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Histochemical study of expression of lectin-reactive carbohydrate epitopes and glycoligand-binding sites in normal human appendix vermiformis, colonic mucosa, acute appendicitis and colonic adenoma

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Summary. In a glycohistochemical analysis of human appendix vermiformis we report the assessment of lectin binding in cells of the Gut Associated Lymphoid Tissue of normal samples and in acute appendicitis using a panel of plant, invertebrate and mammalian lectins with specificity for α-L-Fuc (UEA-I), α-D-Gluc and α-D-Man (Con A), α-D-GalNAc (DBA), GalNAc (SBA, HPA), ß-Gal (RCA-I, 14 kDa=galectin-1) and α-, ß-Gal (VAA). Moreover, we initiate the study of expression of carbohydrate-binding sites in this tissue and in colonic mucosa, employing several types of carrier-immobilized carbohydrate ligands as suitable probes for this purpose.

Within the three populations of macrophages intra-/subepithelial macrophages of the dome region, the lamina propria of the intercryptal region and the follicle-associated epithelium were apparently reactive with most of the lectins and also with mannose and fucose residues of the tested neoglycoproteins. Distinguishing features of germinal center macrophages in relation to intra-/subepithelial phagocytes were the lack of binding of UEA-I and DBA. In comparison to all other types of phagocytes, macrophages of the T-region displayed a rather restricted binding capacity only to Con A and RCA-I. Labeling of macrophages with SBA, HPA and VAA in this location was only rarely found. With respect to dendritic cells no consistently positive reaction was seen for follicular cells, whereas interdigitating cells of the T-region bound Con A, HPA and RCA-I, and, less frequently, SBA.

Lymphocytes in all anatomical subsites of the Gut Associated Lymphoid Tissue, centrocytes, centroblasts and plasma cells had binding sites for Con A and RCA-I in common. Notably, a small number of lymphocytes mostly in the T-region but also in B-cell-rich areas expressed intranuclear binding sites for fucose and mannose residues. Intraepithelial lymphocytes and lymphatic cells of the T-region differed from lymphocytes in other regions by a more frequent expression of VAA-binding sites.

The epithelium of appendix vermiformis and colonic mucosa not only presents lectin binding sites, but also has the capacity to bind carbohydrate structures, as shown by labeled glycoligand-exposing neoglycoproteins. In normal mucosa the extent of binding appeared to be associated with maturation of cells, the surface epithelium showing the most intense staining reaction. This pattern is not detectable in colonic adenoma which reveal increased intensity, when compared to normal mucosa. In contrast to development of hyperplasia, acute inflammation in appendicitis caused no detectable changes of neoglycoprotein binding. Taking our previous assessment on lectin binding in appendicitis into account, we conclude that glycosylation of goblet cell mucus, but not the capacity to bind certain sugar epitopes responds to inflammatory processes, whereas tumorigenesis of colonic adenoma can also affect the binding of neoglycoproteins.

Key words: Appendix, Appendicitis, Lectin, Neoglycoprotein, Colonic adenoma, Gut associated lymphoid tissue

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Introduction

Specific organization (assembly) of highly differentiated lymphoid and non-lymphoid cells in the subregions of human Peyer's patches is a feature of the functional peculiarities of the Gut Associated Lymphoid Tissue (GALT). Human Peyer's patches occurring in the appendix vermiformis are probably functionally and morphologically very similar to intestinal Peyer's

patches (Spencer et al., 1985). Since Peyer's patches are thought to play an important role in the primary immune response within the intestine (Kraehenbuhl and Neutra, 1992), a through investigation of diverse molecular aspects of this area is warranted.

Monitoring the expression of distinct epitopes is a prerequisite for comprehension of the intricate relationship between spatial organization and function. In this respect, the assessment of the recognitive interplay of the sugar part of accessible glycoconjugates with endogenous receptors (lectins) is attracting increasing attention (Gabius, 1987a,b, 1991; Gabius and Gabius, 1993, 1996; Zanetta et al., 1994). Commonly, plant and invertebrate lectins are used to determine whether distinct lectin-reactive carbohydrate sequences are present (Danguy et al., 1994). To go beyond a primarily systematic monitoring with tools of non-mammalian origin, it is reasonable to extend such a panel by the addition of lectins isolated from mammalian tissues, whose specific binding can be indicative for presence of a potential interaction system in situ (Gabius et al., 1993). Therefore, we have included a mammalian galectin (14 kDa) in a selection of exogenous agglutinins with specificities to common constituents of cellular glycoconjugates. In addition to their lectin-dependent visualization the potential ligand properties of such epitopes can be conveniently monitored, when the respective carbohydrate structures are chemically conjugated to a histochemically inert, labeled carrier, establishing a neoglycoconjugate (Lee and Lee, 1994; Bovin and Gabius, 1995; Danguy et al., 1995). These tools enable us to determine the expression of accessible carbohydrate-binding sites which are not negatively affected by the processing of tissue specimen. The types of carbohydrate ligands for this study have deliberately been chosen to be complementary to the specificities of the applied agglutinins to allow comparison.

Previous animal studies have revealed remarkable species-specific variations of glycoconjugate expression within the gut-associated lymphoepithelial tissues (Clark et al., 1993, 1995; Jepson et al., 1995). These glycohistochemically detected variations can have important therapeutic implications in veterinary and human medicine, if orally delivered vaccines and drugs are to be targeted to specific cells of gut-associated lymphoepithelial tissue (e.g. M-cells) via their surface glycoconjugates (Clark et al., 1995). Therefore, we continue the recently initiated glycohistochemical study of gut-associated lymphoepithelial tissues in humans (Brinck et al., 1995).

Following the monitoring of normal tissue samples, we have analyzed the binding of the panel of neoglycoproteins in appendicitis to address the question as to whether inflammatory processes will affect the presence of respective binding sites. In line with this aspect we have also initiated the analysis of glycoligand-binding sites in colonic mucosa and adenoma to reveal, if possible, any alterations of the characteristics within the process of tumorigenesis.

Material and methods

Tissue

21 appendix specimens (12 cases of normal tissue and 9 cases of acute appendicitis) were obtained at the time of surgery from patients which had undergone treatment due to suspected appendicitis. Normal mucosa samples of the sigmoid colon (n=6) and rectum (n=6) were obtained endoscopically form patients investigated for large bowel diseases, in whom no macroscopical or histological colonic lesions had been found. Additionally, samples from 6 tubular and 6 tubulovillous adenomas of the colon sigmoideum were derived from coloscopic examination. Specimens were fixed in 3.6% paraformaldehyde for paraplast-embedded sections. Histological diagnosis was reached by evaluation after conventional hematoxylin and eosin staining.

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 (Deisenhofen, FRG). The ß-galactoside-specific lectin with a molecular weight of 14 kDa from bovine heart (14 kDa= galectin-1) and the galactoside-specific lectin from mistletoe (*Viscum album* agglutinin, VAA), purified as described in detail elsewhere (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).

Neoglycoproteins

The carbohydrate ligand-exposing neoglycoproteins (Lac-BSA-biotin, α-L-Rham-BSA-biotin, α-D-Man-BSA-biotin, Mal-BSA-biotin, α-L-Fuc-BSA-biotin, ß-D-GalNAc-BSA-biotin, ß-D-GlcNAc-BSA-biotin), applied as glycohistochemical tools, were synthesized by diazo coupling to periodate-treated bovine serum albumin (BSA) of the highest commercially available degree of purity, starting from p-aminophenyl derivatives, as described in detail elsewhere (Gabius et al., 1990a,b).

Histochemical processing

The paraplast-embedded sections were routinely processed by rehydration, treatment with 1% hydrogen peroxide solution for 30 minutes to block endogenous peroxidase activity, incubation with 0.1% periodate-treated BSA solutions to saturate unspecific protein-binding sites, incubation with 10 μg biotinylated

lectin/ml (Con A, VAA, RCA-I, 14 kDa) or 40 μg lectin/ml (UEA-I, DBA, SBA, HPA), respectively, or with 100 μg biotinylated neoglycoprotein/ml in phosphate-buffered saline, pH 7.4 containing 0.1% BSA for 4 h at room temperature and, after thorough rinses to completely remove unbound marker, for 1 h with ABC reagents. 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 hematoxylin.

For ascertaining the specificity of lectin-carbohydrate interaction, binding of the individual lectins was inhibited by co-incubation with glyco-inhibitor-containing solution at a sugar concentration of 0.2M (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). Appropriate specificity controls for binding of neoglycoproteins were performed, as described (Gabius and Bardosi, 1991; Danguy et al., 1995). Lack of binding of the label biotin by endogenous biotin-specific proteins, of the carrier protein and any components of the kit reagents, namely the mannose-containing, glycoproteins avidin and horseradish peroxidase, was rigorously excluded.

Within the evaluation of lectin binding, the reactivity was assessed in macrophages of the follicle-associated epithelium, the dome, the lamina propria of the intercryptal region, the germinal centers and the T-region, in dendritic cells of germinal centers and T-regions as well as in lymphatic cells of the follicle-associated epithelium, the dome, the lamina propria of the intercryptal region, the mantle zone of follicles, the germinal centers and the T-region. The extent of staining reactions with neoglycoproteins was evaluated separately in enterocytes of the surface epithelium, in enterocytes of crypts, in goblet cell secretory globules, in adenoma cells of superficial parts of colonic adenoma, in adenoma cells of basal parts of colonic adenoma, in secretory globules of adenoma cells as well as in macrophages, dendritic cells and lymphatic cells of the GALT of the appendix vermiformis with respect to presence of staining and staining intensity. The quantity of labelled cells and the staining intensity were grouped into categories, as given in the footnotes of Tables 1-3. In detail, three independent observers (U.B., R.B. and M.K.) counted the number of labeled cells per 400 cells of each cell type in each subregion and semiquantitatively determined the overall staining intensity according to a 4-grade scale for the intensity of staining due to lectin binding and a 7-grade scale for the intensity of staining due to specific neoglycoprotein binding. A low variability of the results was obtained between the 3 independent observers. This low variability of results was also seen between all evaluated tissue specimens. Only minor quantitative differences of results between observers and all evaluated tissue specimens concerning quantity of labeled cells (mostly below 5%) and staining intensity (identical assessment of more than 80% of cases) allowed us to group the quantity of labeled cells and the staining intensity into categories, as given in detail in Tables 1-3, without further documentation of a statistical analysis.

Results

Binding of lectins to gut associated lymphoid tissue of normal appendix vermiformis (Table 1)

Con A

Con A binds specifically to lymphoid cells and macrophages in all anatomical subsites of the Gut Associated Lymphoid Tissue of human appendix vermiformis. Staining intensity is rather weak in B- and T-lymphocytes of the mantle zone, the T-region and the intercryptal region. Lymphoid cells in the inner two thirds of the germinal center and lymphoid cells of the dome are stained more strongly than germinal center cells in the outer third of the germinal center or lymphocytes in the mantle zone and the T-region. Among lymphoid cells plasma cells display the strongest reactivity with the lectin, comparable to that of macrophages in all anatomical subsites of the GALT (follicle-associated epithelium, intercryptal region, germinal center, T-region). Con A labels interdigitating reticulum cells but apparently not dendritic reticulum cells of germinal centers.

UEA-I

In contrast to lymphocytes, lymphoid cells (centrocytes, centroblasts, plasma cells), dendritic reticulum cells or interdigitating cells, UEA-I binds to macrophages of the follicle-associated epithelium, the intercryptal region and the dome. It is notable that macrophages of the germinal center and the T-region were negative. Endothelial cells were labeled by UEA-I in a heterogeneous manner.

DBA (Fig. 1A,B)

Histochemical reactions with DBA resulted in a staining pattern that apparently closely resembled labeling with UEA-I, with the obvious exception that endothelial cells consistently remained unstained with DBA.

SBA (Fig. 1C,D)

The binding of SBA under the given conditions was confined with rather equal intensity to macrophages of the follicle-associated epithelium, the dome, the intercryptal region and the germinal center as well as to

Table 1. Binding of lectins to subregions of appendiceal Peyer's patches.

LOCATION OF LECTIN BINDING	Con A	UEA-I	DBA	SBA	HPA	VAA	RCA-I	14 kDa
Follicle-associated epithelium								
Lymphocytes	+/2	-/0	-/0	-/0	-/0	(+)/3	+++/3	(+)/1
Macrophages	++/3	++/3	++/2	++/3	++/2	++/3	+++/3	+/1
Dome								
Lymphocytes	+++/2	-/0	-/0	-/0	-/0	(+)/1	+++/4	++/2
Plasma cells	+++/3	-/0	-/0	-/0	+/1	-/0	+++/4	++/2
Macrophages	+++/3	++/2	++/2	+++/3	+++/3	++/3	+++/4	++/2
Intercryptal region								
Lymphocytes	++/1	-/0	-/0	-/0	-/0	(+)/1	+++/4	+/2
Plasma cells	+++/3	-/0	-/0	-/0	+++/2	-/0	+++/4	++/2
Macrophages	+++/3	++/2	++/2	+++/3	+++/3	+/3	+++/4	++/2
Mantle zone								
Lymphocytes	++/1	-/0	-/0	-/0	-/0	(+)/1	+++/4	+/1
Germinal center								
Lymphoid cells (inner two thirds)	+++/2	-/0	-/0	-/0	-/0	(+)/1	+++/4	+/2
Lymphoid cells (outher third)	+++/1	-/0	-/0	-/0	-/0	(+)/1	+++/3	+/2
Macrophages	+++/4	-/0	-/0	+++/3	++/2	+++/4	+++/4	++/2
Dendritic reticulum cells	-/0	-/0	-/0	-/0	-/0	-/0	-/0	-/0
T-region								
Lymphocytes	++/1	-/0	-/0	-/0	-/0	++/2	++/2	-/0
Macrophages	+++/3	-/0	-/0	+/3	+/3	+/1	+++/3	-/0
Interdigitating reticulum cells	+++/3	-/0	-/0	+/2	++/2	(+)/2	+++/2	-/0

The percentage of positive cells 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.

a minority of the macrophages of the T-region and of interdigitating reticulum cells.

HPA (Fig. 1E-G)

HPA binds neither to lymphocytes, centrocytes and centroblasts nor to dendritic reticulum cells. However, plasma cells and macrophages as well as interdigitating reticulum cells can be stained by this lectin. It reacted equally with macrophages of the follicle-associated epithelium and the germinal center. In comparison to the level of these staining reactions, binding of HPA to macrophages of the intercryptal region and the dome was more pronounced. Binding of HPA to macrophages of the T-region was equally strong as that in the intercryptal region and the dome, but it apparently occurred only in 20-40% of the cells. Endothelial cells were intensely labeled by HPA.

VAA

VAA bound in a heterogeneous manner, and with some variability among the tested individual cases, to lymphoid cells mainly of the T-region and the follicle-associated epithelium and to interdigitating reticulum cells. Under the conditions of this comparative study VAA bound to most germinal center macrophages but only to a minority of macrophages in the follicle-associated epithelium, the dome region, the lamina propria and the T-region. Staining intensity was very strong in macrophages of germinal center, strong in the follicle-associated epithelium, the dome region and the lamina propria, but comparatively weak in the T-region. Interdigitating reticulum cells were partly labeled by VAA.

RCA-I

RCA-I showed the smallest extent of selectivity of binding in comparison to the other two galactoside-specific lectins, which is in line with a model for the binding site topologies based on chemical-mapping study with a panel of carbohydrate ligands (Lee et al., 1994). RCA-I bound very strongly to all lymphoid cells in the dome, the mantle region, the inner part of the germinal center and in the intercryptal region and strongly in the outer third of the germinal centers and the follicle-associated epithelium. Lectin-dependent staining intensity of lymphocytes in the T-region was moderate. RCA-I strongly or very strongly bound apparently to all

Fig. 1. A. Normal human appendiceal intercryptal region. DBA. Only macrophages are stained. **B.** Normal human appendiceal germinal center. DBA. No cells are stained. **C.** Normal human appendiceal intercryptal region. SBA. Only macrophages are stained. **D.** Normal human appendiceal germinal center. SBA. Only macrophages are stained. **E, F.** Normal human appendiceal intercryptal region. HPA. Only plasma cells, macrophages and endothelial cells are stained. **G.** Normal human appendiceal germinal center. HPA. Only macrophages are stained. Bar: 6 μm. x 1,350

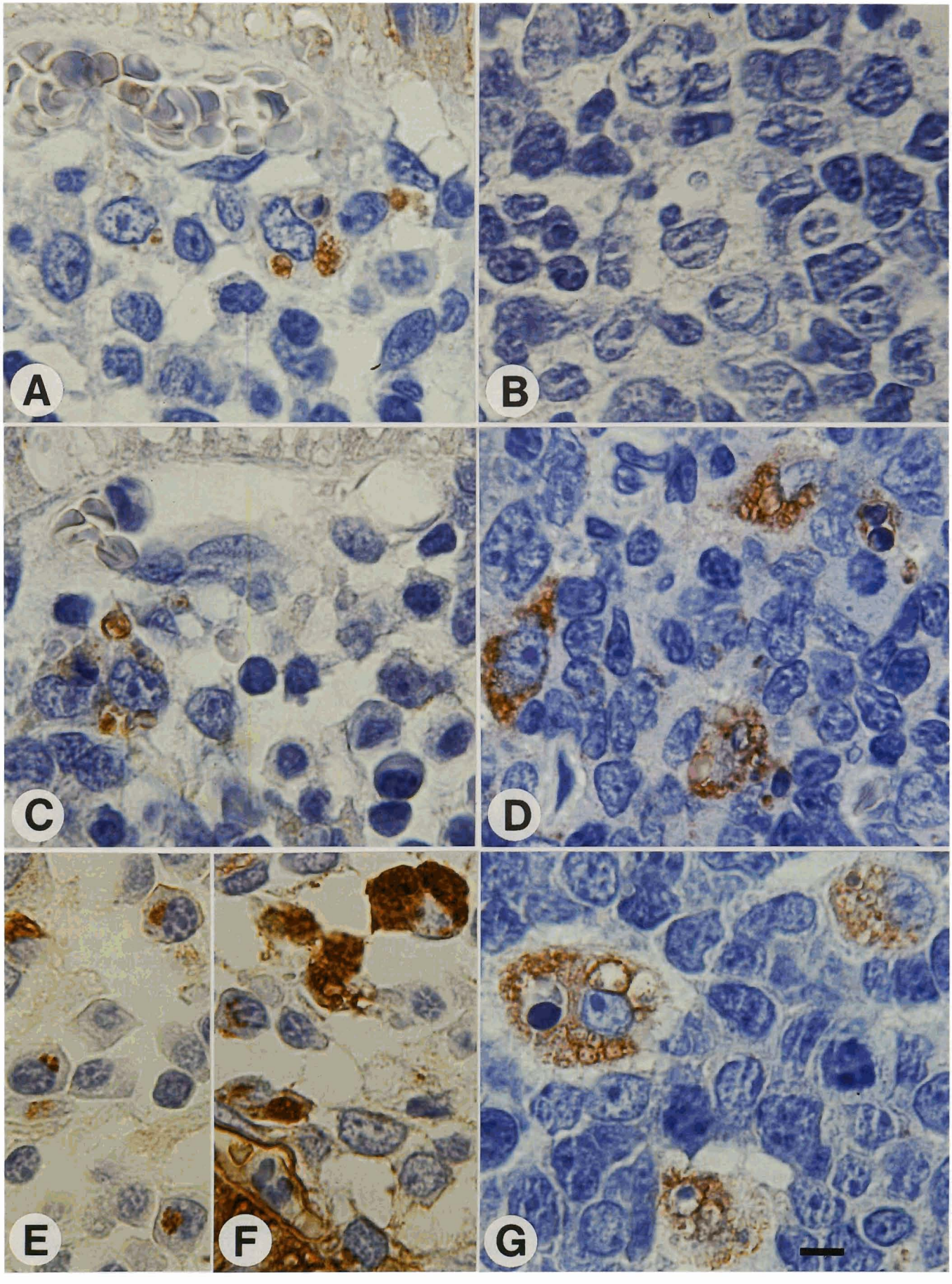
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Table 2. Binding of carrier-immobilized carbohydrate ligands to epithelium and Peyer's patches of normal human appendix vermiformis and in acute appendicitis.

SITE OF NEOGLYCOPROTEIN BINDING	Lac[a]	ß-GalNAc	ß-GlcNAc	α-Man	α-L-Fuc	Mal	α-L-Rham
Epithelial cells							
Surface enterocytes	+++/2	+++/2	+++/3	+++/3	+++/4	+++/1	+++/2
Crypt enterocytes	+++/1	+++/1	+++/2	+++/2	+++/2	+++/1	+++/1
Follicle-associated epithelium							
Lymphocytes	-/0	-/0	-/0	(+)/2	(+)/2	-/0	-/0
Macrophages	-/0	-/0	-/0	(+)/2	(+)/2	-/0	-/0
Dome							
Lymphocytes	-/0	-/0	-/0	(+)/2	(+)/2	-/0	-/0
Plasma cells	-/0	-/0	-/0	++/3	++/3	-/0	-/0
Macrophages	-/0	-/0	-/0	(+)/2	(+)/2	-/0	-/0
Intercryptal region							
Lymphocytes	-/0	-/0	-/0	(+)/2	(+)/2	-/0	-/0
Plasma cells	-/0	-/0	-/0	++/3	++/3	-/0	-/0
Macrophages	-/0	-/0	-/0	(+)/2	(+)/2	-/0	-/0
Mantle zone							
Lymphocytes	-/0	-/0	-/0	+/2	+/2	-/0	-/0
Germinal center							
Lymphoid cells (inner two thirds)	-/0	-/0	-/0	-/0	-/0	-/0	-/0
Lymphoid cells (outer third)	-/0	-/0	-/0	-/0	-/0	-/0	-/0
Macrophages	-/0	-/0	-/0	-/0	-/0	-/0	-/0
Dendritic reticulum cells	-/0	-/0	-/0	-/0	-/0	-/0	-/0
T-region							
Lymphocytes	-/0	-/0	-/0	+/2	+/2	-/0	-/0
Macrophages	-/0	-/0	-/0	-/0	-/0	-/0	-/0
Interdigitating reticulum cells	-/0	-/0	-/0	-/0	-/0	-/0	-/0

The percentage of positive cells is grouped into the categories: -, 0%; (+), 0-20%; +, 20-40%; ++, 40-60%; and +++, 60-100%. The intensity of staining is grouped into seven categories of increasing intensity, ranging from 1 (weak, but significant staining) to 7 (strong staining). Lac: lactose; GalNAc: N-acetylgalactosamine; GlcNAc: N-acetylglucosamine; Man: mannose; Fuc: fucose; Mal: maltose; Rham: rhamnose.

macrophages in the follicle-associated epithelium, the dome, the germinal center and the intercryptal region and most of the macrophages and interdigitating reticulum cells in the T-region.

Galectin-1 (14kDa) (Fig. 2E,F).

In relation to the two plant agglutinins this lectin not only localized distinct ß-galactosides including internal poly-(N-acetyllactosamine)-sequences. Moreover, its mammalian origin enables us to suggest evidence for the display of endolectin-reactive sites with potential physiological relevance. It was thus remarkable that binding of galectin-1 to lymphoid cells in all anatomical subsites was heterogeneous. Staining intensity was moderate in the germinal center, the lamina propria and the dome, but weak in the mantle region and the follicle-associated epithelium. Lymphocytes in the T-region were devoid of detectable 14 kDa-binding sites. Galectin-1 bound moderately to 40-60% of germinal center macrophages, the dome and the lamina propria and weakly to phagocytes of the follicle-associated epithelium. It was the only probe within the applied panel of lectins that also labeled nuclei of cells, as is exemplarily illustrated in Fig. 2E. Together with the documented presence of galectin-1 and also the related galectin-3 in cell nuclei (Gabius et al., 1986; Anderson and Wang, 1992), this observation supports the notion that a recognitive interplay between nuclear lectins and glycoconjugates can be operative (Hubert et al., 1989). In addition to the lectin-dependent characterization of the GALT of appendix vermiformis, complementing our previous study on this aspect which has focused on the epithelial compartment (Brinck et al., 1995), we have employed labeled neoglycoproteins to comparatively analyze the expression of glycoligand-binding sites in this tissue type.

Fig. 2. A. Normal human appendiceal surface epithelium. Lactosylated BSA. Weak to medium staining of cytoplasm and nuclei, excluding mucous droplets of goblet cells. **B.** Superficial part of an adenoma of the large intestine. Lactosylated BSA. Medium to strong staining of cytoplasm and nuclei of epithelial cells. **C.** Normal human appendiceal crypt epithelium. Lactosylated BSA. Weak, but significant staining of epithelial cells, excluding mucous droplets of goblet cells. **D.** Deep (tubular) part of an adenoma of the large intestine. Lactosylated BSA. Strong staining of cytoplasm and nuclei of epithelial cells. **E.** Crypt epithelium and intercryptal region of normal human appendix vermiformis. Mammalian-ß-galactoside-specific lectin, termed galectin-1 or 14 kDa. Cytoplasmic and nuclear staining of epithelial and lymphoid cells. No labeling of mucous droplets of goblet cells. **F.** Crypt epithelium and intercryptal region of normal appendix vermiformis. Inhibition of binding of biotinylated galectin-1 (14 kDa) in the presence of a 50-fold excess of unlabeled lactosylated BSA as well as 0.2M lactose. Bar: 6 μm. x 1,350

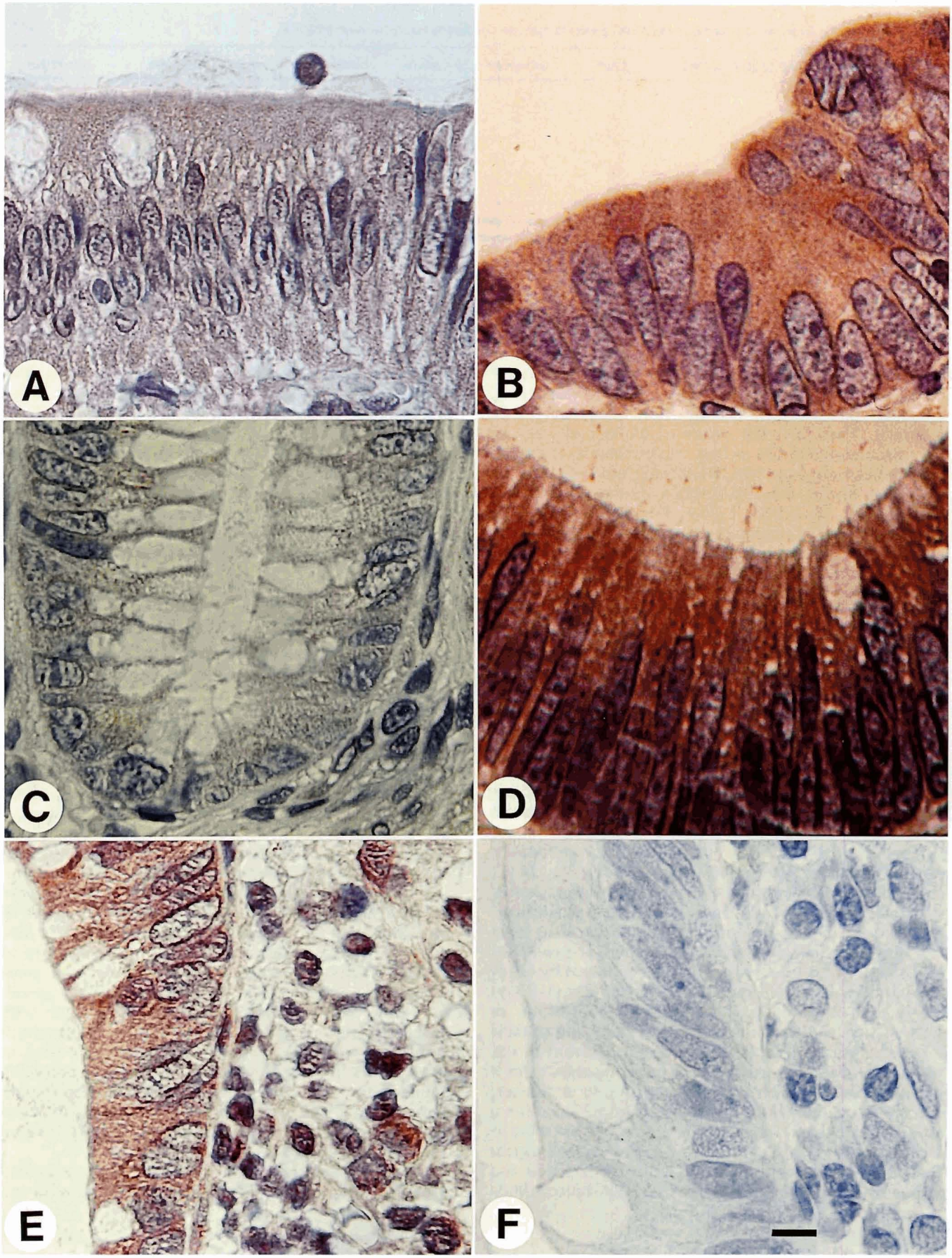
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Table 3. Binding of carrier-immobilized carbohydrate ligands to mucosa and adenoma of the large intestine.

SITE OF NEOGLYCOPROTEIN BINDING	Lac[a]	ß-GalNAc	ß-GlcNAc	α-Man	α-L-Fuc	Mal	α-L-Rham
Mucosa							
Surface enterocytes	2	2	3	3	4	1	2
Crypt enterocytes	1	1	2	2	2	1	1
Adenoma							
Surface enterocytes	5	4	5	6	5	4	5
Crypt enterocytes	6	5	6	7	6	5	6

The intensity of staining reaction is grouped into seven categories of increasing intensity, ranging from 1 (weak, but significant staining) to 7 (strong staining). Lac: lactose; GalNAc: N-acetylgalactosamine; GlcNAc: N-acetylglucosamine; Man: mannose; Fuc: fucose; Mal: maltose; Rham: rhamnose.

Binding of neoglycoproteins to normal tissues (Tables 2 and 3)

The chemical properties of the neoglycoproteins (type of carbohydrate derivative and conjugation, coupling yield and label density) were deliberately kept identical. Therefore, the nature of the ligand was the only parameter allowed to vary. Histochemical reactions with the carbohydrate ligand-exposing markers, namely Lac-BSA-biotin, ß-GalNAc-BSA-biotin, ß-GlcNAc-BSA-biotin, Man-BSA-biotin, Fuc-BSA-biotin and Rham-BSA-biotin, resulted in a characteristic staining pattern of epithelial cells. With respect to the intracellular distribution of reactive sites a certain degree of similarity was observed. The staining intensities, however, differed clearly, emphasizing variations in the abundance of detectable glycoligand-binding sites.

Cytoplasmic and nuclear staining reaction was seen with all applied neoglycoproteins, as is exemplarily shown in Fig. 2A. Within the cytoplasm, specific binding sites were detected in a supranuclear, para-retronuclear and subapical location, whereas the apical cell border and mucus of the goblet cells remained unlabeled (Fig. 2A,C).

Extent of binding of the applied neoglycoproteins with the exception of the maltosylated marker displayed a continuous increase from the base of the glands to the surface epithelium, which exhibited the comparatively highest degree of labeling, as is illustrated in Fig. 2A,C. α-Glucoside-binding sites were equally abundantly present at the surface and the gland epithelium. Comparison of neoglycoproteins with respect to their capacity for labeling the surface epithelium showed Fuc-BSA-biotin to be the strongest marker followed by Man-BSA-biotin and ß-GlcNAc-BSA-biotin; Rham-BSA-biotin, Lac-BSA-biotin and ß-GalNAc-BSA-biotin as well as Mal-BSA-biotin with stepwise reduction of their labeling capacity (Tables 2 and 3). In addition to the routine controls, this grading of staining intensities for a panel of probes which only differed in one parameter, namely the carbohydrate ligand, is clear evidence for a specific protein-carbohydrate interaction. With respect to labeling of the gland epithelium ß-GlcNAc-BSA-biotin, Fuc-BSA-biotin and Man-BSA-biotin showed the strongest staining capacity and Lac-BSA-biotin, Mal-BSA-biotin, ß-GalNAc-BSA-biotin and Rham-BSA-biotin the weakest staining intensity (Tables 2 and 3).

Within the GALT of normal appendix vermiformis, binding of Fuc-BSA-biotin and Man-BSA-biotin was detected in some of the epithelial/subepithelial macrophages of the dome region, the lamina propria of the intercryptal region and the follicle-associated epithelium, but not in phagocytes at any other location. A small number of lymphocytes, mostly in the T-region but also in B-cell rich areas, expressed intranuclear binding sites of fucose and mannose. Additionally, a heterogeneous labeling of plasma cells within their cytoplasm was detected with these two probes (Table 2):

Acute appendicitis (Table 2)

Binding of lectins and neoglycoproteins to GALT and of neoglycoproteins to epithelial cells of appendiceal mucosa remained unchanged under the condition of the acute inflammation.

Binding of neoglycoproteins to colonic adenoma (Table 3)

To reveal any differences in the expression of glycoligand-reactive sites in aberrant cells, we extended this analysis to adenoma cases. A marked alteration of the localization of staining was not detectable, turning our attention to a detailed semi-quantitative comparison.

Cytoplasmic and nuclear staining reaction was assessed with the applied neoglycoproteins, as shown in Fig. 2B,D. Similar to the behavior of normal enterocytes, binding sites for neoglycoproteins in the cytoplasm of adenoma cells were detected in a supranuclear, para-retronuclear and subapical location, whereas the apical cell border was not labeled. Within adenoma cells with extensive mucus production the mucus remained unlabeled with neoglycoproteins, as already seen in goblet cells of normal mucosa. The neoglycoproteins bound more intensely to adenoma cells in deep (basal) parts of gland structures than to adenoma cells in apical gland or villous structures and superficially located adenoma cells (Fig. 2B,D).

Both in superficial and deep parts of the adenoma Man-BSA biotin was the neoglycoprotein with the

highest degree of labeling and Mal-BSA-biotin and ß-GalNAc-BSA-biotin the markers with the lowest degree of labeling. In between, Lac-BSA-biotin, Fuc-BSA-biotin, ß-GlcNAc-BSA-biotin and Rham-BSA-biotin were rather equal in staining (Table 2). It is therefore evident that the availability of accessible binding sites for the tested glycoligand is significantly increased in adenoma cells relative to normal mucosa cells.

Discussion

This study has extended our glycohistochemical analysis of the epithelium of normal human appendix vermiformis (Brinck et al., 1995) to appendiceal Peyer's patches and includes information on the carbohydrate ligand-binding properties of this organ and of colonic mucosa. Comparative examination of cases with colonic adenoma disclosed quantitative differences of neoglycoprotein binding, whereas acute inflammation in appendicitis is associated with changes of lectin binding to mucus of goblet cells only.

In Peyer's plaques of normal appendix vermiformis the ligand density for lectins can differ for cells with respect to cell type and location of cells. Explicitly, the results show that the employed panel of lectins is useful to distinguish three subtypes of macrophages in the GALT of the human appendix vermiformis on the basis of quantitative differences in glycosylation. They comprise cells near the lumen, i.e. macrophages of the follicle-associated epithelium and subepithelial macrophages of the dome and lamina propria, tingible body macrophages of the germinal centers and macrophages of the T-region. Macrophages near the gut lumen consistently express the broadest spectrum of lectin-reactive carbohydrate structures. UEA-I and DBA never bound to any of the tingible body macrophages of the germinal centers or the T-region. UEA-I and DBA appeared to bind preferentially to lysosomal structures. As it is known that foreign antigenic macromolecules taken up from the gut lumen are also transported to macrophages in germinal centers (von Rosen et al., 1981), this reduction of lectin binding may be attributed to degradation of phagocytozed material in deeper portions of Peyer's plaques and/or to altered (physico-) chemical properties of lysosomal membranes.

A second location-dependent peculiarity was observed in macrophages which were either located directly beneath the basal membrane of the follicle-associated epithelium or in the follicle-associated epithelium. These macrophages can bind VAA at the cell surface which is in contact with the basal membrane, lymphocytes or M-cells. Surface binding of VAA was not observed in macrophages of other regions. It is possible, but not proven to suggest that this observed property may be associated with macrophage stimulation in this special environment, e.g. antigen exclusion by M-cells. Lectin binding on the surface of macrophages is of special relevance for phagocytosis, because these binding sites may be involved in adsorption of extracellular macromolecules (with lectin properties), e.g. bacterial surface lectins (Ofek and Sharon, 1988). In addition, glycoconjugates on the surface of macrophages in the follicle-associated epithelium or in a subepithelial position could interact with lectins of other cells like lymphocytes (Gabius, 1987b; Abramenko et al., 1992) which may especially be relevant under the condition of activation.

Our report presents detailed information on lectin binding characteristics of macrophages of human Peyer's patches. In comparison to published data on animal's Peyer's plaques interspecies differences are notable. DBA binding, which has been observed for rat germinal center macrophages (Sminia and van der Ende, 1988, 1991), was not seen, the difference being possibly attributable either to species variability or to tissue processing.

Comparison of our results with data on lectin binding characteristics of macrophages in lymph nodes showed that tingible body macrophages and sinus histiocytes of lymph nodes in contrast to Peyer's plaque macrophages lack SBA-binding sites (Budde et al., 1988). Similar results have been published after examination of rat tissue (Sminia and van der Ende, 1988, 1991). This fact supports the notion of dispersaly accessible carbohydrate residues in macrophages of Gut Associated and non-Gut Associated Lymphoid Tissue both in animals and humans.

The panel of lectins used as probes in this study comprised mitogenic and non-mitogenic lectins. It is striking that under these conditions non-mitogenic lectins (DBA, SBA, HPA) did not bind to lymphocytes, whereas mitogenic lectins (Con A, VAA, RCA-I) did. Remarkably, binding of the mitogenic lectins Con A and RCA-I to glycoligands on lymphocytes can initiate the cascade of growth stimulation after lectin-dependent clustering (Chilson and Kelly-Chilson, 1989). Likewise, signal transduction with enhanced phosphorylation of distinct proteins and phospholipids as well as an increase in Ca^{2+}-availability had been reported for VAA after binding to the cell surface with positive cooperativity (Gabius et al., 1992).

Intraepithelial lymphocytes were characterized by a heterogeneous expression of VAA-binding sites. Since this lymphocyte population is mainly established by CD8+ cytotoxic suppressor-T-lymphocytes (MacDonald and Spencer, 1990) and is closely functionally related to gut epithelium, e.g. by capability of induction of Ia (MHC-class-II) antigens (Auer, 1990; Cerf-Bensussan et al., 1984), VAA-binding sites may also be involved in the regulatory immunological functions of intraepithelial lymphocytes. The elucidation of important immuno-regulatory functions such as enhanced cytokine secretion or superoxide production has already been proven for this lectin (Gabius, 1994).

Marked differences were observed between lectin-binding characteristics of dendritic cells in germinal centers and in the T-region. Binding of lectins to interdigitating cells in the T-region is similar to binding

to macrophages. This observation corroborates the view that interdigitating cells are derived from the mononuclear phagocyte system (Radzun et al., 1984). Our finding that follicular dendritic reticulum cells do not share lectin-binding characteristics of macrophages and of interdigitating cells is in accordance with the theory that they arise from a different cellular origin, perhaps originating from perivascular tissue (Beranek and Masseyeff, 1986).

Remarkable differences exist for the binding patterns of lectins with nominal specificity to the same monosaccharide. This is illustrated for the GalNAc-specific lectins (Fig. 1A-G). In detail, HPA is the only one of the three applied GalNAc-specific lectins that binds in a heterogeneous manner to the Golgi zone of plasma cells and intensely to endothelial cells. Conversely, HPA and SBA stain macrophages of germinal centers quite well, whereas DBA does not bind to these cells. In this context, it is instructive to refer to the observation that even closely related lectins of the same monosaccharide specificity from the organism can exhibit differential binding capacity to homologous lymphocyte populations (Schneller et al., 1995). Development of, for example, the chemical-mapping approach besides the crystallographic analysis has been instrumental for procurement of detailed insights into architectures of the ligand-binding sites to explain the differential behavior at the atomic level (Solís et al., 1996).

Intracellular distribution of carbohydrate ligand-binding sites within enterocytes of appendiceal and colonic mucosa resembled the binding pattern of the mammalian galectin-1 (14 kDa) closer than any other lectin used in this study. This similarity of cellular binding pattern illustrated for lactose-binding sites and the mammalian ß-galactoside-specific lectin (Fig. 2 A,C,E), may serve to point to functional implications of carbohydrate-lectin interactions in situ. Binding of neoglycoproteins excluded the apical cell surface with the striated border, and the secretory vesicles of goblet cells. This appearance may reflect masking of specific receptors in certain locations, which have been demonstrated to contain lactose-binding lectins in an animal study performed by immunohistochemical techniques (Beyer and Barondes, 1982).

The intensity of binding of carrier-immobilized carbohydrate ligands such as ß-galactose, ß-N-acetylgalactosamine, ß-N-acetylglucosamine and α-mannose to epithelial cells of normal mucosa of the large intestine is apparently correlated to the status of maturation of the cells. There is an increase of staining intensity from the bottom of the crypts to the surface epithelium. Such a pattern of labelling has also been found in the case of SBA and RCA-I in the appendix vermiformis, as well as for Con A and 14 kDa in enterocytes of the distal large intestine, hinting to a physiological implication of the presently phenomenological result (Brinck et al., 1995).

Previous studies have shown alterations of glycoconjugates associated with tumorigenesis of colonic adenoma (Boland et al., 1982; Rhodes et al., 1986; Campo et al., 1988; Ho et al., 1988; Lee, 1988; Ota et al., 1988; McGarrity et al., 1989; Orntoft et al., 1991; Dall'Olio and Trere, 1993; Jass et al., 1993; Fucci et al., 1993). The most frequently detected changes of lectin binding in the adenoma comprised increase of reactivity for peanut agglutinin/Amaranthin (Boland et al., 1982; Rhodes et al., 1986; Campo et al., 1988; Lee, 1988; Ota et al., 1988; McGarrity et al., 1989; Orntoft et al., 1991; Sata et al., 1992; Fucci et al., 1993), *Ulex europaeus* agglutinin (Rhodes et al., 1986; Ota et al., 1988; McGarrity et al., 1989; Jass et al., 1993) and *Griffonia simplicifolia* agglutinin-II (Rhodes et al., 1986; Ota et al., 1988). Having synthesized markers to measure the ligand properties of carbohydrate moieties, it is possible to compare glycohistochemically accessible carbohydrate moieties and respective receptors in tissue material with defined morphological alterations. This binding can correlate with morphometric features and with survival of patients in lung and prostate cancer (Kayser et al., 1994, 1995). It appears that increases in lectin-reactive Gal(/Gal-ß-1,3 GalNAc), fucose and GlcNAc moieties, respectively are associated with enhanced presentation of respective carbohydrate ligand-binding capacity in colonic adenoma. It is of interest that the qualitative subcellular binding pattern of neoglycoproteins to adenoma cells closely resembles that of epithelial cells of normal mucosa. The apical surface and mucus of secretory vesicles are free of respective binding sites. Preservation of this phenotype appears to possibly reflect the level of differentiation of adenoma cells. A previous glycohistochemical study (Gabius et al., 1991) has revealed high levels of expression of carbohydrate ligand-binding capacity with specificity for lactose and ß-GalNAc in invasive adenocarcinomas of the large intestine. Using an antibody to either human galectin-1 or to human galectin-3 this result was confirmed on the level of these endolectins (Irimura et al., 1991; Schoeppner et al., 1995). Since galectin-3 expression has been implicated with neoplastic transformation and metastatic progression, the nature of endogenous ligands has been investigated, defining carcinoembryonic antigen, laminin and lysosome-associated membrane glycoproteins as lectin-reactive glycoprotein in colon carcinoma cells (Ohannesian et al., 1995). These results indicate that an increase of certain carbohydrate ligand-binding capacities can be referred to as an apparently early event in the course of progression from adenoma to carcinoma, warranting further studies.

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