffered 1% methyl green solution (pH 4.0).

The specificity of immunostaining was confirmed by control studies involving the replacement of the primary antibody with nonimmune serum from the same donor species (mouse) or omission of the primary antibody, biotinylated secondary antibody, and avidin-biotin-peroxidase complex. These control studies produced no immunostaining. The endothelium and RBCs in blood vessels in sections showed the same ABO(H)-activities as the phenotypes of tissue donors, thus providing suitable internal positive controls.

Detection of Epithelial Mucopolysaccharides

Consecutive tissue sections were stained with Alcian blue (Al · B, pH 2.5) for demonstration of acidic mucopolysaccharides and/or with the periodic acid-Schiff (PAS) reaction for detection of neutral mucopolysaccharides [19].

Results (Tables 1–3)

Esophagus

The intercellular spaces and cytoplasmic margins of normal esophageal mucosae except for the basal layer were distinctly stained with PAS, but not at all with Al · B (Fig. 1a). Also, the same ABO(H)-activities as the phenotypes of tissue donors' RBCs and Le^b-activity were clearly observed in the PAS-positive regions, and a marked polarity in the BGAs' distribution was noted (Fig. 1b). By contrast, in the case of squamous cell carcinoma, PAS-positive regions were restricted to a small number of carcinoma cells and intercellular substances, where only weak activities were found. The distributional polarity of BGAs was completely lost in accordance with irregularity in the distribution of PAS-positive substances.

Stomach

In the normal mucosa, surface mucous cells, mucous neck cells, and some parietal cells gave positive results with PAS-staining. They showed distinct ABO(H)- and Le^b-activities in a Le(a−b+) group, while Le^a-activity was either absent or only very faintly detected in the surface mucous cells or mucous neck cells. As for the Le(a+b−) group, normal surface mucous cells showed no staining reaction for B-activity in one case of group B, Le(a+b−). However,

Table 1. Blood group activity and results of Alcian blue/periodic acid-Schiff (PAS) double staining of normal mucosa from the human alimentary canal

Specimens	Blood group of tissue donors		Number of cases	Blood group activity		Al·B/ PAS double staining	
	Lewis	ABO		ABO(H)	Lewis	PAS	Al·B (pH 2.5)
Esophagus	NT	A	1	A, $O(H)^w$	Le^b[Le^a: vw ~ −]	+++	−
		O	1	O(H)			
Stomach	Le(a−b+)	A	2	A, $O(H)^w$	Le^b	+++	−
		B	1	B, $O(H)^w$			
		O	1	O(H)			
		AB	1	A, B, $O(H)^{vw}$			
	Le(a+b−)	A	2	A^w	$Le^a > Le^b$	+++	−
		B	1	−			
	NT	A	5	A, $O(H)^w$	Le^b or Le^a *	+++	−
		B	5	B, $O(H)^w$			
		O	5	O(H)			
		AB	1	A, B, $O(H)^{vw}$			
Small intestine	Le(a−b+)	A	3	A, O(H)	$Le^a = Le^b$	+ ~ ++	+
	Le(a+b−)	O	1	O(H)	$Le^a = Le^b$	+ ~ ++	+
Ascending and transverse colon	NT	A	3	A, $O(H)^w$	$Le^b > Le^a$ or $Le^a = Le^b$	$+^w$ ~ +	$+^w$ ~ +
		B	2	B, $O(H)^w$			
		O	1	O(H)			
Distal colon [Descending colon ~ Rectum]	Le(a−b+)	A	1	−	$Le^a > Le^b$ or $Le^a = Le^b$	−	+ ~ ++
		O	1	−			
		AB	1	−			
	Le(a+b−)	A	1	−	$Le^a > Le^b$	−	+ ~ ++
	NT	A	3	−	$Le^a > Le^b$ or $Le^a = Le^b$	−	+ ~ ++
		B	1	−			
		O	1	−			
		AB	2	−			

NT, not tested; w, weakly detected; vw, very weakly detected; $Le^a = Le^b$, both Le^a and Le^b activities were equally detected; $Le^a > Le^b$, Le^a activity was dominant; $Le^b > Le^a$, Le^b activity was dominant; Le^a*, only Le^a was found in one of five cases of group O; +++, very distinctly positive; ++, markedly positive; +, positive; $+^w$, weakly positive; −, negative

Table 2. Blood group activity and results of Alcian blue/PAS double staining of intestinal metaplasia of the stomach and tubular adenoma of the colon

Specimens	Blood group of tissue donors		Number of cases	Blood group activity		Al·B/ PAS double staining	
	Lewis	ABO		ABO(H)	Lewis	PAS	Al·B (pH 2.5)
* Intestinal metaplasia of the stomach	Le(a−b+)	A	2	A, $O(H)^w$	$Le^a = Le^b$	$+^w \sim ++$	$+^w \sim ++$
		B	1	B, $O(H)^w$			
		O	1	O(H)			
		AB	1	A, B, $O(H)^{vw}$			
	Le(a+b−)	A	2	A^w	$Le^a = Le^b$	$+^w \sim ++$	$+^w \sim ++$
		B	1	B^w			
	NT	A	5	A, $O(H)^w$	$Le^a = Le^b$	$+^w \sim ++$	$+^w \sim ++$
		B	5	B, $O(H)^w$			
		O	5	O(H)			
		AB	1	A, B, $O(H)^{vw}$			
Tubular adenoma of the colon	NT	A	1 [trans.]	$A, O(H)^{vw}$	$Le^a = Le^b$	$+^w \sim +$	$+^w \sim +$
		B	1 [ascend.]	$B, O(H)^{vw}$	$Le^a = Le^b$	$+^w \sim +$	$+^w \sim +$
		B	1 [sigm.]	−	$Le^b > Le^a$	−	$+ \sim ++$

Refer to the explanatory notes of Table 1. trans., transverse colon; ascend., ascending colon; sigm., sigmoid colon. * ABO(H) activities were heterogeneous in accordance with the heterogeneity of PAS-stainability in ectopic goblet cells

Table 3. Blood group activity and results of Alcian blue/PAS double staining of carcinoma in the human alimentary canal

Specimens	Blood group of tissue donors		Number of cases [Histopathologic classification of carcinoma*]	Blood group activity**		Al·B/ PAS double staining	
	Lewis	ABO		ABO(H)	Lewis	PAS	Al·B (pH 2.5)
Esophagus	NT	A	1 [sq]	A, $O(H)^w$	$Le^a = Le^b$	$+^w \sim +$	−
		O	1 [sq]	O(H)			
Stomach	Le(a−b+)	A	2 [pap, tub_2]	A, $O(H)^w$	$Le^b > Le^a$ or $Le^a = Le^b$	$+^w \sim ++$	−
		B	1 [sig]	B, $O(H)^w$		++	$+^P$, −
		O	1 [tub_1]	O(H)		++	−
		AB	1 [sig]	A [B: −]		++	−
	Le(a+b−)	A	2 [pap, tub_2]	A, $O(H)^w$	$Le^a = Le^b$	++	−
		B	1 [tub_2]	B, $O(H)^w$			
	NT	A	5 [pap, tub_2(2), por(2)]	A, $O(H)^w$	$Le^b > Le^a$ or $Le^a = Le^b$	$+^w \sim ++$	−
		B	5 [pap, tub_1, tub_2(2), por]	B, $O(H)^w$			
		O	5 [tub_1(2), por(2), sig]	O(H)			
		AB	1 [pap]	A, B			
Small intestine	Le(a−b+)	A	1 [well dif. pap]	A,$O(H)^w$	$Le^a = Le^b$	$+ \sim ++$	$- \sim +^w$
Ascending and transverse colon	NT	A	3 [well dif., pap, muc]	A, $O(H)^w$	$Le^a = Le^b$ or $Le^b > Le^a$	$+ \sim ++$	$- \sim +^w$
		B	2 [well dif., tub_1]	B, $O(H)^w$			
		O	1 [muc]	O(H)			
Distal colon [Descending colon ~ Rectum]	Le(a−b+)	A	1 [mod. dif.]	A, $O(H)^w$	$Le^b > Le^a$ or $Le^a = Le^b$	$+ \sim ++$	$- \sim +^w$
		O	1 [mod. dif.]	O(H)			
		AB	1 [well dif.]	A, B			
	Le(a+b−)	A	1 [well dif.]	A, $O(H)^w$	$Le^a = Le^b$	$+ \sim ++$	$- \sim +^w$
	NT	A	3 [tub_1, tub_2, por]	A, $O(H)^w$	$Le^b > Le^a$ or $Le^a = Le^b$	$+ \sim ++$	$- \sim +^w$
		B	1 [tub_2]	B, $O(H)^w$			
		O	1 [well dif.]	O(H)			
		AB	2 [well dif., sig]	A, B***			

* pap, papillary adenocarcinoma; tub_1, well differentiated tubular adenocarcinoma; tub_2, moderately differentiated tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma; sig, signet-ring cell carcinoma; sq, squamous cell carcinoma [16]. $O(H)^w$, weak O(H) activity; $Le^a = Le^b$, both Le^a and Le^b activities were equally detected; $Le^a > Le^b$, Le^a activity was dominant; $Le^b > Le^a$, Le^b activity was dominant; NT, not tested; ++, markedly positive, +, positive; $+^w$, weakly positive; $+^P$, partially positive; −, negative; ** blood group activity: very weak in poorly differentiated adenocarcinomas with few PAS-positive mucins; ***, negative in the case of signet-ring cell carcinoma

no such finding was obtained in a case of group A, Le(a+b−) in which A-activity was noted in the surface mucous cells.

In samples of intestinal metaplasia of the stomach, ectopic goblet cells demonstrated different characteristics for Al · B/PAS stainability from those of normal mucosae and had various proportions of both PAS- and Al · B-positive mucins (Fig. 2a). These cells showed various degrees of ABO(H)-activities, from positive to negative, in good accordance with the intensity of PAS-staining of mucopolysaccharides; cells that were stained mainly with Al · B showed very weak or no staining reaction for ABO(H)-activities (Fig. 2b). However, Le^a was observed in ectopic goblet cells even in a Le(a−b+) group. The regions of intestinal metaplasia in Le(a−b+) were thus clearly distinguished from normal mucosae. In a case of Le(a+b−), however, Lewis-activities in the ectopic goblet cells were almost the same as those in the normal mucosa, where both Le^a- and Le^b-activities were immunostained.

In gastric adenocarcinoma, PAS-positive mucopolysaccharides were distributed at the apical surface and in the intracellular structures (especially the Golgi bodies in the supranuclear region) of tumor cells and in the luminal mucinous secretions of well or moderately differentiated adenocarcinomas (pap, tub) where tumor cells were tightly adherent to each other, showing a kind of regularity in their arrangement and forming papillary or tubular structures (Fig. 3a, b). Furthermore, these tumor cells in both Le(a−b+) and Le(a+b−) groups had ABO(H)- and Lewis-activities, which were compatible with the phenotypes of donors' RBCs, in the PAS-positive sites.

In contrast, poorly differentiated adenocarcinoma (por) had very few PAS-positive components, and it showed very weak or almost negative BGAs.

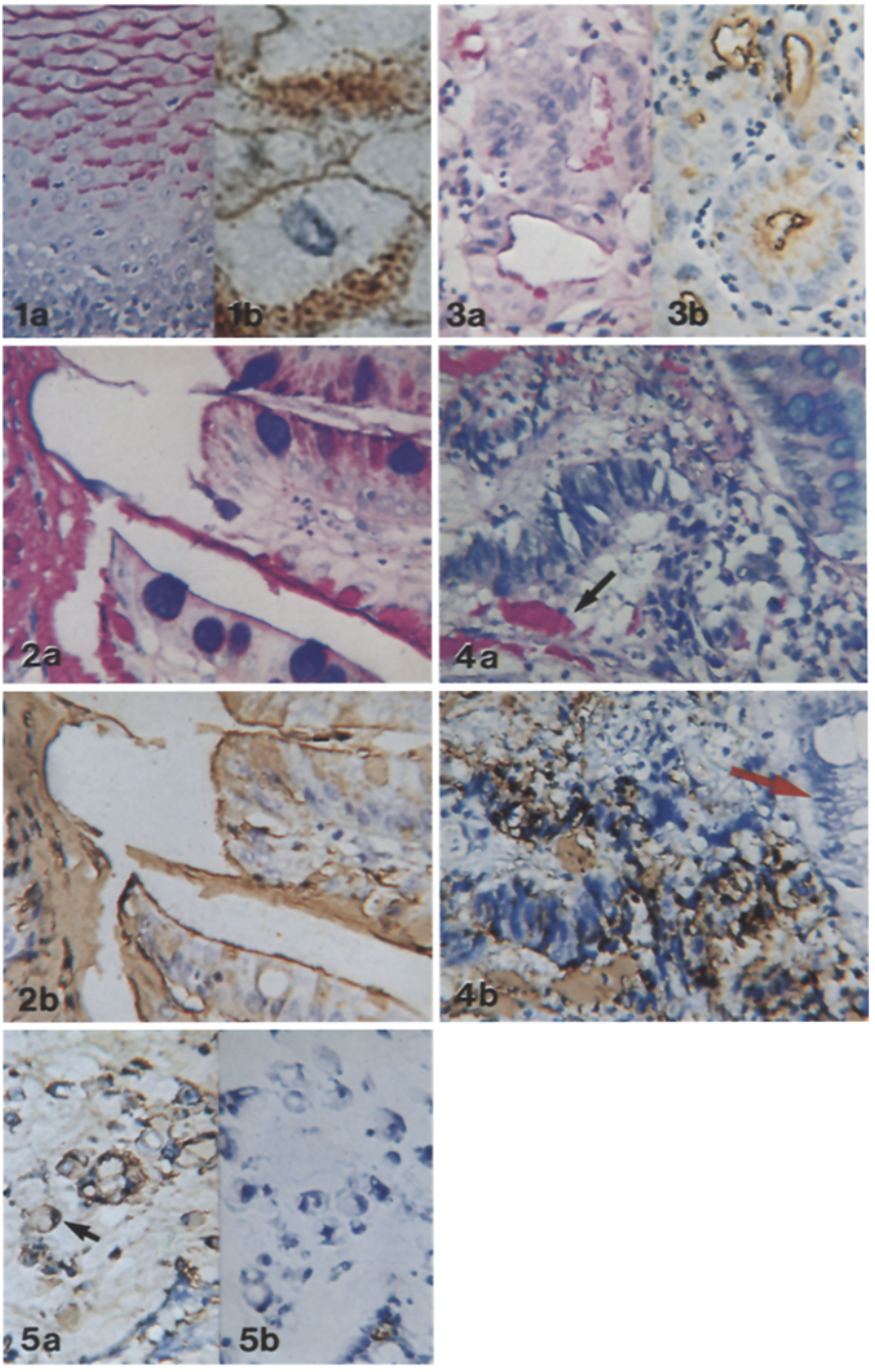
Individually separated signet-ring cells (sig) possessed ABO(H)- and Lewis-activities, which were compatible with the donors' phenotypes, on the PAS-positive mucins in the cytoplasm. On the other hand, signet-ring cells which contained only Al · B-positive and PAS-negative mucins showed no staining reaction for BGAs. Moreover, in another case of signet-ring cell carcinoma from a group AB donor, only A-activity was detected in the PAS-positive mucins.

There was no essential difference in BGAs' expression between early and advanced gastric adenocarcinoma as far as could be determined in this study.

Small Intestine

In the normal mucosae of small intestine, the brush borders and supranuclear regions of absorptive epithelial cells revealed distinct PAS-stainability, and the goblet cells were stained with both PAS and Al · B, thus showing that they had mucins composed of glycoproteins and acidic mucopolysaccharides. In addition, the PAS-positive regions revealed the same ABO(H)-activities as donors and also both types of Lewis-activities, irrespective of the donors' Lewis phenotypes.

In a well differentiated papillary adenocarcinoma of the duodenum of group A, the same A-activity as the donor's phenotype and both types of Lewis-activities were localized in the PAS-positive regions similar to normal mucosae and in the luminal mucinous secretions.


1a
1b
3a
3b
2a
4a
2b
4b
5a
5b

Large Intestine

The goblet cells in normal mucosa of the ascending and the transverse colon had PAS-positive mucins in the cytoplasm, while the epithelial mucins in the descending or sigmoid colon and in the rectum were stained distinctly with Al · B and hardly at all with PAS.

ABO(H)-activities were detected in the ascending and transverse colon up to the left flexure, but they were negative in the portion from the descending colon to the rectum (so-called distal colon). As for Lewis-activities, Le^a was more dominantly distributed than that of Le^b, usually in the distal colon, irrespective of the donors' Lewis phenotypes. However, in a few specimens of the proximal colon, both types of Lewis-activities tended to be demonstrated in some goblet cells and absorptive epithelial cells.

Adenocarcinoma of the colon, which had produced PAS-positive mucins, revealed positive staining for ABO(H)-activities at the PAS-positive apical sur-

Fig. 1. a Demonstration of PAS-positive substances in normal esophageal mucosa. They are located in the intercellular spaces and cytoplasmic margins except for the basal layer. Alcian blue (pH 2.5)/PAS double stain, hematoxylin counterstain (CT-H). × 100. **b** Immunostaining for ABO(H)-activities in normal esophageal mucosa by the avidin-biotin-peroxidase complex (ABC) method. The same activity as the donor's ABO phenotype is noted as a brown reaction product located in the PAS-positive regions shown in **a**. This figure shows O(H)-activity in the specimen from a group O donor. CT-H. × 400

Fig. 2. a Histochemical nature of epithelial mucins in intestinal metaplasia of the stomach, group O, Le(a−b+) donor. On the *left* of this figure, the epithelial mucins in normal gastric mucosae are shown to be exclusively and homogeneously PAS-positive. On the other hand, ectopic goblet cells stained with Alcian blue (pH 2.5) and PAS in different proportions are demonstrated on the *right* and at the *bottom*. Alcian blue/PAS stain, CT-H. × 100. **b** Immunostaining of O(H)-activity by the ABC method with monoclonal anti-H on the same specimen as in **a**. The degree of intensity of O(H)-activity is dependent on the stainability with PAS of the normal mucosae or the ectopic goblet cells. CT-H. × 100

Fig. 3. a Histochemical stainability of well differentiated tubular adenocarcinoma from a group O donor with Alcian blue (pH 2.5)/PAS. The luminal apical surface and supranuclear regions of the tumor cells forming tubular structures are PAS-positive as shown in the figure. CT-H. × 100. **b** Immunostaining of O(H)-activity in the same specimen as in **a**. The activity is stained brown by the ABC method with monoclonal anti-H in the PAS-positive regions of carcinoma cells. CT-H. × 100

Fig. 4. a Alcian blue (pH 2.5)/PAS double staining of the sigmoid colon of a group O donor. On the *upper right* of the figure, the goblet cells in the normal mucosa are stained exclusively with Alcian blue. In contrast, the adenocarcinoma of the sigmoid colon on the *left* has newly produced PAS-positive mucins in the lumen *(arrow)*. CT-H. × 100. **b** Immunostaining of the same specimen as in **a** by the ABC method with monoclonal anti-H. O(H)-activity is markedly noted in the PAS-positive luminal secretions of the carcinoma. However, the activity is completely negative in the normal mucosa *(arrow)*. CT-H. × 100

Fig. 5. a Immunostaining of rectal carcinoma, group AB, by the ABC method with monoclonal anti-A. A-activity is positive in the mucins of nearly all signet-ring cells *(arrow)*. The mucins produced by these cells were also stained with PAS. CT-H. × 100. **b** Immunostaining of the same specimen as in **a** by the ABC method with monoclonal anti-B. There are very few B-positive signet-ring cells in comparison with those possessing A-activity. Both the endothelia of vessels and red blood cells are clearly positive. CT-H. × 100

face and in the luminal secretions even in the carcinoma of distal colon (Fig. 4a, b). The activities detected there were usually compatible with the donors' phenotypes. In addition, Le^b-activity, which was absent from normal mucosae, became apparent especially in adenocarcinoma of the distal colon.

Such a dissociated expression of A- and B-activities as observed in signet-ring cell carcinoma of the stomach was also noticed in the same type of carcinoma of the rectum, group AB (Fig. 5a, b).

These findings obtained through examinations of primary carcinomas of the alimentary canal were also noted in the lymph nodes with metastasis of the tumor cells.

Tubular Adenoma of the Colon

In three cases of tubular adenoma of the colon with mild or moderate dysplasia (groups II–III), the stainability of their epithelial mucins with Al · B and PAS showed no apparent difference from that in normal mucosae of the corresponding colonic portion.

Furthermore, the same ABO(H)- and Lewis-activities as those in normal regions were noted in two cases in the ascending and transverse colon, but in one case of tubular adenoma in the sigmoid colon, Le^a-activity was diminished in intensity when compared with normal mucosae.

Discussion

The results obtained through this study show that ABO(H)-activities are located in the PAS-positive neutral mucopolysaccharides in both normal and diseased tissues of alimentary canal, and that quantitative and/or qualitative changes of these activities are related to the histochemical alterations of epithelial mucins. As for Lewis-activities, they were shown even in the mucins which were stained dominantly by Al · B, for example, those in the normal mucosae of distal colon. In malignancy, however, Lewis-activities were also noted to be distributed at the PAS-positive apical surface of tumor cells, and in the mucinous secretions newly produced by adenocarcinoma cells. Thus, it is speculated that Lewis antigens carrying the immunodeterminants in carcinoma are composed differently from those in normal mucosae, and that this difference has resulted in the changes of stainability with Al · B/PAS.

As described in the Results, normal mucosae in the human upper digestive tract (from the esophagus to the small intestine) contain those epithelial mucins that are remarkably stained with PAS, while there are PAS-negative or very weakly PAS-positive mucins present in the lower portion of large intestine, such as the descending colon, sigmoid colon, and rectum [20–22]. Thus, it was revealed that the presence or absence of BGAs in tissue sections was generally related to histochemical character of mucopolysaccharides, in other words, their stainability with PAS.

This conclusion seems reasonable considering additional findings by other investigators [8–15]: ABO(H)- and Lewis-antigens are present in the form of glyco- or muco-proteins in secretions (saliva, gastric juice, semen, and sweat) or

as glycolipids in the plasma membrane or intracellular structures of RBCs or several kinds of cells in the stomach, intestine, lung, kidney, and pancreas. The immunodeterminants of these antigens are known to be composed of such hexoses and hexosamines as L-fucose, D-galactose, N-acetylgalactosamine, and N-acetylglucosamine, and their vicinal glycols contribute to the PAS-positivity in histochemistry [23].

Weak detection of O(H)-activity in group A, B, or AB donors seems reasonable, since O(H) antigen is the precursor of A- or B-antigen in the biosynthetic pathway [2, 24].

On the basis of histopathologic and biochemical studies [25, 26], such a region of the gastric mucosa containing many ectopic goblet cells, which can be stained with Al · B (pH 2.5), could be regarded in this study as intestinal metaplasia of the stomach, irrespective of whether Paneth's cells were present or not. It became apparent that ectopic goblet cells were positive for BGAs, since they were revealed to contain not only Al · B-positive but also PAS-positive glycoproteins. However, they showed a variety and uneveness concerning BGAs, which was different from the homogenous distribution in the normal mucosa of a Le(a−b+) group. Such differences in the expression seem to be related to compositional changes of mucopolysaccharides and to the precancerous character of intestinal metaplasia of the stomach [25, 26].

In tubular adenoma of the colon with mild or moderate dysplasia and with the same Al · B/PAS stainability as normal mucosae, the finding by which it is perhaps possible to distinguish adenoma from intact mucosa is the decrease of Le^a. It seems, however, necessary to further investigate more cases of tubulovillous or villous adenoma with higher degrees of dysplasia.

As for carcinoma of the alimentary canal (especially gastric carcinoma), no clear relationship between the BGAs' distribution and the degree of histopathologic differentiation could be noted [9], since even signet-ring cells with poor differentiation revealed the activities so long as they had PAS-positive mucins, and some cases of well differentiated adenocarcinoma (such as pap or tub_1) with very few PAS-positive substances or secretions showed almost negative BGAs. In general, it can be said that such BGAs suppressed under normal conditions tended to appear in malignancy in which dedifferentiation might be expected to have occurred [8–15]. In fact, in this study, ABO(H)-activities compatible with the donors' phenotypes were revealed to be located in the PAS-positive components of carcinoma in the distal colon where ABO(H) were negative in normal mucosae.

It is of considerable interest that signet-ring cell type carcinoma of stomach or rectum of group AB contained PAS-positive mucins that reacted only with anti-A, but not with anti-B. This was confirmed by using polyclonal in addition to monoclonal antibodies. This kind of dissociation in the expression of A and B antigens has been reported in the cancer of stomach [27] and uterus [28], and it may be speculated that *B* gene is inhibited or that novel mucins with A-activity are produced in tumor cells [29], and that *A* and *B* genes are regulated independently.

As for the biologic functions of BGAs, it seems likely that they are closely involved in cell-to-cell contact and recognition [4, 5], since they were apparently

located in the PAS-positive intercellular spaces (e.g. in the intercellular bridges) of normal esophageal epithelia as in this study. By contrast, however, BGAs were noticed exclusively in the cytoplasm of those PAS-positive signet-ring cells that were not in contact with each other or not forming any kind of papillary or tubular structure.

In summary, it seems apparent that a knowledge of the distribution of BGAs defined by monoclonal antibodies is not only applicable to blood group examination in forensic serology [30], but also provides a very useful method for detecting both primary tumors and metastatic carcinoma cells in lymph nodes and for studying the biologic nature of the cells, which can be shown by applying Al · B/PAS double staining.

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