J Clin Pathol 1986;**39**:1331–1334

Glycoprotein abnormalities in colonic carcinomata, adenomata, and hyperplastic polyps shown by lectin peroxidase histochemistry

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SUMMARY A technique for lectin-peroxidase histochemistry was adapted for the study of formalin fixed paraffin embedded colonic tissue. Ten lectins with differing carbohydrate binding specificity were tested against 20 normal rectal biopsy specimens and tissue from 19 colonic carcinomata, 19 tubular or tubulovillous adenomata, and 19 hyperplastic polyps. None of the normal rectal biopsy specimens bound the lectins peanut agglutinin (PNA), Griffonia simplicifolia II (GSII), and Ulex europaeus I (UEAI), whereas 18 carcinomata, 12 adenomata, and 18 hyperplastic polyps showed affinity for one or more of these lectins.

Hyperplastic colonic polyps are shown to possess similar abnormalities in glycoprotein structure to malignant and adenomatous colonic tissue. This may simply indicate a non-specific reaction to changed rates of cell proliferation but might represent a more fundamental association between hyperplastic polyps and adenocarcinomas.

There has been considerable interest in recent years in the use of lectins to detect changes in glycoprotein structure in malignant tissue. Lectins are glycoproteins found both in plants and animals that bind to specific carbohydrate moieties. Peanut agglutinin (PNA), which recognises βD Gal 1–3 D gal NAc, has been shown to bind to malignant breast,[1–3] lymphoid,[4] and colonic tissue.[5–7] Colonic adenomata may also bind PNA, and it has been suggested that PNA positive adenomata may have an increased risk of malignant transformation.[8] This is difficult to reconcile with the report that hyperplastic polyps are even more likely to be PNA positive[8] than adenomata.

Previous reported studies of lectin histochemistry in colonic disease have been performed using fluorescein tagged lectins. The disadvantage of this technique, as with all other fluorescein histochemical techniques, is that the sections have to be reported before the fluorescence fades, and this limits the size of study performed. Previous lectin and histochemical studies of colonic tissue have used either fairly small numbers of tissue samples or only a few (one to five) lectins. Peroxidase tagged lectins do not have this disadvantage, although not all workers have had success with them.[9] We have found that the technique for lectin peroxidase histochemistry described by Kuhlmann *et al*[10] works reliably with formalin fixed paraffin embedded colonic tissue and uses very low concentrations of lectin. This has allowed us to perform a larger study of lectin histochemistry of colonic carcinoma, adenomata, and hyperplasia using 10 peroxidase tagged lectins selected on the basis of their differing carbohydrate specificities.

Accepted for publication 30 June 1986

Material and methods

Tissue was obtained from 19 colonic carcinoma resection specimens, 19 tubular and tubulovillous adenomata, and 19 hyperplastic polyps that had been removed colonoscopically. Rectal biopsy specimens from 20 patients with irritable bowel syndrome (all sigmoidoscopically and histologically normal) were studied. Most of these tissue samples were gathered prospectively and in all cases tissue had been fixed in 10% formaldehyde for 12–24 hours at room temperature, washed, dehydrated and paraffin embedded. Eleven 5 μm sections were cut from each specimen (10 for lectin binding and one for routine haematoxylin and eosin staining), placed on acetone cleaned slides, deparaffinated in xylene and passed from absolute ethanol into 0·01 M phosphate buffered saline (PBS), pH 7·2. Lectin-peroxidase binding was performed

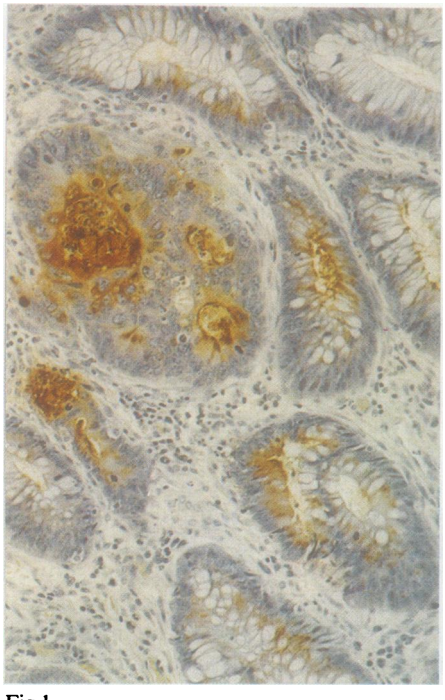

Fig 1

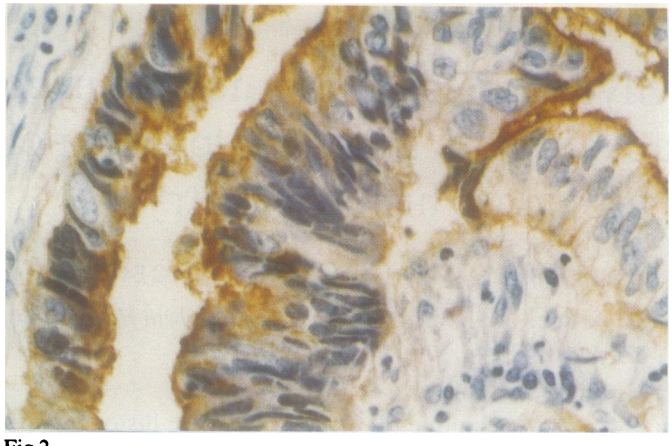

Fig 2

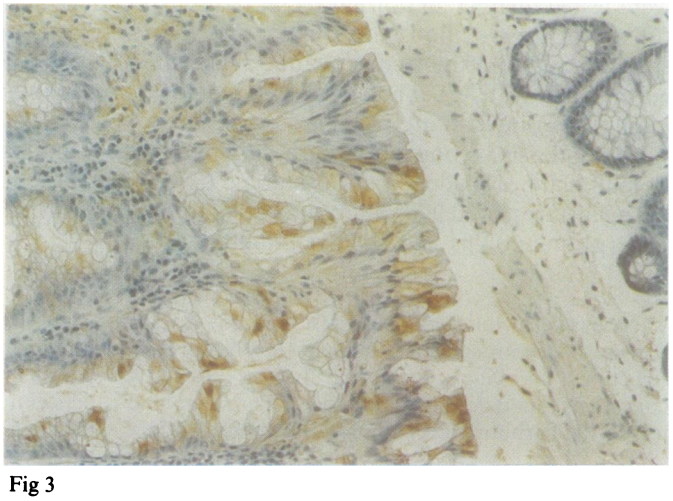

Fig 3

Fig 1 *Colonic carcinoma and adjacent mucosa showing positivity with PNA peroxidase (Haematoxylin and PNA peroxidase) × 150.*

Fig 2 *Colonic adenoma showing surface positivity with UEAI peroxidase (Haematoxylin and UEAI peroxidase.) × 250.*

Fig 3 *Colonic hyperplastic polyp showing positivity with PNA peroxidase. Nearby normal mucosa negative (Haematoxylin and PNA peroxidase.) × 250.*

using the method of Kuhlmann *et al*[10] with slight modifications. Endogenous peroxidases were inhibited by treatment with 1% hydrogen peroxide in PBS for one hour. Sections were then washed in buffer, which varied according to the lectin used (table 1). They were then incubated in one of the lectin peroxidase conjugates under test (0·002 mg lectin conjugate/ml of appropriate buffer) for 24 hours at 4°C. Ten lectins were used (table 1). Peroxidase tagged lectins were obtained from EY Laboratories, United States. After incubation sections were washed three times in PBS (five minutes each wash) and peroxidase activity of bound lectin-peroxidase conjugates was shown by incubation in 3,3′ diaminobenzidine and H_2O_2.[11] Sections were then washed, counterstained lightly with haematoxylin, dehydrated and mounted. Parallel experiments, in which lectin binding was inhibited by pre-incubation of each lectin with its appropriate binding sugar, confirmed the specificity of lectin binding.

Table 1 *Lectins used in this study*

Origin	*Abbreviation*	*Buffer*	*Specificity*
Peanut	PNA	0·01 M PBS pH 7·2	D Gal B (1 → 3) Gal NAc (T antigen)
Gorse seed	UEA_1	,,	α-L-fucose (H antigen)
Griffonia seed	GSII	,,	DGlc NAc (TK antigen)
Wheat germ	WGA	,,	Neu NAc, Glc NAc
Soy bean	SBA	,,	α-D-Gal NAc, D-Gal
Horse gram	DBA	,,	α-D-Gal Nac, D-Gal
Osage orange seed	MPA	,,	α-D-Gal
Griffonia seed	GSI	0·01 M PBS pH 7·2 + 0·001 M $CaCl_2$	α-D-Gal
Jack Bean	Con A	0·005 M Tris saline pH 7·0 + 0·01 M $CaCl_2$ + 0·01 M $MgCl_2$	α-D-Man, α-D-Glc
Horseshoe crab	LPA	0·05 Tris saline pH 8·0 + 0·001 M Ca Cl_2	Neu NAc

Results

All biopsy specimens bound WGA (galactose and sialic acid binding) as expected. No normal rectal mucosa bound PNA (βgal 1–3β gal NAc binding), UEA (α-fucose binding), or GSII (glc NAc binding), whereas a high proportion of abnormal biopsy specimens bound these lectins (table 2). PNA and GSII both showed a similar pattern of binding with supranuclear labelling of abnormal cells as well as labelling of free mucus and cell walls. UEA showed a distinctive pattern with strong labelling of the border of abnormal cells and less mucin staining. Mucosa adjacent to carcinoma cells often exhibited the same lectin binding abnormalities (table 2). This abnormality was usually confined to the three or four crypts nearest to the carcinoma. Mucosa adjacent to adenomata or hyperplastic polyps, however, showed normal lectin binding in all but four cases (three adenomata had adjacent mucosa positive for PNA but not UEA or GSII, and one hyperplastic polyp had adjacent mucosa positive for PNA and GSII but not UEA). In all other cases the distinction between lectin binding of the normal and diseased mucosa was striking. Hyperplastic polyps showed a similar pattern of lectin binding to the carcinomas and adenomata. The lectins SBA, DBA, MPA and GSI bound varying proportions of the biopsy specimens tested, but no obvious differences between the groups were shown by these lectins, although a high proportion of hyperplastic polyps bound SBA and GSI (table 2), ConA and LPA did not bind to any of the biopsy specimens.

Discussion

There are now a considerable number of published papers showing that peanut lectin has a strong affinity for malignant or premalignant epithelium, but this study confirms the findings of a previous report—that is, the peanut lectin binding by hyperplastic polyps,[8] which are not usually thought to be premalignant. Binding of this lectin usually indicates the presence of an exposed galactose at the end of the glycoprotein side chain, which would imply some degree of desialylation or lack of sialylation, as the colonic mucosal and cellular glycoproteins are usually sialylated—that is, they contain sialic acid as the terminal residue on the glycoprotein side chains. As a corollary to this, previous studies have shown that treating red blood cells with sialidase renders them agglutinable by the peanut lectin.[12] The peanut lectin positivity of colonic carcinomata therefore seems to contradict the findings of previous studies, which have shown an increased sialic acid content in tumours or transitional mucosa.[13] This apparent paradox could perhaps be explained if non-sialylated galactose had been added subterminally as a side branch on the glycoprotein side chains while leaving the terminal sialic acid residues in situ. Such increased branching of glycoprotein side chains has been reported previously in malignant tissue.[14]

The (UEAI) and (GS II) positivity found in benign and malignant colonic polyps implies the presence of exposed fucose (UEAI binding) and NAc glucosamine (GSII binding). Previous studies have

Table 2 *Lectin binding to normal rectal mucosa and colonic carcinomata, adenomata, and hyperplastic polyps*

	PNA	*UEAI*	*GSII*	*WGA*	*SBA*	*DBA*	*MPA*	*GSI*	*ConA*	*LPA*
Normals (n = 20)	0	0	0	20	6	12	18	5	0	0
Carcinoma (n = 19)	17	15	14	19	8	3	19	5	0	0
Adjacent mucosa (n = 16)	9	0	1	16	6	5	16	0	0	0
Adenomatous polyps (n = 20)	8	8	3	20	2	1	20	11	0	0
Hyperplastic polyps (n = 19)	16	15	17	19	18	10	19	17	0	0

shown a relative decrease in fucose and N-ac glucosamine content of colonic carcinoma tissue, so it is likely that the UEA2 and GSII positivity reflects increased exposure of fucose and NAc glucosamine, possibly due to shortening of the hexose side chains, rather than an absolute increase in these sugars.[15] Whereas the peanut lectin binding is predominantly intracellular and probably reflects a change in mucus synthesis within the Golgi apparatus, the UEAI binding is almost exclusively directed to the cell surface and must reflect changes in structural glycoproteins.

It is striking that hyperplastic colonic polyps show the same changes in lectin binding as those in colonic carcinomas and adenomata. One possibility that cannot be excluded is that hyperplastic polyps may represent either a very early stage in the malignant process or reflect a change in cellular function that is an essential part of that process. This latter point of view has been advanced recently by Jass, who pointed out some other functional similarities between hyperplastic and malignant colonic tissue[16] including the production of carcinoembryonic antigen (CEA) by hyperplastic polyps.[16-19]

This study has shown that lectin peroxidase histochemistry can be performed simply and reproducibly on formalin fixed paraffin embedded tissue. The very low concentration of lectins used in this technique confers considerable advantage both in terms of cost and specificity of binding. This study confirms that there are abnormalities in the hexose content of mucus and structural glycoproteins, which are common to hyperplastic, adenomatous, and carcinomatous colonic epithelium. Further study of these biochemical abnormalities may lead to a better understanding of the association between hyperplasia and malignant change.

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