Lectin Staining of Neoplastic and Normal Background Colorectal Mucosa in Nonpolyposis and Polyposis Patients

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A lectin histochemistry approach was adopted for comparative assessment of a colon cancer risk. Binding of Ulex europaeus agglutinin-I (UEA-I), peanut agglutinin (PNA), Griffonia simplicifolia agglutinin-II (GSA-II), and Dolichos biflorus agglutinin (DBA) was investigated in tumor and background tissue from a total of 34 adenoma and 44 cancer patients and compared with reaction patterns in control and familial adenomatous polyposis (FAP) patients. Adenoma patients with UEA-I positive rectal mucosa were found to have a 33.3 percent familial history of large bowel cancer, which was significantly higher ($P < 0.05$) than the respective 4.0 percent figure for patients with negative rectal mucosa. In the cancer patients, an even stronger correlation was noted, with a 63.2 percent UEA-I positive family history association being recorded, as opposed to 4.0 percent in the negative rectal mucosa patients ($P < 0.01$). Thus, the results suggest that, apparently, normal rectal background mucosa of individuals genetically at high risk for colon and rectal cancer demonstrates a specific lectin binding ability similar to that of FAP patients and that the simple method using UEA-I staining of rectal biopsy specimens can be of practical use in identification of high-risk colorectal cancer. [Key words: Lectin; Familial adenomatous polyposis; Background mucosa; Colorectal cancer; Colorectal adenoma]

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It has been noted that changes in glycoconjugates accompany cell differentiation[1] and that this feature may be useful as a cellular marker for malignant transformation. Lectins, which are accurate probes for sugar residues, have thus been applied mostly as a supportive measure to identify malignant cells;[2–8] among the wide range available, Ulex europaeus agglutinin-I (UEA-I), peanut agglutinin (PNA), and Griffonia simplicifolia agglutinin-II (GSA-II) are generally regarded as particularly suitable histologic tumor cell markers for the colon and rectum.[2–8] Conversely, Dolichos biflorus agglutinin (DBA) binds to goblet-cell mucin in normal colon and rectal mucosa[4, 9, 10] but not to the majority of colonic cancers.[4, 7] A recent report, however, revealed that the normal rectal mucosas of FAP patients also were stained by UEA-I.[11] To establish a practical aid for identification of other individuals at high risk for colorectal cancer, the present comparative investigation of endoscopic biopsy material staining with the above four lectins for diagnostic applicability was performed.

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PATIENTS AND METHODS

Patient Population

All patients had been examined by routine colonoscopy in the second Department of Surgery at the Hyogo College of Medicine during the period 1985 to 1987. Clinical details for tumor and background mucosa studies are summarized in Tables 1 and 2, respectively.

Thirty-six adenoma specimens were obtained by endoscopic polypectomy from 34 patients. A total of 31 specimens from 44 patients with colorectal cancer who were operated on in our clinic were also investigated. Included were one patient in the adenoma group and five patients in the cancer group with a family history of high cancer incidence over two generations. Background tissue was sampled from all of these tumor patients.

Control patients were as follows: five autopsied patients who died of cardiac disease apparently not associated with colorectal changes and 31 patients who underwent colonoscopic examination without evidence of neoplastic lesions. Twenty-five of

Table 1.
Numbers of Patients and Specimens Investigated in the Tumor Tissue Study

Type of Lesion	Method of Sampling	No. of Patients (Male:Female)	Mean Age (yr) (Range)	No. of Specimens			
				RC	LC	Rect	Total
Adenoma (nonpolyposis)	Polypectomy	34 (23:11)	63.4 (39–80)	11	14	11	36
Cancer (nonpolyposis)	Surgery	31 (17:14)	61.9 (23–83)	8	12	11	31

RC = right colon; LC = left colon; Rect = rectum.

Table 2.
Breakdown of Patient/Subject Types in the Background Mucosa Study

Group	Method of Sampling	No. of Cases (Male:Female)	Mean Age (yr) (Range)	Site of Sampling	
				Colorectal	Rectal
Control subjects	Autopsy	5 (1:4)	59.8 (37–86)	5	—
	Biopsy	31 (16:15)	45.6 (28–67)	15	16
	Total	36 (17:19)	52.7 (28–86)	20	16
Adenoma patients	Biopsy	34 (26:8)	57.5 (39–80)	10	24
Cancer patients	Biopsy and/or surgery	44 (29:15)	60.1 (23–83)	10	34
FAP patients	Surgery	10 (6:4)	38.7 (19–59)	10	—

these had presented with positive occult blood stools, abdominal pain, or diarrhea, and the remaining six were undergoing complete medical examinations. This group included two patients who were investigated because of a family history of high cancer incidence over two generations.

Tumor Tissue

The 36 adenomas included 32 tubular and four tubulovillous lesions. Twenty-seven displayed moderate atypia, and nine demonstrated mild atypia. The 31 cancer specimens included 22 of Dukes' A, 4 of B, and 5 of C. Twenty-three of these adenocarcinomas were well differentiated, and eight were moderately well differentiated.

Background Mucosa

Control Subjects. In the autopsy cases, generally two mucosal specimens were obtained from five subsites of the large bowel, including the ascending, transverse, descending, and sigmoid colon and the rectum. In 15 of the biopsy cases, mucosal specimens were obtained using a punch method from all five subsites: one each from the colonic subsites and three from the rectum (one each from the Houston valves). In the other 16 patients, biopsy sampling was from the rectum only.

Adenoma Patients. The numbers of polyp lesions per patient ranged from one to seven, averaging 2.9 lesions. Mucosal specimens were obtained by colorectal biopsy in 10 patients and by rectal biopsy only in 24.

Cancer Patients. Background specimens were obtainable from both the colon and rectum, from either endoscopic biopsy or resected material, in ten cancer patients. Only rectal specimens were obtained in the other 34 patients. Mucosal sampling in resected colons was made from areas at least 5 cm from the margin of the cancer lesion.

FAP Patients. Four profuse- and six sparse-type FAP patients were sampled. The entire colon and rectal mucosas were removed in four patients by proctocolectomy and in the remaining six by restorative proctocolectomy. Multiple mucosal sampling was made throughout the large bowel. Cancer was associated in four patients.

Histologic Staining and Evaluation

Tissue specimens were fixed in 10 percent buffered-Formalin (pH 6.9), washed, dehydrated, paraffin embedded, cut at 4 μ, and stained according to the ABC method of Hsu and Raine,[12] with some minor alterations. Sections were incubated in a solution of trypsin (Type II; Sigma Chemical Co.,

St. Louis, MO) 1 mg/ml in Tris saline, pH 7.5, with 6.8 mM $CaCl_2$ for 30 minutes, at 37°C. After washing for 5 minutes in water, endogenous peroxidase was blocked with methanol containing 0.3 percent H_2O_2 at room temperature for 30 minutes. Thereafter, they were washed three times in phosphate-buffered saline (PBS) and incubated at room temperature for 15 minutes with 1 percent bovine serum albumin. Sections were then incubated with biotin-labeled lectins (Bio-lectins) (Table 3) obtained from Vector Laboratories (Burlingame, CA) at 4°C for 24 hours, washed three times in PBS, and incubated with avidin-peroxidase complex for 1 hour. Binding sites were demonstrated with 3,3′-diaminobenzidine (DAB) for 5 minutes at room temperature. After being washed in tap water, the sections were counterstained with hematoxylin and cover-slipped. Bio-PNA sections were preincubated in neuraminidase (Vibrio cholerae, Type V; Sigma) at 0.27 U/ml in acetate buffer, pH 5.5, with 0.68 mM $CaCl_2$ for 1 hour, at 4°C. Bio-DBA sections were not digested and were incubated at 4°C for 1 hour. The specificities of UEA-I, PNA, GSA-II, and DBA binding were tested, respectively, by adding 0.2 M Fuc, Gal, GlcNAc, and GalNAc to the incubation medium.

Adjacent sections of all material were stained with hematoxylin and eosin for routine histology.

The stained preparations were examined under the light microscope. In the normal mucosa, peroxidase staining of goblet-cell mucins was considered to be specific to the lectins. Specimens were evaluated as positive if more than half the goblet-cell vacuoles of the total crypts per field demonstrated lectin binding.

Family Study

Family history of colorectal cancer was assessed for patients and control subjects by means of chart reviewing or interviewing. When the patient had one or more first-degree relatives having colorectal cancer, the proband was defined as "positive family history." Statistical comparisons were made using the χ^2 and McNemar tests.

RESULTS

Tumor Tissue

In adenomas, UEA-I, PNA, and DBA binding appeared mainly in goblet-cell mucins whereas GSA-II staining was found in the cellular supranuclear regions. There were no significant differences in positivity rates between the lectins or any relationship to degree of atypia or location. In the cancers, staining was mainly in the cell apex and of secretory product, and the intensity varied between samples. UEA-I staining was expressed more strongly than PNA and GSA-II. UEA-I, GSA-II, and PNA were positive in most of the cancer specimens, while DBA was negative in the majority. There were no differences in the positivity rates with lesion location. The lectin binding of tumor tissues is summarized in Table 4.

Background Mucosa

In background mucosa, UEA-I was primarily limited to the goblet-cell mucin in the upper two-thirds of the crypts. At the base of the crypts, UEA-I staining was negative or weakly positive at the cell apex. PNA and GSA-II showed similar lectin-binding patterns with weak supranuclear staining of the cytoplasm. In several patients, weak positivity was observed for both goblet-cell mucin and the cell apex region in the deeper portion of the crypts. DBA reacted homogeneously with goblet-cell mucin throughout the crypts.

Of the four lectins (Table 5), only UEA-I showed any significant differences in binding ability with colon as opposed to rectal location, the mucosal epithelium in the former site demonstrating stronger staining, with little binding in the rectum. On the other hand, the rectum was positive for UEA-I in many FAP patients and in about half the cancer patients.

Table 3.
Lectins Used in this Study

Lectin Origin	Abbreviation	Dilution	Sugar Specificity
Ulex europaeus agglutinin-I	UEA-I	1:500	α-L-Fuc
Peanut agglutinin	PNA	1:100	β-D-Gal—(1 → 3)-D-GalNAc
Griffonia simplicifolia agglutinin-II	GSA-II	1:100	α,β-D-GlcNAc
Dolichos biflorus agglutinin	DBA	1:100	α-D-GalNAc

Table 4.
Positive Rate for Binding of Various Lectins in Colorectal Tumor Tissue from Different Sites

	Study Group							
	Adenoma				Cancer			
	RC	LC	Rect	Total	RC	LC	Rect	Total
UEA-I	9/11 (81.8%)	12/14 (85.7%)	10/11 (90.9%)	31/36 (86.1%)	8/8 (100.0%)	12/12 (100.0%)	10/11 (90.9%)	30/31 (96.8%)
PNA	10/11 (90.9%)	12/14 (85.7%)	11/11 (100.0%)	33/36 (91.7%)	8/8 (100.0%)	12/12 (100.0%)	11/11 (100.0%)	31/31 (100.0%)
GSA-II	7/11 (63.6%)	12/14 (85.7%)	7/11 (63.6%)	26/36 (72.2%)	8/8 (100.0%)	12/12 (100.0%)	11/11 (100.0%)	31/31 (100.0%)
DBA	10/11 (90.9%)	11/14 (78.6%)	8/11 (72.7%)	29/36 (80.6%)	1/8 (9.1%)	2/12 (16.7%)	1/11 (12.5%)	4/31 (12.9%)

RC = right colon; LC = left colon; Rect = rectum.

Table 5.
Positive Rate for Binding of Various Lectins in Colorectal Background Mucosa from Different Sites

	Study Group											
	Control			Adenoma			Cancer			FAP		
	RC	LC	Rect	RC	LC	Rect	RC	LC	Rect	RC	LC	Rect
UEA-I	20/20 (100.0%)	14/20 (70.0%)	8/36 (22.2%)	10/10 (100.0%)	8/10 (80.0%)	9/34 (26.5%)	10/10 (100.0%)	9/10 (90.0%)	19/44 (43.2%)	10/10 (100.0%)	9/10 (90.0%)	7/10 (70.0%)
PNA	0/20 (0.0%)	0/20 (0.0%)	2/36 (5.6%)	2/10 (20.0%)	2/10 (20.0%)	3/34 (8.8%)	2/10 (20.0%)	1/10 (10.0%)	9/44 (20.5%)	6/10 (60.0%)	6/10 (60.0%)	6/10 (60.0%)
GSA-II	0/20 (0.0%)	0/20 (0.0%)	3/36 (8.3%)	0/10 (0.0%)	0/10 (0.0%)	5/34 (14.7%)	1/10 (10.0%)	0/10 (0.0%)	7/44 (15.9%)	6/10 (60.0%)	6/10 (60.0%)	6/10 (60.0%)
DBA	20/20 (100.0%)	20/20 (100.0%)	36/36 (100.0%)	10/10 (100.0%)	10/10 (100.0%)	34/34 (100.0%)	44/44 (100.0%)	44/44 (100.0%)	44/44 (100.0%)	10/10 (100.0%)	10/10 (100.0%)	10/10 (100.0%)

RC = right colon; LC = left colon; Rect = rectum.

In the FAP patients, UEA-I was positive in the rectum of all four profuse-type cases and in three (50.0 percent) of six cases of the sparse-type. PNA was positive in all profuse-type and in two (33.3 percent) sparse-type cases. GSA-II showed exactly the same staining characteristics regarding positivity rate with site or polyp density, and DBA was positive for all regions in all cases of both profuse and sparse-types.

Family History

Relationships between family history of colorectal cancer and the lectin-binding ability of rectal background mucosa are summarized in Table 6. A "positive family history" was concluded in seven patients (19.4 percent) from the control group, four (11.8 percent) from the adenoma group, and 13 (29.5 percent) from the cancer group. Among the four lectins tested, only UEA-I showed a statistical association between binding affinity of rectal mucosa and positive family history, the correlation for UEA-I-positive patients in the adenoma group being 33.3 percent, significantly higher ($P < 0.05$) than the 4.0 percent figure for UEA-I-negative patients. In the cancer group, the relationship was even clearer, 63.2 percent of the UEA-I-positive patients demonstrating a family history, as opposed to a mere 4.0 percent in the UEA-I-negative patients ($P < 0.01$).

DISCUSSION

The present comparative investigation of PNA, UEA-I, GSA-II, and DBA lectin binding in the large intestine of control, FAP, and tumor patients demonstrated a clear correlation between a positive UEA-I reaction and familial cancer history. Thus, while the binding of PNA, GSA-II, or DBA in any of the groups was not significantly related to a

Table 6.
Correlation Between Family History and Lectin Binding in Rectal Mucosa from Control, Adenoma, and Cancer Patients

Lectins	Control (n = 36)		Adenoma Patients (n = 34)		Cancer Patients (n = 44)	
	Family history		Family history		Family History	
	+	−	+	−	+	−
UEA-I	3[a]/8[b] (37.5%)	4[a]/28[c] (14.3%)	3/9 (33.3%)	1/25 (4.0%)*	12/19 (63.2%)	1/25 (4.0%)**
PNA	0/2 (0%)	7/34 (20.6%)	0/3 (0%)	4/31 (12.9%)	4/9 (44.4%)	9/35 (25.7%)
GSA-II	1/3 (33.3%)	6/33 (18.2%)	1/5 (20.0%)	3/29 (10.3%)	4/7 (57.1%)	9/37 (24.3%)
DBA	7/36 (19.4%)	—	4/34 (11.8%)	—	13/44 (29.5%)	—

* $P < 0.05$; ** $P < 0.01$.
[a] A numerator shows number of positive family history.
[b] A denominator shows number of positive lectin staining.
[c] A denominator shows number of negative lectin staining.

history of large bowel cancer development, that of UEA-I in patients with adenomas or cancers was significantly higher in familial cancer history patients.

It is of particular interest that, in our study, affinity for UEA-I staining was evident for goblet-cell mucin of normal-appearing crypts of the rectum in 22.2 percent of the control group, in 26.5 percent of the adenoma group, and in 43.2 percent of the cancer group. Jacobs and Huber[13] and Yuan *et al.*,[10] using UEA-I, reported low positivity rates for normal rectal epithelium of 12.5 percent and 11.1 percent, respectively, while Yonezawa *et al.*,[6] Bresalier *et al.*,[9] and Rhodes *et al.*,[7] described normal rectal epithelium as lacking UEA-I binding sites. The differences in positive rates between these and our studies are presumably due, at least in part, to subject selection. The present results would suggest less or no involvement of large intestinal cancer or family history of large bowel cancer in their series. Certainly this is the case for the Jacobs and Huber[13] study, which concentrated on inflammatory disease.

The various regions of the large intestine differ not only light-microscopically and electron-microscopically,[14] but also in histochemical characteristics,[15] as demonstrated by high-iron diamine-Alcian blue (HID-AB) staining. In rats, Freeman *et al.*,[16] concluded that differences in lectin affinity for large intestinal mucosa in each region directly reflect differences in cellular protein components of individual cells. In humans, most blood group substances are present throughout the colon in the embryo and fetus[17] but disappear from the distal colon in the adult.[18,19] They can reappear, however, under conditions related to cancer. In the present FAP series, UEA-I binding sugars were present in the distal colon and rectum of all profuse-type and in 50 percent of the sparse-type cases. Particularly in the former, not only the adenoma tissue, but also the intervening mucosa between polyps, was positive, in line with the results of Yonezawa *et al.*[11] and supporting the view that antigen expression in background mucosa of FAP resembles tumor tissue more than normal tissue. The present results for UEA-I binding would indicate that background mucosa of other high-risk patients may demonstrate a similar shift. Matsushita *et al.*[20] reported that the UEA-I binding glycoproteins in cancer tissue of the proximal colon were smaller in molecular weight than those in the normal mucosa by means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the Western blotting method. It remains to be seen whether the UEA-I binding glycoproteins in the background rectal mucosa of the colorectal cancer high-risk group or FAP are identical with those in tumor tissue. Muto *et al.*[21] earlier used Periodic acid-thionin Schiff/Potassium hydroxide/Periodic acid-Schiff (PAT/KOH/PAS) staining for effective diagnosis of FAP, reporting that, in the background mucosa of FAP cases, the position of acylation differed at $C_{7,8,9}$ of sialomucin from that of normal colorectal mucosa. Lipkin and Deschner[22,23] demonstrated in their autoradiography cytokinetic studies that, by using [^{3}H]-thymidine, the intestinal gland proliferating cell layers extended to the surface of the mucosa in FAP patients, but not in controls. They concluded, therefore, that suppression of DNA synthesis is absent in these patients, indicating the onset of abnormal cell differentiation prior to morphologic changes in apparently normal mucosa. Expression of UEA-I might be interpreted similarly.

The biologic significance of the phenomenon of re-expression of sugar antigens by colonic tumor tissue remains unclear; however, it may be of clinical importance as a marker for neoplastic transformation in the colon and rectum. Thus, detection of background rectal epithelial expression of UEA-I binding may be useful in early diagnosis and localization of neoplastic transformation. The case of performance of rectal biopsy and the simplicity of the screening technique for UEA-I binding suggest its introduction for better definition of high-risk colorectal cancer.

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