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Regional distribution of glycoconjugates in normal, transitional and neoplastic human colonic mucosa

A histochemical study using lectins

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Summary. Regional distribution of glycoconjugates in normal and neoplastic colonic mucosa was studied by means of eight lectins: *Dolichos biflorus* (DBA), *Glycine max* (SBA), *Triticum vulgare* (WGA), *Arachis hypogaea* (PNA), *Griffonia simplicifolia-I* (GS-I), *Canavalia ensiformis* (Con A), *Limax flavus* (LFA), and *Ulex europaeus-I* (UEA-I). The lectin binding patterns were examined in 40 normal colonic mucosa (NM) (12 proximal (P) and 28 distal (D)), 38 carcinomas (15 P and 23 D), and 31 transitional mucosa (TM) (9 P and 22 D). Sections of NM located 5 cm and 10 cm distant from the tumour and sections from the resection margins (more than 10 cm from the tumour) of the surgical specimens were also studied in 19 cases (6 P and 13 D). In NM, regional differences between the proximal and distal colon were detected with most lectins. DBA, SBA and LFA bound mainly to the goblet cell mucin of the distal colon, while GS-I and UEA-I labelling predominated in proximal colonic mucosa. The lectin reactivity in carcinomas was: DBA 26%, SBA 63%, PNA 95%, GS-I 66%, UEA-I 76%, WGA 100%, Con A 92% and LFA 42%. No regional differences were observed in the lectin patterns of proximal and distal colonic carcinomas nor was any relationship detected between lectin reactivities and Dukes stage, size or histological type of tumours. Transitional mucosa of both the proximal and distal colon showed an increase in PNA-binding and loss of DBA and SBA. LFA and UEA-I reactivity in proximal TM was similar to that observed in proximal NM. Distal TM showed a decrease in LFA labelling and the appearance of UEA-I reactivity in goblet cell mucin in 5 cases (23%). The reactivity of the other lectins was as with NM. The only change in normal mucosa distant from tumours was a focal increase in PNA reactivity in 4 cases. These findings suggest that carcinomas from different colonic regions have a more uniform distribution of carbohydrates than the respective NM.

Key words: Normal colonic mucosa – Transitional mucosa – Colonic carcinoma – Glycoconjugates – Lectins

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Introduction

Morphological and functional differences in the epithelium of the distal and proximal colon in both humans and animals have been described (Trump et al. 1984). Likewise, colonic carcinomas arising in proximal and distal regions differ in their epidemiology, morphology and biological behaviour (Haenszel and Correa 1971; Berg and Godwin 1974; Welch and Donaldson 1979). Glycoconjugates are major components of colonic epithelial cells, and regional differences in their composition and distribution between distal and proximal colonic mucosa have been demonstrated by biochemical and histochemical techniques (Filipe and Branfoot 1976; Freeman et al. 1978; Reid et al. 1984). These studies have also shown that the neoplastic transformation of colonic mucosa is associated with important modifications in its glycoconjugate content and metabolism (Kim and Isaacs 1975; D'Gorman and LaMont 1978; Montero and Segura 1980; Reid et al. 1984). Furthermore, signifi-

cant changes in mucosubstances have been detected in mucosa adjacent to carcinomas (transitional mucosa) (Filipe and Branfoot 1976) and, to a lesser degree, in normal mucosa distant from tumours (Shamsuddin et al. 1981; Wood et al. 1985). However, few studies have compared the distribution of glycosubstances in proximal colon carcinomas with the distribution of those in distal colon carcinomas. Membrane glycoconjugates play an important role in functions related to neoplastic transformation and malignant behaviour of cells (Hakomori 1981; Dennis and Laferte 1987). The differences observed between proximal and distal colonic carcinomas may be associated with different carbohydrate distribution.

Lectins are a group of proteins or glycoproteins of non-immune origin that can bind carbohydrates in a very specific way (Goldstein et al. 1980). Their specificity in binding carbohydrates is higher than that obtained with other histochemical techniques. For this reason, they have been used to investigate the presence and distribution of glycoconjugates in tissues (Damjanov 1987). Thus, lectins supply new information which can be compared with that obtained from biochemical and histochemical studies.

Several studies have described the lectin-binding patterns of normal colonic mucosa and their modification in neoplastic (Klein et al. 1981; Boland et al. 1982; Cooper 1982; Örntoft et al. 1985; Kellokumpu et al. 1986; Campo et al. 1988) and inflammatory diseases (Jacobs and Huber 1985; Pihl et al. 1985; Ahnen et al. 1987). However, most of these studies have concentrated on distal colonic carcinomas and on "normal" mucosa obtained from surgically-removed specimens bearing carcinomas. Few studies have described the regional differences between normal (Jacobs and Huber 1985; Lee 1987) and neoplastic mucosa (Yonezawa et al. 1982; Bresalier et al. 1985) and the number of lectins used has been limited. The profile of the major colonic carbohydrates detected by biochemical studies (Allen 1978; Clamp 1980) has not been covered. In this study we have used eight lectins to investigate the regional distribution of several carbohydrates in normal colonic mucosa and their modifications in a) carcinomas arising in the proximal and distal colon, b) mucosa adjacent to carcinomas (transitional mucosa (TM)), and c) normal mucosa distant from tumours.

Materials and methods

Thirty-eight surgically removed colonic carcinomas and 40 samples of normal mucosa (NM) were studied. Colonic mucosa contiguous to the carcinomas (transitional mucosa (TM)) could be examined adequately in 31 cases. In addition, sections of histologically normal mucosa located 5 cm and 10 cm distant from the tumour and sections from the resection margins (more than 10 cm from the tumour) of the surgical specimens were obtained from 19 cases of large bowel carcinoma.

Table 1. Cases examined

	Total cases	Proximal colon	Distal colon
Normal mucosa	40	12	28
Transitional mucosa	31	9	22
Normal mucosa distant from tumours	19	6	13
Carcinomas	38	15	23
Well-differentiated	6	4	2
Moderately-differentiated	22	5	17
Poorly-differentiated	3	1	2
Colloid	7	5	2

Normal mucosa samples were obtained endoscopically from patients investigated for large bowel diseases in whom no macroscopical or histological colonic lesions had been found. The clinical disorders in these patients were iron-deficiency anaemia of unknown origin, functional bowel disease, and change in bowel habit. The histological grading of tumours was established according to previous criteria (Morson and Sobin 1976; Blenkinsopp et al. 1981). Thus, 6 tumours were well-differentiated adenocarcinomas, 22 moderately-differentiated, 3 poorly-differentiated and 7 colloid carcinomas. The distribution of these tumours according to Dukes' system was Dukes A: 1 case, Dukes B: 22 cases and Dukes C: 15 cases. The blood group of patients with carcinomas was obtained from patient and blood bank records: 11 were group A, 8 group B, 10 group 0 (H) and 9 patients were not tested. The regional distribution of the different cases studied is summarized in Table 1. "Proximal colon" specimens included those from caecum, ascending and transverse colon, whereas descending, sigma and rectum samples were included as "distal colon".

All surgical and endoscopic specimens were fixed in neutral 10% formalin overnight and embedded in paraffin. Serial 5 μm sections were cut and one section of each case was stained with haematoxylin-eosin for microscopical study.

Eight lectins were used in this study (Table 2): *Glycine max* (SBA), *Triticum vulgare* (WGA), *Arachis hypogaea* (PNA), *Griffonia simplicifolia,* agglutinin-I (GS-I), *Canavalia ensiformis* (Con A), *Limax flavus* agglutinin (LFA) from E-Y Labs (San Mateo, Calif); *Ulex europaeus* agglutinin-I (UEA-I) and *Dolichos biflorus* agglutinin (DBA) from Vector (Burlingame, Calif). These lectins were conjugated with fluorescein isothiocyanate (FITC isomer I from Sigma, St Louis, MO) using the method described by Clarke and Hoggart (1982). Lectins were used at a concentration of 200–250 μg/ml dissolved in phosphate buffered saline (PBS).

Sections were deparaffinized, hydrated, rinsed in PBS and incubated with lectin in a moist chamber for 45 min at room temperature. They were then washed with PBS and counterstained with a solution containing 1 g toluidine blue, 20 ml 90% ethanol, 1 ml acetic acid and 180 ml PBS. Sections were mounted in Fluoprep (Biomerieux, Charboniers les Bains, France). Staining specificity was tested by adding 0.2 M of the appropriate inhibitor sugar to the lectin incubation medium.

Some sections from normal mucosa, transitional mucosa, and carcinomas were treated with saponification and neuraminidase prior to being incubated with LFA. Saponification was

Table 2. Fluorescein isothiocyanate (FITC)-conjugated lectins

Lectin	Source	Simple sugar specificity
Dolichos biflorus agglutinin (DBA)	Horse gram (*Dolichos biflorus*)	α-D-GalNAc
Soybean agglutinin (SBA)	Soybean (*Glycine max*)	α-D-GalNAc[a]
		β-D-GalNAc[a]
		α-D-Gal
Wheat germ agglutinin (WGA)	*Triticum vulgare*	β-D-GlcNAc[a]
		Neu5Ac
Limax flavus agglutinin (LFA)	*Limax flavus*	αNeu5Ac[a]
		αNeu5Gc
Peanut agglutinin (PNA)	*Arachis hypogaea*	β-D-Gal(1–3)GalNAc[a]
		α-D-Gal
Griffonia simplicifolia agglutinin-I (GS-I)	*Griffonia simplicifolia*	α-D-Gal
Ulex europaeus agglutinin-I (UEA-I)	Gorse seed (*Ulex europaeus*)	α-L-Fuc
Concanavalin A (Con A)	Jack bean (*Canavalia ensiformis*)	α-D-Man[a]
		α-D-Glc
		α-D-GlcNAc

Abbreviations: GalNAc, N-acetylgalactosamine; Gal, galactose; GlcNAc, N-acetylglucosamine; Neu5Ac, N-acetylneuraminic acid; Neu5Gc, N-glycolylneuraminic acid; Fuc, fucose; Man, mannose; Glc, glucose

[a] Main sugar specificity

Table 3. Summary of lectin reactivity in normal colonic mucosa

	Cases	DBA	SBA	PNA	GS-I	UEA-I	WGA	LFA	Con A
Proximal colon	12	12 (100%)	12 (100%)	4 (33%)*	10 (83%)	12 (100%)	12 (100%)	4 (33%)	12 (100%)
Distal colon	28	28 (100%)	27 (96%)	0 (0%)	1 (4%)	8 (29%)*	28 (100%)	27 (96%)	24 (86%)

* Only the glycocalyx area of superficial columnar cells was slightly stained

carried out with 0.5% potassium hydroxide in 70% ethanol for 15 min (Reid et al. 1973). *Clostridium perfringens* neuraminidase (Boehringer Mannheim, FRG) was used at 3 U/ml according to the procedure of Leathem and Atkins (1983). The slides were incubated with neuraminidase at 37° C in a humidified environment for 12 h. Following these treatments, the sections were incubated with LFA using the usual method.

All the samples were analysed with a Leitz Dialux-20 microscope equipped with epifluorescence illumination. Carcinomas were considered positive when lectin reactivity was observed in more than 25% of the section.

Contingency tables devised to study differences in lectin reactivity among the proximal and distal colonic regions and changes in lectin binding among NM, TM and carcinomas were drawn up using the Chi-square test or the Fisher's exact probability test. The possible influence of all the variables (patients' blood group, Dukes stage, size and histological type of tumours) together on lectin binding to carcinomas was analysed by means of Loglinear models (procedure HILOGLINEAR of SPSS^x) (Norusis 1985).

Results

The reactivity obtained with each lectin in NM, TM and carcinomas is summarized in Tables 3, 4 and 5.

Regional differences in binding patterns between normal mucosa of the proximal and distal colon were observed with most lectins (Table 3). Striking differences were obtained with UEA-I, GS-I and LFA. In the proximal colon, UEA-I (Fig. 1a) and GS-I (Fig. 1b) bound to goblet cell mucin and the glycocalyx area in 100% and 83% of the cases respectively. In the distal colon, however, UEA-I did not stain goblet cell mucin and weak reactivity was only seen in the glycocalyx area of columnar cells in 8 cases (29%). GS-I labelled goblet cell mucin and the glycocalyx area in only 1 case from the distal colon (4%). Goblet cells and the glycocalyx area of the distal colonic mucosa were slightly stained with LFA in nearly all cases (96%), while in the proximal colon only 4 cases (33%) were slightly stained. LFA reactivity was increased in sections from both the proximal and distal colon by previous saponification. Moreover, the negative cases of proximal colon became LFA reactive after KOH treatment. Neuraminidase digestion diminished or eliminated LFA labelling in both colonic areas (Fig. 1c–e).

DBA and SBA showed similar patterns throughout the colonic mucosa. Although all cases from the proximal and distal regions were stained with both lectins, the distribution in the crypts dis-

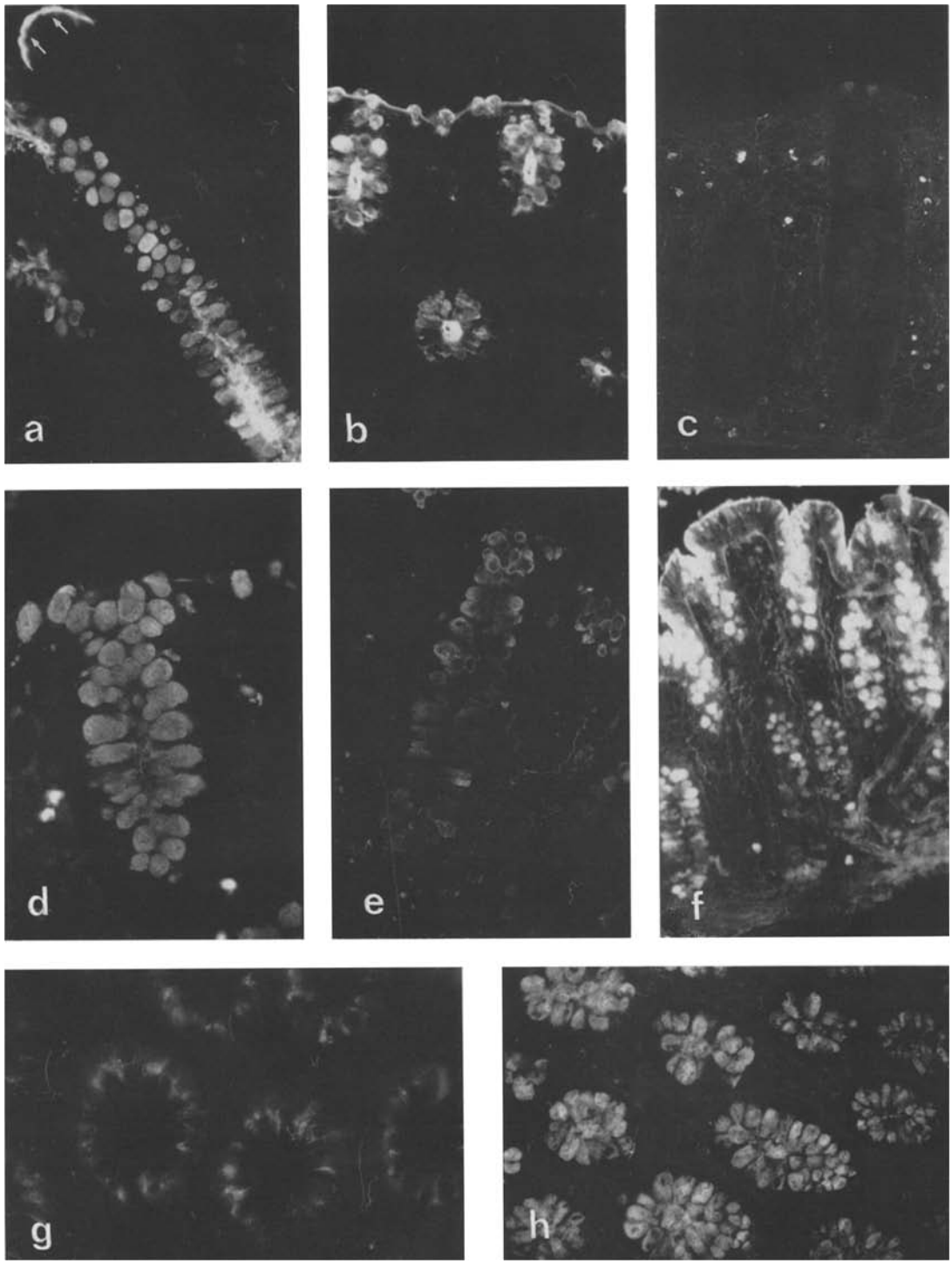

Fig. 1. (a–h). Paraffin sections of proximal (**a–e**) and distal (**f–h**) colonic mucosa incubated with various lectins conjugated with fluorescein isothiocyanate (FITC). (**a**) UEA-I in proximal colon showing an intense label in goblet cell mucin and glycocalyx area (*arrows*). ×200. (**b**) In the proximal colon GS-I also stains goblet cell mucin and glycocalyx area. ×200. (**c–e**) Sections of proximal colon incubated with LFA (**c**), LFA after saponification (**d**) and LFA after the sequence KOH-neuraminidase (**e**). The LFA negative proximal colonic mucosa (**c**) becomes LFA reactive after saponification (**d**). Neuraminidase diminished the LFA labelling (**e**). (**c**) and (**e**) ×175; (**d**) ×275. (**f**) DBA stains goblet cell mucin and glycocalyx area of distal colonic mucosa. ×145. (**g**) PNA is slightly reactive in the supranuclear area of glandular cells, but citoplasms are negative. ×275. (**h**) Con A stains goblet cell mucin of distal colonic mucosa. ×175

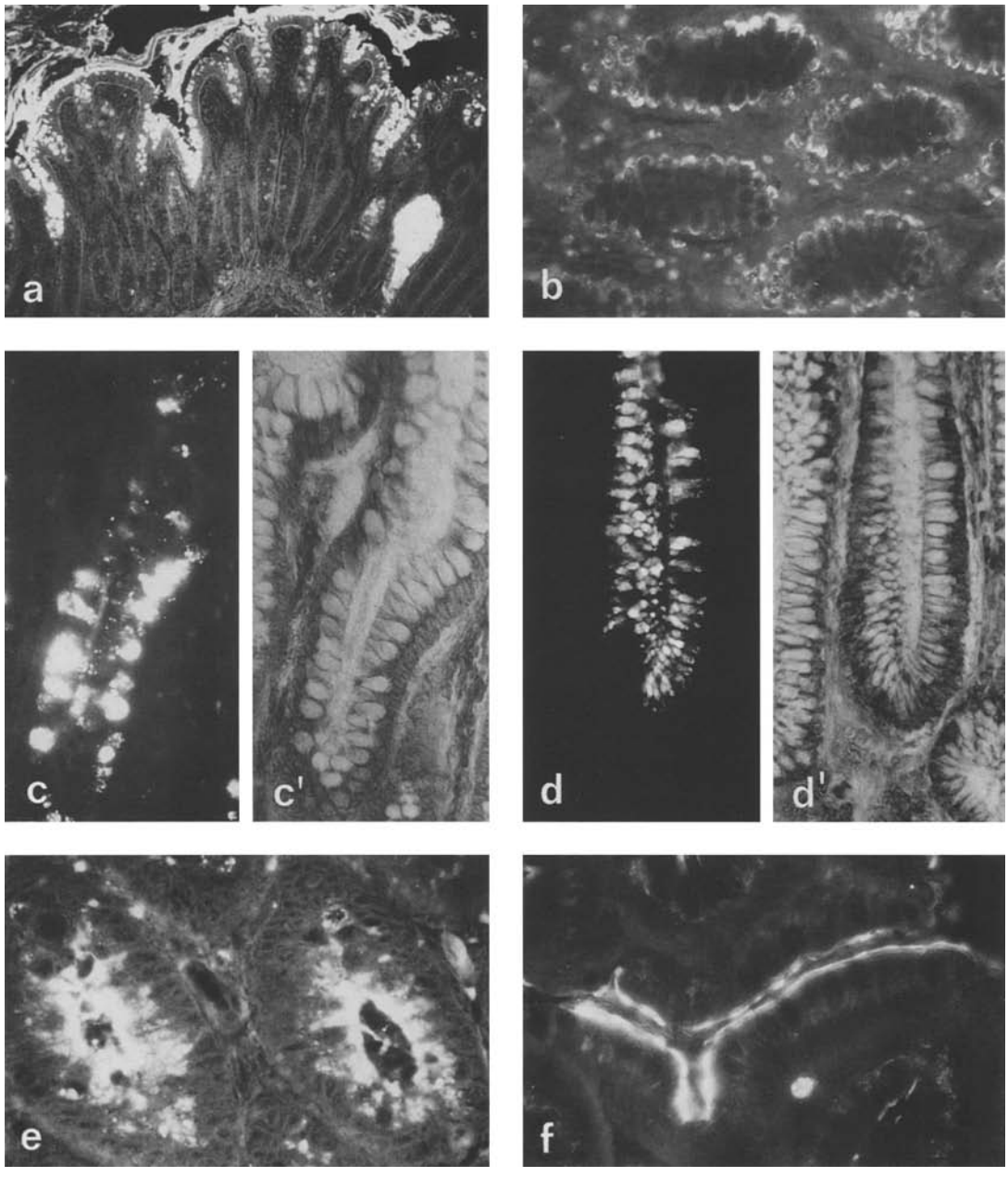

Fig. 2. (a–f). Paraffin sections of transitional mucosa (**a–d**) and normal colonic mucosa distant from tumours (**e** and **f**) incubated with various lectins conjugated with FITC and counterstained with toluidin blue (**c'** and **d'**). (**a**) DBA labelling in transitional mucosa adjacent to a distal colon carcinoma. Only the goblet cell mucin of the upper crypt and the glycocalyx area are stained (compare with Fig. 1f). ×70. (**b**) SBA in a distal transitional mucosa. Only the supranuclear area of glandular cells is reactive. ×175. (**c**) PNA in transitional mucosa binds goblet cell mucin. ×175. (**d**) GS-I reactivity in goblet cell mucin of a distal transitional mucosa. ×175 (**e**) and (**f**) Sections of normal colonic mucosa distant from tumours. PNA reactivity is observed in glandular cells (**e**) and glycocalyx area (**f**). ×275

closed regional differences. In the proximal colon, DBA and SBA stained mainly mucin of the goblet cells situated at the top of the crypt, while the lower part was negatively or very slightly stained. In the distal colon, this gradient of reactivity was less evident (Fig. 1f). The glycocalyx area was similarly stained in both proximal and distal colonic mucosa (Fig. 1f).

Goblet cell mucin of the whole colon was unreactive with PNA. Only the glycocalyx area was weakly stained in 4 cases of proximal colonic mucosa (33%). Supranuclear labelling was irregularly

Table 4. Summary of lectin reactivity in transitional mucosa

	Cases	DBA	SBA	PNA	GS-I	UEA-I	WGA	LFA	Con A
Proximal colon	9	2 (22%)	4 (44%)	6 (67%)	4 (44%)	6 (67%)	9 (100%)	5 (56%)	8 (89%)
Distal colon	22	6 (27%)	7 (32%)	10 (45%)	4 (18%)	5 (23%)	22 (100%)	7 (32%)	17 (77%)

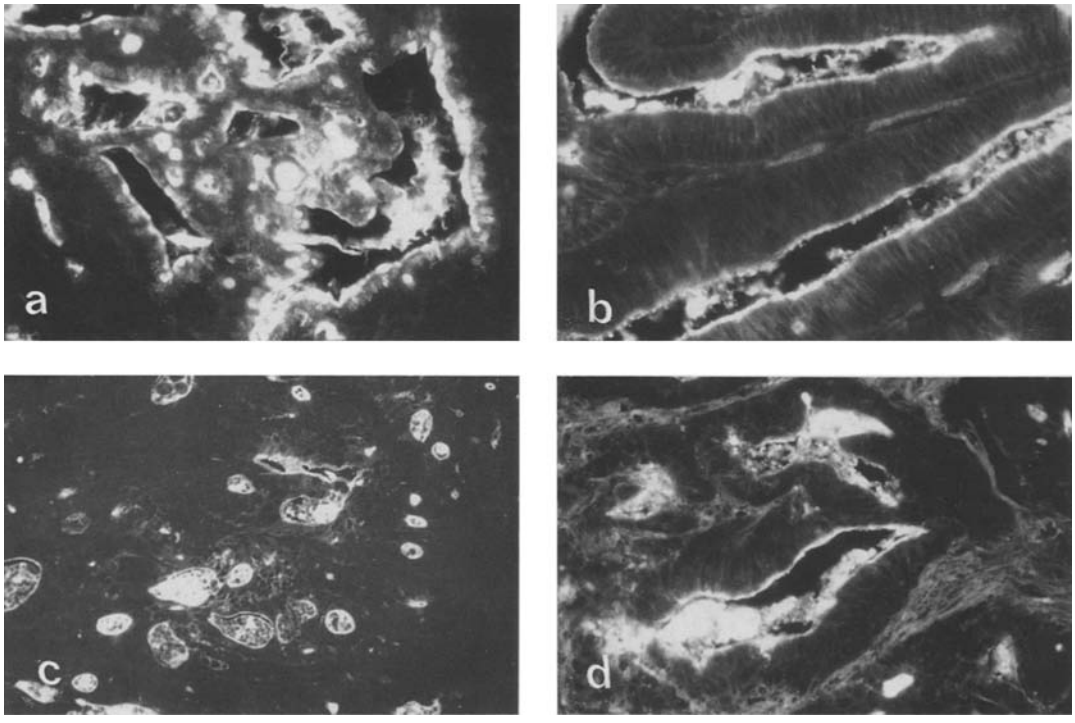

Fig. 3. (a–d). Paraffin sections from colonic carcinomas incubated with PNA-FITC (**a**), UEA-I-FITC (**b**), GS-I-FITC (**c**) and WGA-FITC (**d**). All lectins show labelling in the luminal surface of the neoplastic glands and in the intraluminal material. (**a, b**) and (**d**) ×175; (**c**) ×70

observed in some crypts throughout the colon (Fig. 1g).

WGA and Con A revealed no regional differences in distribution. WGA labelling was very intense in goblet cell mucin, the glycocalyx area and the supranuclear region. Con A stained goblet cell mucin (Fig. 1h) and the glycocalyx area irregularly.

Transitional mucosa of both the proximal and distal colon showed a strong decrease in DBA (Fig. 2a) and SBA (Fig. 2b) labelling and an increase in PNA reactivity in comparison with NM. PNA-positive goblet cell mucin was detected in 6 proximal (67%) and in 10 distal (45%) cases of TM (Fig. 2c and c'). Furthermore, 2 proximal and 1 distal cases of TM revealed strong reactivity in the glycocalyx area, but their goblet cell mucin was unreactive. The supranuclear region was stained more strongly than in normal mucosa. GS-I in TM showed few differences from NM patterns in both the proximal and distal colon. The decrease of GS-I reactivity in proximal TM and the small increase of GS-I binding in distal TM (Table 4; Fig. 3d and d') did not attain statistical significance. The supranuclear region of TM was stained by GS-I more strongly than in NM. In proximal TM UEA-I shows a binding pattern similar to that observed in NM from the proximal colon. Although a loss of UEA-I reactivity was detected in some cases, it was not statistically significant. In contrast with the UEA-I binding pattern in NM from the distal colon, 5 cases (23%) of distal TM showed a slight UEA-I reactivity in goblet cell mucin. Furthermore, in all 5 cases UEA-I labelling in the glycocalyx area occurred.

LFA reactivity in proximal TM was similar to that of NM, but a decrease was detected in TM of distal colonic carcinomas. Prior saponification did not modify the LFA binding pattern in TM, and neuraminidase treatment decreased or elimi-

Table 5. Summary of lectin reactivity in colonic carcinomas

	Cases	DBA	SBA	PNA	GS-I	UEA-I	WGA	LFA	Con A
Proximal colon	15	5 (33%)	8 (53%)	15 (100%)	8 (53%)	10 (67%)	15 (100%)	8 (53%)	14 (93%)
Distal colon	23	5 (22%)	16 (70%)	21 (91%)	17 (74%)	19 (83%)	23 (100%)	8 (35%)	21 (91%)
Total	38	10 (26%)	24 (63%)	36 (95%)	25 (66%)	29 (76%)	38 (100%)	16 (42%)	35 (92%)

nated its reactivity when it was positive. No differences were observed in WGA and Con A labelling between TM and NM.

Normal mucosa located at a distance from tumours showed a similar lectin binding pattern to that of control NM. Only in two cases from the distal colon (15%) were PNA-positive cells found in the sections at 5 cm, 10 cm and more than 10 cm distant from the tumours (Fig. 2e). Two additional samples from the distal colon (15%) showed a PNA reactivity in the glycocalyx area, but no positive goblet cell mucin was found (Fig. 2f). All these cases revealed PNA-staining of goblet cell mucin and the glycocalyx area in the respective TM.

No regional differences could be found in the lectin binding patterns of proximal and distal colonic carcinomas (Table 5). Tumours in both locations showed a similar decrease in DBA and SBA reactivity and an increase in PNA labelling (Fig. 3a). The strong UEA-I (Fig. 3b) and GS-I (Fig. 3c) labelling of distal colonic carcinomas contrasted with the low reactivity in normal and transitional distal mucosa. WGA (Fig. 3d) and Con A were positive in nearly all cases. In distal colon, carcinomas showed a decrease in LFA reactivity compared with NM. On the other hand, no statistical relationship could be established using Loglinear models between the lectin profile of carcinomas and the patients' blood group, Dukes stage, tumour size and histological type of tumours.

Discussion

In this study we have demonstrated the presence of significant differences in the distribution of several carbohydrates between the proximal and distal regions of the human colon. In contrast, proximal and distal colonic carcinomas show similar lectin binding patterns in both locations. UEA-I and GS-I reacted strongly with NM from the proximal colon, whereas these lectins did not react with NM from the distal colon. However, LFA, DBA and SBA reacted more weakly with NM from the proximal colon than in NM from the distal colon. Similar findings were obtained with UEA-I and DBA in previous studies (Yonezawa et al. 1982; Bresalier et al. 1985). All these lectins recognize terminal non-reducing carbohydrates of glycoconjugates. These results suggest that the main terminal carbohydrates in the proximal colonic mucosa are fucose and galactose while in distal colonic mucosa sialic acid and N-acetylgalactosamine predominate. Biochemical and histochemical studies have previously disclosed a higher content of fucose in proximal colonic mucosa and a higher content of sialic acid in distal colonic mucosa (Hoskins and Zamcheck 1963; Reid et al. 1984). These two sugars compete with each other to occupy the end position in oligosacharide chains of glycoconjugates (Paulson et al. 1978). The regional variations detected in the present study with UEA-I and LFA are in accordance with these biochemical findings.

Histochemical and biochemical studies have demonstrated the presence and heterogeneity of sialic acids in colonic mucosa (Rogers et al. 1978). In this study, LFA did bind, though weakly, with NM from the distal colon in almost all cases. In the proximal colon however, LFA binding with NM was negative in 67% of the cases. After submitting the slides to saponification, LFA reactivity became stronger and all cases of the proximal colon were positive. Since KOH treatment is considered to remove O-acyl ester from sialic acids (Reid et al. 1973), LFA might mainly bind non-acylated sialic acids. Shulte et al. (1984) also observed a class of sialic acids that only reacted with LFA after saponificaton and they suggested that O-acetylation at C_4 may hinder LFA binding. Human colonic mucosa has been shown to have a high content of O-acylated sialic acids (Reid et al. 1984) which would explain the low LFA reactivity without previous saponification.

In spite of the regional differences observed in the lectin binding patterns of normal colonic mucosa, carcinomas arising in proximal and distal colon showed similar lectin reactivity. Studies of blood group antigens in normal colonic mucosa have also demonstrated significant regional differences in the expression of these carbohydrates (Wi-

ley et al. 1981). However, the expression of blood group antigens in colonic carcinomas is not related to the site of the primary tumour (Wiley et al. 1981; Compton et al. 1987). These findings suggest that the carbohydrate distribution of carcinomas from different colonic regions might be more uniform than that found in normal mucosa from the same regions.

The lectin binding pattern of carcinomas was generally characterized by a loss of labelling with DBA and SBA and by labelling with PNA, UEA-I, GS-I, Con A, and WGA. LFA showed irregular distribution. The most striking differences between carcinomas and their corresponding NM were revealed by PNA, DBA and SBA in the two colonic regions and by UEA-I, GS-I and LFA in the distal colonic mucosa. As previous studies have shown with distal colonic carcinomas (Boland et al. 1982; Cooper 1982; Kellokumpu et al. 1986; Campo et al. 1988), tumours from the proximal colon exhibit a similar increase in PNA binding and a decrease in DBA-labelling. Bresalier et al. (1985) observed a relationship between DBA-labelling in proximal colonic carcinomas and the size of the tumours. In the present study, however, DBA-labelling, as with the binding of the other lectins, was found to be unrelated to either size, histological type or Dukes stage of tumours. The appearance of UEA-I and GS-I binding in distal colonic carcinomas, given the absence of labelling with these lectins in the corresponding NM, is a phenomenon similar to that observed with blood group antigens in this same colonic region (Wiley et al. 1981; Cooper and Haesler 1978). Blood group antigens are present in the distal region of the fetal colon and their expression in carcinomas is considered to be an oncofetal characteristic. However, UEA-I and GS-I are only irregularly expressed in human fetal colon (Urbanski et al. 1983) and so their reactivity in distal carcinomas might have a different significance.

Transitional mucosa (TM) has morphological and histochemical characteristics which differ from those of normal mucosa (Filipe and Branfoot 1976). Studies in distal colonic carcinomas have shown that TM presents a lectin reactivity similar to that observed in the tumours. This pattern is characterized by an increase in PNA binding and a loss of DBA- and SBA-labelling (Boland et al. 1982; Cooper 1982; Campo et al. 1988). Our study has confirmed these findings and has shown, moreover, that these lectins in TM of proximal colonic regions disclose a similar pattern to those in TM of distal colonic regions. It is interesting to note that although UEA-I was unreactive in goblet cell mucin of distal NM, in distal TM this cellular compartment displays a slight UEA-I reactivity in 23% of the cases studied. GS-I in TM from both regions follows the pattern of the corresponding NM: proximal TM was frequently GS-I positive while distal TM was usually unreactive with this lectin.

The most significant histochemical change in TM is the replacement of sulphomucins by sialomucins (Filipe and Branfoot 1976). However, the low reactivity obtained in TM with LFA, a sialic acid-binding lectin (Miller et al. 1982), in our study apparently contradicts this fact. Shulte et al. (1984) have also detected histochemically the existence of sialic acid residues which do not bind to LFA or to the KOH-LFA sequence. The reason for the lack of LFA binding to this subset of sialic acids is still unclear (Shulte et al. 1984).

Normal mucosa distant from tumours has been shown to have morphological and histochemical differences from NM (Shamsuddin et al. 1981). Örntoft et al. (1985) observed PNA and T-antigen reactivity in some crypts of mucosa remote from carcinomas. This PNA reactivity was associated with morphologically abnormal glands. Similarly, our studies revealed an abnormal PNA lectin reactivity in the mucosa distant from tumours, but only in four cases. The reactivity of other lectins was as in NM controls. Whether these changes have a premalignant or a reactive significance is still not clear, further study being required into benign lesions without premalignant significance.

In conclusion, the distribution of the carbohydrates recognized by these lectins shows important variations throughout the normal colonic mucosa. Carcinomas, however, express a similar carbohydrate reactivity in proximal and distal colonic mucosa. The changes in lectin reactivity observed in TM and in normal mucosa distant from tumours share some patterns with tumours while maintaining some NM lectin reactivity.

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References

Ahnen DJ, Warren GH, Greene LJ, Singleton JW, Brown WR (1987) Search for a specific marker of mucosal dysplasia

in chronic ulcerative colitis. Gastroenterology 93:1346–1355
Allen A (1978) Structure of gastrointestinal mucus glycoproteins and the viscous and gel-forming properties of mucus. Br Med Bull 34:28–33
Berg JW, Godwin JD (1974) The epidemiologic pathology of carcinomas of the large bowel. J Surg Oncol 6:381–400
Blenkinsopp WK, Stewart-Brown S, Blesowsky L, Kearney G, Fielding LP (1981) Histopathology reporting in large bowel cancer. J Clin Pathol 34:509–513
Boland CR, Montgomery CK, Kim YS (1982) Alterations in human colonic mucin occuring with cellular differentiation and malignant transformation. Proc Natl Acad Sci USA 79:2051–2055
Bresalier BS, Boland CR, Kim YS (1985) Regional differences in normal and cancer-associated glycoconjugates of the human colon. J Natl Cancer Inst 75:249–260
Campo E, Condom E, Palacin A, Quesada E, Cardesa A (1988) Lectin binding patterns in normal and neoplastic colonic mucosa. A study of *Dolichos biflorus* agglutinin, Peanut agglutinin and Wheat germ agglutinin. Dis Colon Rectum 31:892–899
Clamp JR (1980) Gastrointestinal mucus. In: Wright R (ed) Recent advances in gastrointestinal pathology. Saunders Co, London, pp 47–58
Clarke AE, Hoggart RM (1982) The use of lectins in the study of glycoproteins. In: Marchalonis JJ, Warr GW (eds). Antibody as a tool. John Wiley and Sons, Chichester, pp 347–401
Compton C, Wyatt R, Konugres A, Ehrenthal D, Durda P (1987) Immunohistochemical studies of blood group substance H in colorectal tumors using a monoclonal antibody. Cancer 59:118–127
Cooper HS (1982) Peanut lectin binding sites in large bowel carcinomas. Lab Invest 47:383–390
Cooper HS, Haesler WE Jr (1978) Blood group substances as tumor antigens in the distal colon. Am J Clin Pathol 69:594–598
D'Gorman TA, LaMont JT (1978) Glycoprotein synthesis and secretion in human colon cancers and normal colonic mucosa. Cancer Res 38:2784–2789
Damjanov I (1987) Biology of disease. Lectin cytochemistry and histochemistry. Lab Invest 57:5–20
Dennis JW, Laferte S (1987) Tumor cell carbohydrate and the metastatic phenotype. Cancer Metastasis Rev 5:185–204
Filipe MI, Branfoot AC (1976) Mucin histochemistry of the colon. Curr Top Pathol 63:143–178
Freeman HJ, Kim Y, Kim YS (1978) Glycoprotein metabolism in normal proximal and distal rat colon and changes associated with 1,2-dimethylhydrazine-induced colonic neoplasia. Cancer Res 38:3385–3390
Goldstein IJ, Hughes RC, Monsigny M, Osawa T, Sharon N (1980) What should be called a lectin? Nature 285:66
Haenszel W, Correa P (1971) Cancer of the colon and rectum and adenomatous polyps. A review of epidemiologic findings. Cancer 28:14–24
Hakomori S (1981) Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. Ann Rev Biochem 50:733–764
Hoskins LC, Zamcheck N (1963) Studies on gastric mucins in health and disease. Ann NY Acad Sci 106:767–774
Jacobs LR, Huber PW (1985) Regional distribution and alterations of lectin binding to colorectal mucin in mucosal biopsies from controls and subjects with inflammatory bowel disease. J Clin Invest 75:112–118
Kellokumpu I, Karhi K, Andersson LC (1986) Lectin-binding sites in normal, hyperplastic, adenomatous and carcinomatous human colorectal mucosa. Acta Pathol Microbiol Immunol Scand Sect A 94:271–280
Kim YS, Isaacs R (1975) Glycoprotein metabolism in inflammatory and neoplastic diseases of the human colon. Cancer Res 35:2092–2097
Klein PJ, Osmers R, Vierbuchen M, Ortman M, Kanio J, Unlenbruck G (1981) The importance of lectin binding sites and carcinoembryonic antigen with regard to normal, hyperplastic, adenomatous and carcinomatous colonic mucosa. Rec Res Cancer Res 79:1–9
Leathem AJC, Atkins NJ (1983) Lectin binding to paraffin sections. In: Bullock GR, Petrusz P (eds) Techniques in immunocitochemistry. Academic Press, London, pp 39–67
Lee YS (1987) Lectin reactivity in human large bowel. Pathology 19:397–401
Miller RL, Collawn JF, Fish WW (1982) Purification and macromolecular properties of a sialic acid-specific lectin from the slug *Limax flavus*. J Biol Chem 257:7574–7580
Montero C, Segura DI (1980) Retrospective histochemical study of mucosubstances in adenocarcinomas of the gastrointestinal tract. Histopathology 4:281–291
Morson BC, Sobin LH (1976) Histological typing of intestinal tumours. International Histological Classification of Tumours No 15. WHO, Geneva
Norusis MJ (1985) SPSS^x Advanced statistics guide. McGraw-Hill, New York
Örntoft TF, Mors NPO, Eriksen G, Jacobsen NO, Poulsen HS (1985) Comparative immunoperoxidase demonstration of T-antigens in human colorectal carcinomas and morphologically abnormal mucosa. Cancer Res 45:447–452
Paulson JC, Prieels JP, Glasgow LR, Hill RL (1978) Sialyl- and fucosyltransferases in the biosynthesis of asparaginyl-linked oligosaccharide in glycoproteins: Mutually exclusive glycosylation by β-galactoside α2–6 sialytransferase and N-acetylglucosamide α1–3 fucosyltransferase. J Biol Chem 253:5617–5624
Pihl E, Peura A, Johnson WR, McDermott FT, Hughes ESR (1985) T-antigen expression by peanut agglutinin staining relates to mucosal dysplasia in ulcerative colitis. Dis Colon Rectum 28:11–17
Reid PE, Culling CFA, Dunn WL (1973) Saponification-induced increase in the periodic acid Schiff reaction in the gastrointestinal tract. Mechanism and distribution of the reactive substance. J Histochem Cytochem 21:473–482
Reid PE, Culling CFA, Dunn WL, Ramey CW, Clay MG (1984) Chemical and histochemical studies of normal and diseased human gastrointestinal tract. I. A comparison between histologically normal colon, colonic tumours, ulcerative colitis and diverticular disease of the colon. Histochem J 16:235–251
Rogers CM, Cooke KB, Filipe MI (1978) Sialic acids of human large bowel mucosa: O-acylated variants in normal and malignant status. Gut 19:587–592
Shamsuddin AKM, Weis L, Phelps PC (1981) Colon epithelium. IV. Human colon carcinogenesis. Changes in human colon mucosa adjacent to and remote from carcinomas of the colon. J Natl Cancer Inst 66:413–419
Shulte BA, Spicer SS, Miller RL (1984) Histochemical localization of sialoglycoconjugates with a sialic acid-specific lectin from the slug *Limax flavus*. Histochem J 16:1125–1132
Trump BF, Phelps PC, Shamsuddin AM (1984) Cellular pathobiology of human large intestine. In: Wolman SR, Mastromarino AJ (eds) Progress in cancer research and therapy. Raven Press, New York, pp 23–49
Urbanski SJ, Levine ME, Borgs P, Bruce WR, Krepinsky JJF (1983) Colonic mucus in human fetal development. Oncodevelop Biol Med 4:363–370

Welch JP, Donaldson GA (1979) The clinical correlation of an autopsy study of recurrent colorectal cancer. Ann Surg 189:496–502

Wiley EL, Mendelsohn G, Eggleston J (1981) Distribution of carcinoembryonic antigens and blood group substances in adenocarcinoma of the colon. Lab Invest 44:507–513

Wood CB, Dawson PM, Habib NA (1985) The sialomucin content of colonic resection margins. Dis Colon Rectum 28:260–261

Yonezawa S, Nakamura T, Tanaka S, Sato E (1982) Glycoconjugate with *Ulex europaeus* agglutinin-I-binding sites in normal mucosa, adenoma, and carcinoma of the human large bowel. J Natl Cancer Inst 69:777–785

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