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Lectin-binding sites in the epithelium of normal human appendix vermiformis and in acute appendicitis

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Summary. By using histochemical methods, the binding pattern of various lectins in the epithelium of normal human appendix vermiformis was assessed. In addition to plant and invertebrate sugar receptors with nominal monosaccharide specificity for α-L-Fuc (UEA-I), α-D-Man and α-D-Gluc (Con A), α-D-GalNAc (DBA), D-GalNAc (SBA, HPA) ß-D-Gal (RCA-I) and D-Gal (VAA), a mammalian ß-galactoside-specific lectin (MW, 14 kDa) was included in the applied panel. The apical surface of enterocytes presented binding sites for RCA-I on all cells, binding sites of UEA-I, DBA, SBA, HPA and VAA heterogeneously and no binding sites of Con A and 14 kDa. Binding sites of DBA, SBA, HPA, VAA and RCA-I within enterocytes were located primarily focally in a supranuclear position, whereas Con A and 14 kDa bound to the cytoplasm both in apical and basal cell parts.

In the follicle-associated epithelium more enterocytes expressed SBA- and VAA-binding sites than in the crypt epithelium. No differences between the lectin-binding pattern of M-cells and enterocytes were found in the follicle-associated epithelium. Intraepithelial macrophages were heterogeneously positive for the full panel of applied lectins. In contrast, intraepithelial lymphatic cells expressed binding sites only for RCA-I and less prominently for Con A, VAA and 14 kDa. Goblet cell mucus contained lectin-binding sites in a heterogeneous manner: binding sites for Con A were not detected in goblet cells for DBA, SBA, VAA and 14 kDa in less than 20%, for UEA-I in 20-40%, for HPA in 40-60% and for RCA-I in 60-100% of the goblet cells. Secreted mucus differed in its lectin-binding capacity from intracellular goblet cell mucus selectively by an increase of UEA-I, SBA- and RCA-I-binding sites and a lack of 14 kDa-binding sites. Comparative study of lectin binding to goblet cell mucin in another region of the large intestine, namely the rectosigmoid, demonstrated that DBA, SBA and 14 kDa bound mainly to the distal colon, while UEA-I and VAA labelling was selectively found in appendiceal goblet cell mucin.

Comparing the lectin-binding pattern in normal appendix epithelium and in appendicitis, the percentage of goblet cells expressing DBA- and SBA-binding sites in mucus globules was found to be about 4 times higher in appendicitis than in normal appendix. These results demonstrate that the expression of lectin-binding sites in appendiceal goblet mucin is specifically altered in appendicitis, indicating that there are selective changes of glycosylation of mucin in goblet cells mainly of the lower and middle crypt segment. Changes of lectin-binding pattern in appendicitis are discussed in connection with histochemical findings in inflammatory bowel disease.

Key words: Appendix, Appendicitis, Lectin

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Introduction

The human appendix vermiformis harbours a morphologically and functionally highly organized part of the gut-associated lymphoid system (Bockman, 1983; Spencer et al., 1985). The epithelium of the appendix vermiformis represents the receptor part of this appendiceal lymphatic organ (Owen and Jones, 1974). In this organ endogenous glycoconjugates are functionally involved in binding and uptake of intraluminal antigenic material and potentially pathogenic microorganisms (Neutra et al., 1987). Additionally, the epithelial lining of the appendix vermiformis establishes a protective coating against pathogenic noxes from the intestinal lumen (Sisson et al., 1971; Fischer et al., 1984). In particular, mucin glycoproteins secreted by the goblet cell population function as an important part of «non-specific» defence in addition to a complex array of immunological mechanisms (Filipe, 1979; Podolsky, 1989), thus warranting the thorough analysis of their glycocomposition and of any disease-associated alterations. Plant lectins and, recently, isolated mammalian lectins have already proved their value for histochemical detection of glycoconjugates in pathology (Gabius et al., 1991, 1993; Gabius and Gabius, 1993;

Danguy et al., 1994). Consequently, such a carbohydrate epitope-specific probe of mammalian origin will be employed in this stuy.

It is generally accepted that structural alterations in the secreted mucus are directly related to certain gastrointestinal diseases (Filipe and Branfoot, 1976; Filipe, 1979; Filipe and Fenger, 1979; Ehsanullah et al., 1982a,b, 1985; Reid et al., 1984). To addres the issue as to whether the expression of lectin-binding sites in human appendix vermiformis in appendicitis may change, we studied the epithelial lining of normal human appendix vermiformis with histochemical techniques using a panel of plant and mammalian lectins as tools. Comparative histochemical examination of appendicitis was performed to demonstrate inflammation-related changes of the glycoconjugate expression in the human appendix vermiformis.

Materials and methods

Tissue

21 appendix specimens were obtained at the time of surgery from patients operated on for suspected appendicitis. Normal mucosa samples of the sigmoid colon (n=6) and rectum (n=6) were obtained endoscopically from patients investigated for large bowel diseases, in whom no macroscopical or histological colonic lesions had been found. Specimens were fixed in 3.6% paraformaldehyde for paraplast-embedded sections. Histological diagnosis was confirmed by evaluation after conventional haematoxylin and eosin staining. Appendix specimens comprised 12 cases of normal appendix (control) and 9 cases of acute appendicitis.

Lectins

Biotinylated derivatives of concanavalin A (Con A), *Ulex europaeus* agglutinin-I (UEA-I), *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), *Helix pomatia* agglutinin (HPA) and *Ricinus communis* agglutinin-I (RCA-I) were obtained from Sigma Co. (Deisenhofen, FRG). The ß-galactoside-specific lectin with a molecular weight of 14 kDa from bovine heart (14 kDa) and the galactoside-specific lectin from mistletoe (*Viscum album*, VAA), purified as described (Gabius, 1990), were biotinylated with biotinyl-N-hydroxysuccinimide ester in the presence of lactose to protect the active site (Bardosi et al., 1990; Gabius et al., 1992).

Histochemical processing

The sections were processed by rehydration, treatment with 1% hydrogen peroxide to block endogenous peroxidase activity, incubation with 0.1% periodate-treated BSA solutions to saturate unspecific protein-binding sites, incubation with 10 µg/ml biotinylated lectins (Con A, VAA, RCA-I, 14 kDa) or 40 µg/ml lectin (UEA-I, DBA, SPA, HPA), respectively, in phosphate-buffered saline, pH 7.4, containing 0.1% BSA for 4 h at room temperature and then for 1 h with ABC reagents, after through rinses. The formation of the coloured product, visualizing the probe-binding sites, was carried out by incubation for 30 minutes with the following solution: 15 mg 3-amino-9-ethylcarbazole dissolved in 3.75 ml dimethylformamide and added to 71.25 ml 0.1M sodium acetate buffer (pH 5.2), to which 0.75 ml 3% hydrogen peroxide solution was pipetted. Counterstaining was carried out with haematoxylin.

To ascertain the specificity of lectin-carbohydrate interaction, binding of the individual lectins was inhibited by co-incubation with a mixture of 0.2M sugar (inhibition of Con A by α-D-mannose; UEA-I by α-L-fucose; DBA, SBA and HPA by N-acetyl-D-galactosamine; VAA, RCA-I and 14 kDa by ß-lactose).

The extent of staining reactions was evaluated separately in enterocytes of the surface epithelium, in enteroyctes of crypts, in goblet cell secretory globules, in intraluminal mucus of the crypts, in intraepithelial macrophages (identified by the presence of intracytoplasmic inclusions) and in intraepithelial lymphocytes with respect to quantity of stained cells and intensity of staining. The quantity of stained cells (0=0%, (+)=0-20%, +=20-40%, ++=40-60%, +++=60-100%) and the staining intensity were grouped into categories, as given in detail in Table 1. Staining reactions in intracellular regions (apical cell surface, subapical region, supranuclear region, para-retronuclear region) of enterocytes were evaluated separately.

Results

Normal appendix vermiformis (see Table 1)

Histochemical reactions with Con A, UEA-I, DBA, SBA, HPA, VAA, RCA-I and 14 kDa in the epithelium of the human appendix vermiformis resulted in a characteristic staining pattern of epithelial and non-epithelium-derived cells (lymphocytes, macrophages) with respect to intensity of cellular staining, quantity of stained cells, and intracellular distribution of lectin-reactive sites as well as with respect to different cell types and intracellular and secreted mucus, respectively.

Con A (Fig. 1A)

Histochemical reactions with Con A in enterocytes of crypts and the surface epithelium resulted in a rather diffuse granular cytoplasmic staining, excluding the striated border. Intracellular and secreted mucus produced by goblet cells were free of Con A-binding sites. Staining of non-epithelial cells (lymphocytes and macrophages) was heterogeneous and weaker than in enterocytes.

UEA-I (Fig. 1B)

UEA-I bound intensely to the apical surface of most

Table 1. Binding of lectins to epithelium of normal appendix vermiformis.

LOCATION OF LECTIN BINDING	CON-A	UEA-I	DBA	SBA	HPA	VAA	RCA-I	14kDa
Surface enterocytes								
apical cell surface	-/0	+++/4	+/4	++/3	+++/2	+/3	+++/4	-/0
subapical cytoplasm	+++/4	+++/3	++/3	+++/3	+++/3	++/1	+++/4	+++/2
supranuclear cytoplasm	+++/4	+++/4	++/3	+++/3	+++/3	++/2	+++/4	+++/2
para-retronuclear cytoplasm	+++/4	++/3	(+)/2	-/0	(+)/3	-/0	(+)/1	+++/2
Crypt-enterocytes								
subapical cytoplasm	+++/4	+++/3	++/3	+/2	+++/3	(+)/2	+++/3	+++/2
supranuclear cytoplasm	+++/4	+++/3	++/3	+/2	+++/3	(+)/3	+++/3	+++/2
para-retronuclear cytoplasm	+++/4	++/3	(+)/1	-/0	(+)/3	-/0	(+)/1	+++/2
Goblet cells								
Intracellular mucus	-/0	+/2	(+)/3	(+)/3	++/3	(+)/2	+++/2	**(+)/1**
Luminal mucus	-/0	+++/3	(+)/3	+/3	++/3	(+)/2	+++/4	-/0
Intraepithelial macrophages	++/3	++/3	++/2	++/3	++/2	++/3	+++/3	+/1
intraepithelial lymphocytes	+/2	-/0	-/0	-/0	-/0	(+)/3	+++/3	(+)/1

The percentage of positive structures (cellular subsites, cells, mucus) is grouped into the categories: -, 0%; (+); 0-20%;+, 20-40%; ++, 40-60%; and +++, 60-100%. The intensity of staining reaction is grouped into the categories: 0, no staining; 1, weak, but significant staining; 2, medium staining; 3, strong staining; and 4, very strong staining.

enterocytes. In the cytoplasm of enterocytes a focal (patchlike) binding of UEA-I was observed in the subapical cell region and the Golgi region. The para-retronuclear region of enterocytes was either free of binding sites or rather diffusely stained. The Golgi region of enteroyctes in the surface epithelium was mostly more strongly positive than the Golgi region of enterocytes in crypts. Intracellular mucus was only moderately stained in a minority of goblet cells. Lectin-binding to mucus secreted by the goblet cells into the crypt lumen was more intense than to intracellular mucus. Intraepithelial lymphocytes were generally free of binding sites of UEA-I. Macrophages binding UEA-I intensely, were found in the epithelium restricted to the follicle-associated epithelium.

DBA (Figs 1C, 2B)

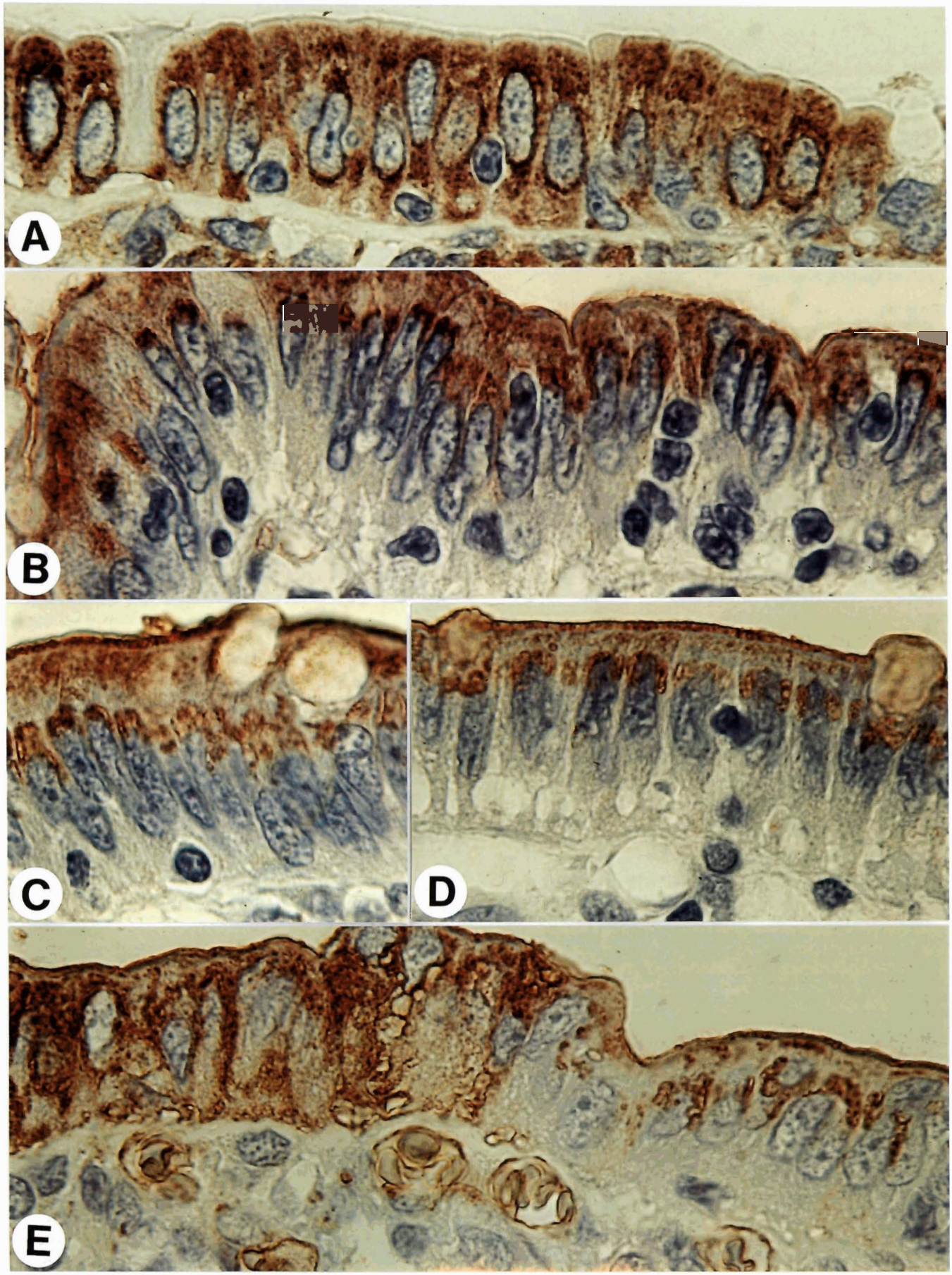
Binding of DBA to the apical surface of enterocytes was heterogeneous. In the cytoplasm of enterocytes an intense, partly vesicular, partly granular binding of DBA focally in about half of the cells was observed, whereas para-retronuclear staining was rather weak, granular and focal; overall restricted to about 16% of the cells. Para-retronuclear staining of enterocytes in crypts was even weaker than in the surface epithelium. Approximately 14% of the goblet cells contained secretory vacuoles with detectable binding sites for DBA. These goblet cells were restricted to the surface epithelium and the upper third of the crypts. Likewise, mucus with detectable DBA-binding sites in the crypt lumina was only observed in the upper third of the crypts. Intraepithelial lymphocytes were not labelled by DBA. Within intraepithelial macrophages DBA-binding sites were found focally associated with phagocytosed material.

SBA (Figs. 1D, 2A)

Binding of SBA to the apical surface of enterocytes was non-uniform, i.e. restricted to about half of the enterocytes. Enterocytes expressing SBA-binding sites at their apical surface usually also had respective binding sites in the cytoplasm. As noted for the surface, expression of binding sites in the cytoplasm of enterocytes was heterogeneous. Thus, only 70% of the enterocytes had respective binding sites, located selectively and focally within the subapical cytoplasm and the Golgi region, where vesicular dye precipitates were observed. Para-retronuclear SBA-binding sites were neither observed in the surface epithelium nor in the crypt epithelium. Staining reactions with SBA in the crypts were both weaker and restricted to fewer enterocytes compared to respective staining reactions in the surface epithelium. About 15% of the goblet cells contained secretory vacuoles with detectable binding sites for SBA. In the crypt lumina about a quarter of the mucus contained respective binding sites. Intraepithelial lymphocytes were not labelled with SBA. Within intraepithelial macrophages SBA-binding sites were associated with phagocytosed material.

HPA (Fig. 1E)

Binding of HPA to the apical surface of enterocytes and in their cytoplasm was heterogeneous, observed in more than 60% and 70% of the cells, respectively. The intracellular binding sites were located mostly selectively and focally within the subapical cytoplasm and the Golgi region, where fine vesicular dye precipitates were observed. In some appendix-cases epithelial cells of the crypts and the surface epithelium near the crypt openings exhibited a strong HPA-binding to the basolateral cell membrane and focally to the para-retronuclear cytoplasm. This cellular staining pattern did not occur in the follicle-associated epithelium. Thus, the border between follicle-associated epithelium and crypt-associated epithelium was clearly marked histochemically (Fig. 1E). About half of the goblet cells


A
B
C
D
E

contained secretory vacuoles with detectable HPA-binding sites. HPA-dependent staining was also noticed in about half of the secreted mucus in the crypt lumina (strong staining). Intraepithelial lymphocytes were free of HPA-binding sites, whereas macrophages of the follicle-associated epithelium demonstrated moderate staining reactions with HPA focally in granules of the cytoplasm.

VAA (Fig. 1F)

Binding of VAA to the apical surface of enterocytes was heterogeneous. In the cytoplasm of enterocytes a weak to moderate vesicular binding of VAA focally in about 50% of the enterocytes of the surface epithelium was observed. Staining intensity in the subapical cytoplasm was weaker than in the Golgi region. Para-

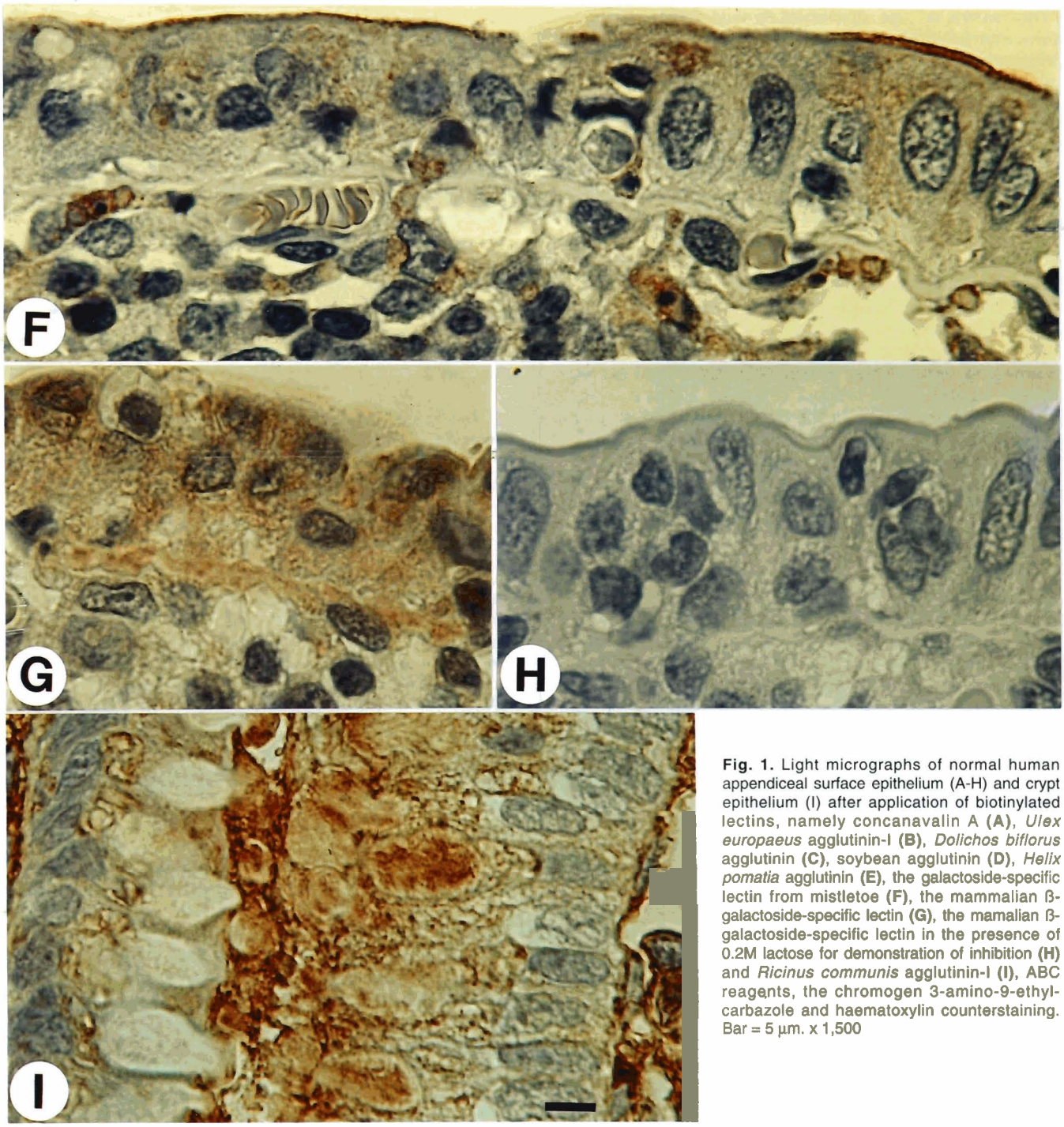


Fig. 1. Light micrographs of normal human appendiceal surface epithelium (A-H) and crypt epithelium (I) after application of biotinylated lectins, namely concanavalin A **(A)**, *Ulex europaeus* agglutinin-I **(B)**, *Dolichos biflorus* agglutinin **(C)**, soybean agglutinin **(D)**, *Helix pomatia* agglutinin **(E)**, the galactoside-specific lectin from mistletoe **(F)**, the mammalian ß-galactoside-specific lectin **(G)**, the mamalian ß-galactoside-specific lectin in the presence of 0.2M lactose for demonstration of inhibition **(H)** and *Ricinus communis* agglutinin-I **(I)**, ABC reagents, the chromogen 3-amino-9-ethyl-carbazole and haematoxylin counterstaining. Bar = 5 µm. x 1,500

retronuclear VAA-binding sites in enterocytes were not detectable under the given conditions. The same staining pattern was assessed in enterocytes of cyrpts. However, binding sites could only be detected in about 10% of the enterocytes. VAA-binding to secretory vacuoles could only be detected in few goblet cells. The staining intensity was moderate. Similarly, mucus secreted by goblet cells into the crypt lumina contained only focally-detectable VAA-binding sites. The lymphoid cell population of the epithelium was heterogeneous concerning the expression of VAA-binding sites. Macrophages in the follicle-associated epithelium clearly contained VAA-binding sites.

RCA-I (Fig. 1I)

RCA-I bound strongly to the apical surface of enterocytes. In the cytoplasm of enterocytes a focal granular binding of RCA-I was observed for the subapical and the Golgi region, whereas RCA-I binding sites in the para-retronuclear cell region were very rarely seen. Staining of enterocytes in the surface epithelium was stronger than in the crypt epithelium. About 90% of the goblet cells contained detectable RCA-I-binding sites within the secretory vacuoles. Comparison of RCA-I binding to intracellular goblet cell mucus and secreted mucus in the crypt lumina revealed a more intense histochemical reaction in the crypt lumina. Most of the lymphocytes (70%) and macrophages contained RCA-I-binding sites.

14kDa (Fig. 1G)

In contrast ot the other lectins, obtained from plants or invertebrates, the binding pattern of this mamalian ß-galactoside-binding protein serves as an indication for the presence of glycoligands with affinity to endogenous S-type lectins. Histochemical reactions with 14 kDa in enterocytes of crypts and the surface epithelium resulted in a moderate rather diffuse, in some cells focally intensified «patchy» staining, excluding the striated border. No clear difference concerning staining intensities, staining pattern and quantity of stained cells was observed between the surface epithelium and the crypt epithelium. 14 kDa was the only lectin within the applied panel that also labelled nuclei (in 10-20% of cells). 14 kDa labelled the basal membrane of the epithelium heterogeneously. Only a few single goblet cells contained detectable 14 kDa-binding sites within their secretory vacuoles. No 14 kDa-binding could be detected in mucus secreted into the cryptal lumen. 14 kDa-binding was seen in a few intraepithelial lymphocytes and some macrophages.

Acute appendicitis

Histochemical reactions with Con A, UEA-I, HPA and the three galactoside-binding lectins VAA, RCA-I and 14 kDa in the epithelium of acutely inflamed appendix vermiformis resulted in a characteristic staining pattern which was identical to the staining pattern in normal appendix vermiformis.

The binding pattern of DBA and SBA to appendix-epithelium, however, differed when sections of acute appendicitis and normal appendix were compared (Fig. 2A-D). Whereas DBA- and SBA-binding to enterocytes and non-epithelium-derived cells (lymphocytes, macrophages) was unchanged in acute inflammation, alterations of lectin binding to goblet cell mucus were found in acutely inflamed appendix.

Goblet cells exposing binding sites of DBA and SBA in their mucus droplets were more numerous in acute appendicitis (DBA: 54%, SBA: 59% of the goblet cells) than in normal appendix (DBA: 14%, SBA: 15% of the cells). Similarly, the quantity of DBA- and SBA-positive secreted mucus in the crypt lumina was higher in appendicitis than in normal appendix. These quantitative changes in appendicitis were apparently due to an increase of DBA- and SBA-reactivity of goblet cells in the lower and middle parts of the crypts.

Comparison of lectin binding in the normal human appendix vermiformis to lectin binding in the distal large intestine

Con A

In the distal large intestine Con A binding to enterocytes in crypts was weaker than that to surface enterocytes, whereas in the appendix Con A binding to crypt enterocytes was as strong as that to surface enterocytes.

UEA-I

In the distal large intestine UEA-I binding to crypt enterocytes was as strong as that to surface enterocytes, whereas in the appendix UEA-I binding to surface enterocytes was more intense than to crypt enterocytes. In the distal large intestine UEA-I binding sites were found neither in the goblet cell mucus nor in the secreted intraluminal mucus, whereas 20-40% of goblet cells in the appendix had UEA-I binding sites. Extracellular intraluminal mucus in the crypts of the appendix was stained even more strongly than in the goblet cells.

DBA

Whereas DBA binding to crypt enterocytes in the distal large intestine was weaker than to surface enterocytes, in the appendix binding of DBA in the supranuclear part of enterocytes was as strong in the surface epithelium as in the crypts. DBA binding to goblet cell mucus in the rectosigmoid was observed in 90% of the cells. In the appendix it was limited to approximately 14% of the goblet cells, located selectively in the surface epithelium and upper third of the crypts. In the rectosigmoid also, goblet cells free of DBA-binding secretory globules were detected in the crypt ground, suggesting that DBA-binding sites are an indicator of mucus production in «highly differentiated» cells.

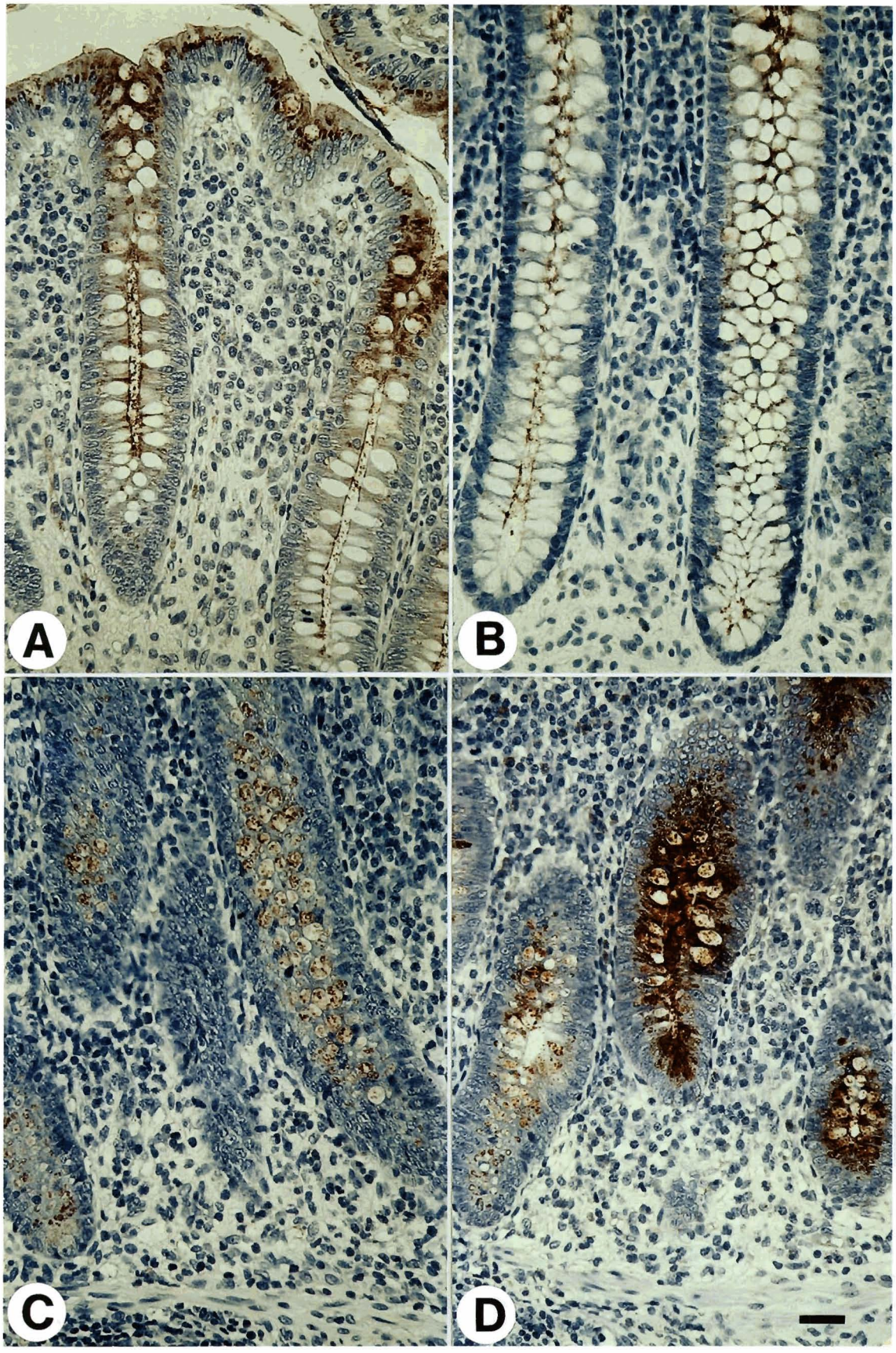


Fig. 2. Light micrographs of normal human appendiceal crypts (A,B) and appendiceal crypts in acute appendicitis (C, D) after application of biotinylated soybean agglutinin **(A, C)** and biotinylated *Dolichos biflorus* agglutinin **(B, D),** ABC reagents, the chromogen 3-amino-9-ethylcarbazole and haematoxilin counterstaining. Bar= 24 µm. x 330

SBA

In the rectosigmoid, binding of SBA was equally weak in surface and crypt enterocytes. In contrast, in the appendix SBA binding was stronger in the surface enterocytes than in the crypts. Evident differences were noted between the number of SBA-binding goblet cells in the appendix and in the rectosigmoid; namely 80% compared to 15% of the cells were positive.

HPA

Binding of HPA to the epithelial lining of the rectosigmoid differed from the appendiceal binding pattern by a greater number of HPA-binding goblet cells (approximately 95% in the rectosigmoid versus 50% in the appendix).

VAA

VAA binding to enterocytes of the distal large bowel was weaker than in the appendix vermiformis. In the rectosigmoid, binding of VAA to the Golgi region was a weak as that to the subapical region of the enterocytes, whereas in the appendix binding to the Golgi region was pronounced compared to the subapical region. VAA-binding sites could neither be detected in the goblet cell mucus nor in the secreted mucus. In the appendix, the galactoside-binding mistletoe lectin bound both to intracellular and to secreted mucus.

RCA-I

In the distal large intestine no differences between the binding intensity of RCA-I in intracellular and in secreted mucus were detected, whereas in the appendix binding to secreted mucus was stronger than to intracellular mucus.

14 kDa

In the rectosigmoid, 14 kDa binding to surface enterocytes was more pronounced than to crypt enterocytes, whereas in the appendix vermiformis no differences were noted. The quantity of 14 kDa-binding goblet cells was much higher in the rectosigmoid than in the appendix vermiformis (10% versus 80%). In the rectosigmoid, 14 kDa-binding sites were detected in the secreted mucus, whereas this lectin failed to detect any suitably glycoligands in the appendix vermiformis.

Discussion

This study has analyzed the lectin-binding pattern in the epithelium of normal human appendix vermiformis. Comparative investigation of acute appendicitis revealed differences of lectin binding in mucus droplets of goblet cells and in secreted (luminal) mucus in the inflammatory state.

In the epithelium of normal human appendix vermiformis lectins differ in their affinity for different cellular components of enterocytes. SBA- and VAA-binding sites are restricted to the supranuclear and subapical cell regions. UEA-I-, DBA-, HPA- and RCA-I-binding sites are located both in the supranuclear and subapical cell regions and, quantitatively different, in the basal cell region. Binding sites of all these lectins (SBA, VAA, RCA-I, UEA-I, DBA, HPA) are focal in the cells, thus suggesting mainly binding to organelles supposedly of the Golgi apparatus and the smooth endoplasmic reticulum in the subapical cell region (Lee, 1987). Con A and 14 kDa bound to glycoligands in the cytoplasm of the supranuclear, subapical and basal cell regions. The preference of Con A for cytoplasmic staining can be attributed to the presence of oligomannose-like structures in the rough endoplasmic reticulum (Laurila et al., 1978; Fuhrman and Bereiter-Hahn, 1984).

Binding of lectins with similar specificity to a monosaccharide can exhibit dissimilarities. Taking galactoside-binding lectins like VAA and ricin as an example, the participation of structural features of the subterminal sugar moiety and anomeric linkage type significantly differ within this group (Lee et al., 1992, 1994). Both applied plant lectins (RCA-I, VAA) primarily recognize the terminal galactose residue with graded influence of the subterminal carbohydrate moiety and the anomeric linkage, whereas the mammalian lectin strongly binds to the Gal-ß-1,4-GlcNAc - disaccharide (Lee et al., 1992, 1994). Obviously, the similar nominal specificity to galactose will not guarantee a comparable extent of binding to cellular galactose-containing glyco-conjugates, emphasizing the need to employ the tissue lectin for any functional correlations. It is interesting to note, with respect to the subcellular staining pattern, that from this group of galactose-binding lectins the mammalian lectin exhibited nuclear staining. In line with the biochemical data, RCA-I and VAA, exhibiting a comparatively low degree of selectivity to galactosides, stained macrophages and lymphocytes fairly well in contrast to the tissue lectin. As emphasized for galactoside-binding lectins, the GalNAc-specific lectins SBA, DBA and HPA similarly revealed notable differences in their binding pattern to certain cell types, namely enterocytes and goblet cells, reflecting measurable levels of dissimilarities in the fine structural ligand recognition.

Lectins differ distinctly in their affinity to the apical surface of enterocytes including M-cells, as indicated by different staining intensities, heterogeneity of binding to enterocytes or lack of apical surface binding. The apical surface of enterocytes is formed by microvilli with mucus closely attached to it (Forstner et al., 1973; Etzler, 1979). As lectin-binding sites at the apical surface of intestinal epithelial cells may be used as adhesion sites by bacteria, protozoa, or viruses (Gitler et al., 1985), lectin-binding sites in this location may play a role in intestinal infection (Sharon, 1987). Galactose residues, visualized in this study on the apical surface of M-cells and other enterocytes, could for example provide

suitable adhesion sites for *Entamoeba histolytica* with known suitable specificity of surface lectins (Petri et al., 1987) and preferential pathogenicity in the human appendix vermiformis. In this respect heterogeneity of enterocytes may be of interest. Having observed a correlation of presence of lectin-binding sites of DBA, SBA and VAA at the apical surface of enterocytes with the presence of respective binding sites in the supranuclear cell region, it can be assumed that the heterogeneity of respective lectin-binding sites at the apical surface may be due to differences of synthetic metabolism of enterocytes rather than due to accidental local variations of mucin-binding in the tissue specimen.

Remarkable quantitative differences exist for lectin binding between the enterocytes of crypts and the surface epithelium. Since enterocytes of the surface epithelium are derived from the crypt epithelium (Wolf and Bye, 1984) and build up a functionally specialized cover - the so called follicle-associated epithelium (Bockman, 1983) - it is reasonable to suggest that these differences may be differentiation-related. Apparently, these differences mainly affect the binding sites of SBA and VAA, which appear more frequently in enterocytes of the follicle-associated epithelium than in enterocytes of the crypt epithelium and of HPA which does not bind to the basolateral membranes of the follicle-associated epithelium.

Specialized cells of the follicle-associated epithelium, so-called M-cells, serve as antigen receptors of the gut-associated lymphatic system (Neutra et al., 1987). No clear-cut differences concerning the lectin-binding affinity of the apical cell surface of M-cells (in the vicinity of lymphocytes and macrophages) and non-M-cells (enterocytes not in contact with lymphocytes and macrophages) were found in our study. In these respects our findings are similar to previous results gained in histochemical studies on M-cells in the distal small intestine of mice and rats (Owen and Bhalla, 1983) and rabbits (Neutra et al., 1987). As M-cells elicit a mucosal immune response by transferring macro-molecules and bacteria to intraepithelial macrophages and lymphocytes (Owen, 1977; Wolf and Bye, 1984), we have studied the presence of glycosubstances in intraepithelial macrophages and lymphocytes which might be recognized by respective bacterial lectins in vivo. It is noteworthy that SBA binding to bone marrow-derived macrophages or their precursors differs markedly, eliciting differentiation-enhancing responses (Krugluger et al., 1994). Our staining reactions demonstrate that intraepithelial macrophages, lymphocytes and adjacent M-cells express a specific lectin-binding pattern with certain similarities and differences. As we have gathered preliminary information on variation of the lectin-binding pattern of macrophages in the gut-associated lymphoid tissue in relation to the location in different anatomical subsites of the appendix vermiformis, one can assume that differences of lectin-binding pattern might be related to environmental influences including exposure to phagocitable material.

Remarkable quantitative differences are assessed between the lectin-binding capacity of intracellular goblet cell mucus and extracellular secreted mucus in the crypt lumina. These differences may be due to mucin degradation by bacteria of the gut (Sato and Spicer, 1982). This study shows that goblet cell mucus in appendicits displays lectin-binding activities quantitatively different from goblet cell mucus in normal human appendix vermiformis. Similar changes of glycosylation of goblet cell mucus (increase of DBA and SBA binding, but not of HPA binding to goblet cell mucus) have been assessed in inflammatory bowel disease (Yoshioka et al., 1989). Thus, appendicitis can be regarded as an appropriate model system, demonstrating that changes of mucus glycosylation can be related to acute inflammation rather than a mixture of chronic and acute inflammatory processes as in inflammatory bowel disease. However, the qualitatively identical change of mucus glycosylation (in terms of lectin binding) as an inflammatory bowel disease is not necessarily related to malignant change, as has been controversely discussed in inflammatory bowel disease (Ahnen et al., 1987). The effects of mucosal inflammation on expression of appendiceal mucin lectin-binding sites has to our knowledge not previously been investigated. In our study in normal control appendices the percentage of goblet cells carrying DBA- or SBA-binding mucus is less than one third in all of 12 investigated specimens (ranging from less than 1% to 30%) and these goblet cells are restricted to the surface epithelium and to the upper third of the crypts. This appendiceal binding pattern of DBA is identical to the pattern in other subsites of the proximal large intestine, as reported in previous studies (Bresalier et al., 1985) and ascertained in the coecum (unpublished observation), but clearly differs from the distal large intestine, as observed by us and also reported previously (Bresalier et al., 1985). Although the precise functional implications of the observed alterations are at present not obvious, such changes clearly reflect regulation of glycosylation, warrating further studies.

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