Lectin-peroxidase conjugates in histopathology of gastrointestinal mucosa

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Summary

Peroxidase (HRP) conjugates of *Dolichos biflorus* agglutinin (DBA), *Arachis hypogaea* agglutinin (PNA) and *Ulex europaeus* agglutinin (UEA I) were used as specific molecular probes in order to describe histological patterns of carbohydrate moieties in glycoconjugates of gastric mucosa. Specimens of normal mucosa, metaplasias and carcinomas were fixed, embedded in paraffin and stained with lectins by a postembedding technique.

In normal mucosa, DBA reacted with surface epithelial, parietal and antral gland cells; neck cells did not react. In single goblet cell metaplasia, with few exceptions, goblet cells showed DBA binding. In contrast, mainly negative goblet cells were seen in intestinal metaplasia. In gastric carcinomas of intestinal or diffuse types, heterogenous staining patterns were observed.

Large numbers of mucus-producing surface epithelial, neck and antral gland cells stained with PNA-HRP conjugates. Parietal cells did not react. Among the chief cells only few showed PNA binding. In single goblet cell metaplasia, equal numbers of goblet cells were positive or negative, whereas goblet cells in intestinal metaplasia were mainly unstained. Carcinomas contained both PNA-positive and PNA-negative cells; PNA-positive cells occurred preferentially in the tumor periphery.

UEA I-HRP conjugates stained the bulk of surface epithelial and neck cells; chief cells and antral gland cells were mainly positive, too. Parietal cells did not react. In single goblet cell metaplasia and intestinal metaplasia, UEA I-positive as well as UEA-negative goblet cells occurred. Striated cells exhibited a great variety of lectin reactivity.

Under both normal conditions and in carcinoma develoment, qualitative and quantitative differences in the composition of glycoconjugates must be expected.

Key words: Lectins – Glycoconjugates – Gastric mucosa – Histopathology

Introduction

Structure-function relationships in normal gastrointestinal mucosa, especially in disease, are not readily diagnosed by use of routine histopathological staining methods. The introduction of histochemical stainings such as periodic acid-Schiff and Alcian blue at pH 2.5 (Mowry 1963), Alcian blue at pH 1.0 (Lev and Spicer 1964) and high iron-diamine/Alcian blue (Spicer 1965) was a significant advance in staining mucins by their chemical properties. Moreover, several special methods which induce defined chemical or enzymatic alterations in tissue sections have been developed for the staining of reactive groups in epithelial mucins (review by Filipe 1979). However, the complexity and poor reproducibility of these methods has prevented their routine application. Apart from this, clear-cut specificity according to the criteria of modern biochemistry and immunology, cannot be achieved. Detailed data on the complex composition of mucins, their pathways of synthesis and excretion and their morphological relationship to normal ontogeny and disease are still scanty and subject to controversy.

Gastrointestinal mucins are glycoconjugates containing various amounts of different carbohydrate moieties. Because lectins possess a high affinity and a narrow range of specificity for defined sugar residues, lectins appear to provide a potential approach for their histological identification. Apart from the use of lectins as agglutination reagents in ABH blood group typing, they became of major interest in cell biology when Burger and Goldberg (1967) described a "tumor-specific" determinant on neoplastic cell surfaces detectable by a wheat germ agglutinin. In the meantime a large number of lectins with differing specificities are now known and found in a variety of plants, invertebrates and vertebrates (Lis and Sharon 1973; Simpson etal. 1978; Uhlenbruck et al. 1979; Barondes 1981). Some of them have been applied especially as serological tools for the study of carbohydrate receptors on the cell surface of normal, transformed and malignant cells or for cell fractionation (Burger 1969; Inbar and Sachs 1969; Sela etal. 1970; Inbar et al. 1972; Reisner and Sharon 1980).

In a similar way to labelled antibodies in immunohistological techniques, lectins conjugated with fluorescein, ferritin, enzymes etc. can be used. Consequently, attempts have been made to localize carbohydrate moieties in cell surfaces (Nicolson and Singer 1971; Huet and Garrido 1972; Gonatas and Avrameas 1973) and in intracellular Spaces (Etzler and Branstrator 1974; Freeman etal. 1980; Stoward etal. 1980; Suzuki etal. 1981; Sato and Spicer 1982; Boland et al. 1982 a) at both light and electron microscopic levels. Thus, cellular details are visualized which due to molecular specificity, differ from those detectable by immunohistology. In these studies, animal tissues were mainly employed.

It is expected that new information will be gathered about the carbohydrate composition of glycoconjugates in gastrointestinal mucosae by lectin-labelling experiments. This might be useful for histopathological diagnosis. In the present study, we have taken differently fixed and paraffm embedded tissues from normal and diseased human gastric mucosa. Peroxidase conjugated lectins from *Arachis hypogaea* with specificity for D-galactose → N-acetyl-galactosamine (β-D-Gal-(l → 3)-D-GalNAc), from *Dolichos biflorus* with specificities for D-galactose (D-Gal) and N-acetyl-galactosamine (α-D-GalNAc) and from *Ulex europaeus* with specificity for L-fucose (α-L-Fucose) were used in postembedding staining technique for the localization of the respective sugar residues in tissue sections.

Material and Methods

Biochemicals. Peroxidase (HRP) conjugates of *Arachis hypogaea* agglutinin, *Dolichos btflorus* agglutinin and *Ulex europaeus* agglutinin I were obtained from Medac GmbH (Hamburg, FRG). The respective inhibitors, i.e. D-galactose and lactose (Lotan et al. 1975), D-galactose (Etzler and Kabat 1970) and L-fucose (Matsumoto and Osawa 1969), were purchased from Merck (Darmstadt, FRG). Lectins, their specificities and inhibitors used for control studies are summarized in Table 1.

Table 1. List of lectins used in this study

Origin of lectins	Abbreviation	Specificity	Inhibitor
Arachis hypogaea (peanut)	PNA	β-D-Gal-$(1 \rightarrow 3)$-D-GalNAc, D-Gal	D-Gal Lactose
Dolichos biflorus (horse gram)	DBA	α-D-GalNAc, D-Gal	D-Gal
Ulex europaeus I (gorse seeds)	UEA I	α-L-Fucose	L-Fucose

Tissues. Human gastric mucosae of blood groups A, B, AB and 0 were obtained through surgery. Nine stomachs were resected from individuals with carcinoma and one was from an ulcer patient. For classification of single goblet cell metaplasia and of intestinal metaplasia see Konjetzny (1928) and Goldman and Ming (1968). For gastric cancer, the nomenclature of Lauren (1965) was used. Stomachs were opened along the larger curvature. From each stomach, multiple samples were taken; corpus and antrum were processed separately. Specimens were either fixed in 10% formaldehyde for 12-24 h at room temperature, washed, dehydrated and embedded in paraffin according to classical procedures in histopathology or fixed in 99% ethanol - 1% acetic acid for 12-15 h at 0° - 4° C, dehydrated in absolute ethanol, cleared in benzene and embedded in paraffin (Kuhlmann 1975; Wurster et al. 1978).

Histology and Lectin Staining. Routine histology was performed on sections with H.E., PAS and Alcian blue at pH 2.5.

For lectin staining, 5-7 μm thick sections were mounted on acetone cleaned slides, deparaffinated in xylene and passed from absolute ethanol into phosphate-buffered saline (0.01 M phosphate buffer pH 7.2 plus 0.15 M NaCl; PBS). Prior to incubation in lectin conjugates, endogenous peroxidases were irreversibly inhibited by treatment with 1% hydrogen peroxide in PBS for 1 h (Kuhlmann 1975). Slides were then washed in PBS and incubated with either of the above described HRP-labelled lectins (0.001-0.02 mg/ml PBS) for 24 h at 4° C. Unreacted conjugates were washed off by three successive washings for 5 min each in PBS. Peroxidase activity was revealed by incubation in 3,3'-diaminobenzidine and H_2O_2 (Graham and Karnovsky 1966). After washings in PBS, sections were dehydrated and mounted under coverglass.

Controls. Specificity of lectin binding was checked on serial sections by incubation as above in the HRP-labelled lectins which have been supplemented with either of the respective inhibitors: 0.2 M D-galactose and 0.2 M lactose for PNA; 0.2 M D-galactose for DBA; 0.2 M L-fucose for UEA I (Table 1). Each procedure was followed by washings in PBS and the enzyme Substrate.

Results

Three carbohydrate specific lectins have been chosen as probes in order to obtain information about their respective binding patterns in human gastric mucosa. Experiments with variously fixed tissue specimens indicated that fixations based on either formaldehyde or ethanol-acetic acid could be employed with equal success.

Specificity controls with selected inhibitors showed that typical serological lectin binding studies could be readily adapted to tissue sections for postembedding lectin-stainings. Prior to incubation in lectin-HRP conjugates, endogenous peroxidases must be irreversibly inhibited in order not to interfere with staining derived from the lectin conjugates. This step was done by treatment of sections with 1 % hydrogen peroxide for 1 h.

Histological localizations with HRP conjugates of DBA, PNA and UEA I are shown in Figs. 1-4. All observations with the different types of mucosa cells are summarized in Table 2. In Table 3, data on lectin stainings in individual carcinomas of the intestinal type are shown separately.

Table 2. Localization of lectin-binding in normal gastric mucosa and in metaplasia

Localization	DBA	PNA	UEA I
Surface epithelial cells	mainly + [a] few −	+ and −	+
Neck cells	−	+ and −	+
Parietal cells	+	−	−
Chief cells	−	mainly − few +	mainly + few −
Antral gland cells	+ and −	mainly + few −	mainly + few −
Single goblet cell metaplasia	mainly + few −	+ and −	+ and −
Intestinal metaplasia goblet cells	mainly − few +	mainly − few +	+ and −
columnar cells	−	mainly − few +	+ and −

[a] Intensity of reactions: − No; + clear positive; + and − positive as well as negative cell areas; see text

Table 3. Detection of lectin-binding in gastric carcinomas

Lectin	Human gastric carcinomas of intestinal type (5 cases)				
	29243/76[a]	5406/78	8778/79	2922/80	6409/80
DBA	−	−	−	mainly − few +	−
PNA	mainly − few +	mainly − few +	mainly − few +	−	+ and −
UEA I	−	+	mainly − few +	−	mainly − few +

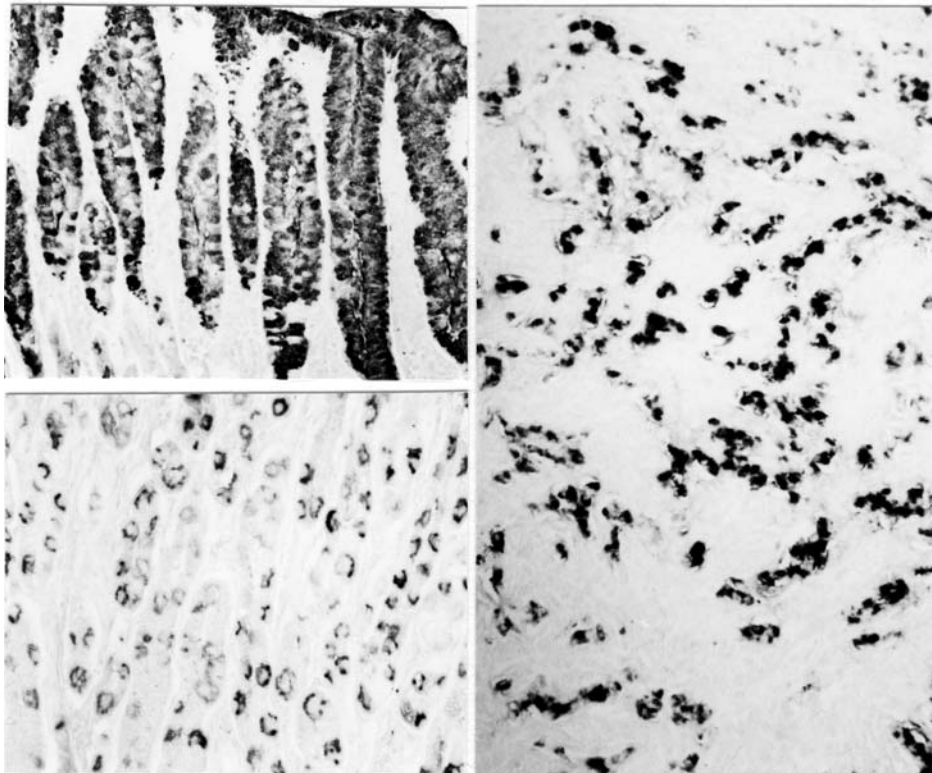[a] Histopathological specimen number

Fig.1 a, b. Gastric body mucosa with DBA binding. **a** Surface epithelial cells. Original x 160. **b** Parietal cells. Original x 250

Fig. 2. Gastric carcinoma of the diffuse type with PNA binding tumor cells besides not reacting tumor cells. Original x 160
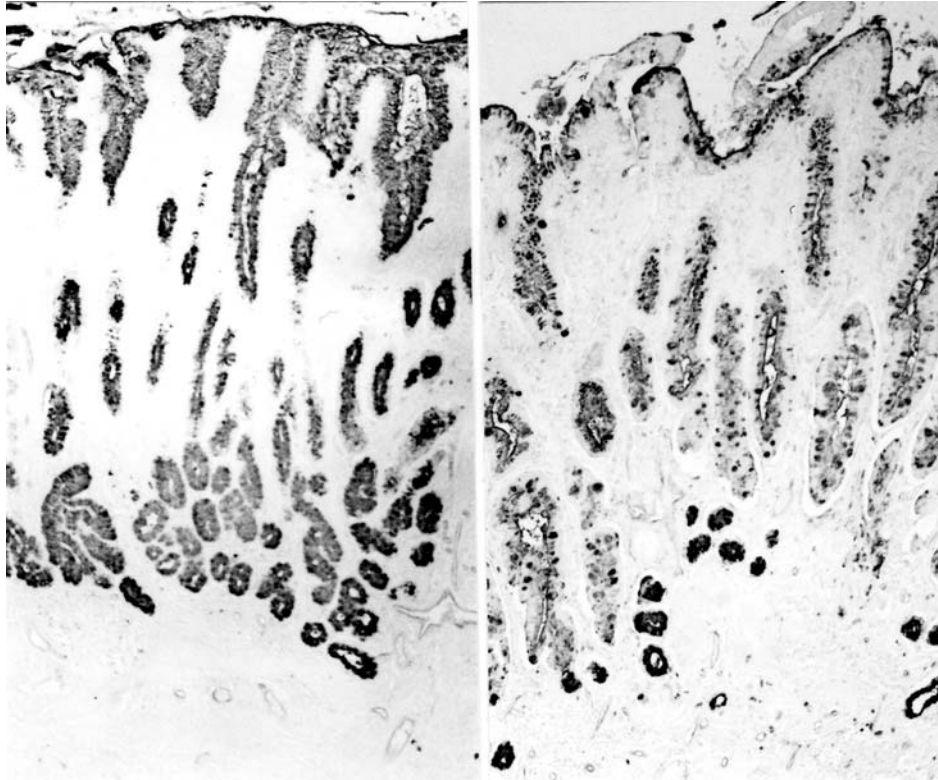
Fig.3. Gastric antral mucosa with UEA I binding by surface epithelial cells, neck cells and antral gland cells. Original x 63

Fig. 4. Gastric antral mucosa with chronic inflammatory changes. Binding of UEA I by many striated columnar cells and goblet cells of intestinal metaplasia and by residual antral gland cells. Original x 63

Discussion

Combined analysis of mucosal cells by biochemistry, immunology and histology provides detailed data about their heterogenous molecular composition. Knowledge of the latter is a prerequisite for the defmition of cell-associated markers which are useful for comprehensive histopathology. For this purpose, immuno-analysis of mucosae and application of immuno-histological methods have great promise (for references see Häkkinen et al. 1968; Kuhlmann et al. 1970; Rapp et al. 1979; Bara et al. 1980). Nevertheless, it can be expected that the use of lectins for the description of carbohydrate moieties in mucosal glycoconjugates will also be of importance. Successive enzymatic digestions of tissue components were not tried in these experiments. Thus, mainly terminal carbohydrate groups were stained rather than inner chain ones. It is the aim of subsequent studies to perform selective digestions in order to localize such moieties.

Cellular DBA Binding

Intracellular DBA reactivity was observed in the cytoplasm of most of the surface epithelial cells. However, in some sections non-reactive cells of the same morphology were also seen. Neck and chief cells did not contain DBA binding substances. In contrast, parietal cells showed positive reactivity in all preparations. In antral gland cells, an irregular staining pattern was observed throughout. Interestingly, all the three mucus producing cell types (i.e. surface epithelial cells, neck cells and antral gland cells) which are usually PAS positive due to neutral glycoconjugates (Lev 1966) showed a different DBA binding behaviour. Thus, PAS

and DBA staining are independent of each other and it remains to be seen to what extent the latter reflect different functional states or maturation stages.

In single goblet cell metaplasia in the stomach surface epithelium, goblet cells with few exceptions contained DBA reactivity. The observation of similar histochemical behaviour in both single goblet cell metaplasia and normal intact surface epithelial cells may reflect identical secretory function. In contrast to single goblet cell metaplasia, mainly negative goblet cells were seen in intestinal metaplasia; only few goblet cells reacted with DBA. Striated columnar cells were always negative. For goblet cells in rat small intestine, Etzler and Branstrator (1974) observed heterogenous DBA staining inasmuch as not all goblet cells reacted. They attributed the different staining behaviour to maturation of goblet cells in which mucins with different carbohydrate portions might be synthesized.

Of five carcinomas of intestinal type, cells from four tumors showed no DBA reactivity at all. In one carcinoma, DBA staining was predominantly negative with a few intermingled positive cells. DBA staining appeared to be more heterogenous in carcinomas of the diffuse type. Only in the tissue specimens of one carcinoma were all the carcinoma cells present negative; other carcinomas were composed of both positive and negative cells. The heterogenous results are similar to those reported for colon carcinoma (Boland et al. 1982 a) and which were considered to be due to molecular heterogeneity of mucins synthesized by the cancer cells.

DBA specifically recognizes terminal nonreducing GalNAc residues which are found in blood group A (Etzler and Kabat 1970). As described above, GalNAc could be detected in a variety of mucosal cells. Because such observations were made in gastric mucosa specimens of donor blood groups A, B, AB and 0, DBA staining patterns did not merely reflect blood group reactivity.

Cellular PNA Binding

Large numbers of mucus-producing surface epithelium, neck and antral gland cells exhibited cytoplasmic affmity for PNA, but unreactive cells could also be observed. Parietal cells did not stain with PNA-HRP conjugates. Among the chief cells only a few showed lectin binding.

In Single goblet cell metaplasia, approximately equal numbers of goblet cells were either PNA-positive or PNA-negative. This distribution represented a similar pattern to that found in the surface epithelial cells. On the other hand, goblet cells in intestinal metaplasia were mainly unstained. Also, few of the striated columnar cells stained with PNA-HRP conjugates. In comparison with DBA experiments, considerable variations in positive and negative PNA stainings were also observed in the different mucus-producing cell types. As already mentioned, variations in lectin binding are the result of changes in glycoconjugate composition associated with cell function. Detailed conclusions cannot be drawn at present.

Carcinomas of the intestinal and diffuse types contained both PNA-positive and PNA-negative cells of which PNA-positive cells occurred preferentially in the tumor periphery adjacent to normal mucosa. One carcinoma of intestinal type was completely negative. This tumor was the only one which contained few DBA-reactive cells. In other carcinomas of the human gastrointestinal tract, PNA binding might behave very differently. Thus, colonic carcinomas were described as having positive PNA reactivity in secretory active tumor cells whereas goblet cells in normal colonic mucosa did not (Boland et al. 1982a). These observations may reflect significant differences in goblet cell differentiation and function in the various regions of the gastrointestinal tract. With respect to glycoconjugate synthesis (at least for carbohydrates) data on gastric cancer and on colon cancer are not directly comparable.

PNA is known to possess high affinity for D-galactose and D-galactose → N-acetyl-galactos-amine. The latter represents the *Thomsen-Friedenreich*-antigen. This cryptic antigen can be exposed after neuraminidase treatment of normal cells, but may also occur in breast, gastric and colonic carcinomas (Springer et al. 1975; Hanfland and Uhlenbruck 1978; Boland et al. 1982b). Because no enzymatic digestions were performed with our material, it remains to be clarified if the described detection of intracellular galactose-containing glycoconjugates will represent terminal saccharides or carbohydrate structures in incompletely glycosylated glyco-conjugates. In our studied human material, the observed PNA binding appears not to be due to reaction with blood group antigens.

Cellular UEA I Binding

The bulk of surface epithelial and neck cells showed homogenous cytoplasmic lectin staining. Chief cells and antral gland cells were mainly positive, whereas parietal cells remained negative. UEA I is a fucose-specific lectin and its staining pattern was not surprising since epithelial secretory glycoconjugates contain a limited variety of oligosaccharides, one of which is fucose. The latter was suggested as a possible terminal sugar (Sato and Spicer 1982).

The staining pattern in single goblet cell metaplasia was comparable to that described for PNA and will not be repeated. In intestinal metaplasia, the same observations prevailed. In different specimens, striated cells exhibited a great variety of lectin reactivity, showing (a) lectin binding or lack of binding in a given section; (b) no lectin binding at all; or (c) positive reactivity of all cells in apical portions. This diversity of reactions is not understood at present.

In gastric carcinomas of intestinal and diffuse types, UEA I lectin staining also revealed a heterogenous picture, but identical patterns were not detected. For example, an UEA I-posi-tive carcinoma of intestinal type was DBA-negative, while populations of the same carcino-ma showed positive as well as negative PNA reactions.

UEA I can be employed for the characterization of H(0) blood group in which fucose occurs as terminal oligosaccharide. In our UEA I binding studies, gastric specimens from donors of either A, B, AB or 0 blood group reacted in an analogous way. Hence, blood group depen-dency of the histological lectin bindings were not observed irrespective of the use of normal gastric mucosa or carcinomas.

In conclusion, heterogenous staining patterns occurred with a given lectin in the different cell populations of gastric mucosa as well as heterogenous staining patterns in a given cell population for the various lectins employed. These data indicate qualitative and quantitative differences in the composition of glycoconjugates (e.g. mucins) under both normal conditions and carcinoma development. Intracellular lectin binding did not appear to depend on donor blood groups. With numerous mucosal specimens of either of the above mentioned blood groups, comparable results were obtained. A systematic study with further lectins of distinct specificities is required. The influence of blood group specificities, including the cryptic ones, on the histological lectin stainings should always be checked. It is necessary to expose hidden carbohydrate groups in glycoconjugates by selective enzymatic digestion of terminal carbo-hydrates so that better information about the molecular composition of the former is obtained. Such data can be important in assessing alterations in glycoconjugate synthesis in the course of cell differentiation (including malignancy). It is suggested that the combination of immunohistology and lectin labelling can provide more data than the single use of one of these techniques.

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