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Detection of Inflammation- and Neoplasia-Associated Alterations in Human Large Intestine Using Plant/Invertebrate Lectins, Galectin-1 and Neoglycoproteins

Key Words
Inflammation
Adenoma
Large intestine
Lectin
Galectin
Neoglycoprotein

Abstract
Commonly, plant and invertebrate lectins are accepted glycohistochemical tools for the analysis of normal and altered structures of glycans in histology and pathology. Mammalian lectins and neoglycoproteins are recent additions to this panel for the detection of lectin-reactive carbohydrate epitopes and glycoligand-binding sites. The binding profiles of these three types of probes were comparatively analyzed in normal, inflamed and neoplastic large intestine. In normal colonic mucosa the intracellular distribution of glycoconjugates and carbohydrate ligand-binding sites in enterocytes reveals a differential binding of lectins with different specificity and of neoglycoproteins to the Golgi apparatus, the rough and smooth endoplasmic reticulum and the apical cell surface. The accessible glycoligand-binding sites and the lectin-reactive carbohydrate epitopes detected by galectin-1 show the same pattern of intracellular location excluding the apical cell surface. Lectin-reactive carbohydrate epitopes detected by plant lectins of identical monosaccharide specificity as the endogenous lectin [*Ricinus communis* agglutinin-I (RCA-I), *Viscum album* agglutinin (VAA)], however, clearly differ with respect to their intracellular distribution. Maturation-associated differences and heterogeneity in glycohistochemical properties of epithelial cells and non-epithelial cells (macrophages, dendritic cells, lymphocytes) are found. Dissimilarities in the fine structural ligand recognition of lectins with nominal specificity to the same monosaccharide have been demonstrated for the galactoside-specific lectins RCA-I, VAA and galectin-1 as well as the N-acetylgalactosamine (GalNAc)-specific lectins *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA) and *Helix pomatia* agglutinin in normal mucosa and in acute appendicitis. Acute inflammation of the intestinal mucosa found in acute phlegmonous appendicitis is associated with selective changes of glycosylation of mucin in goblet cells mainly of lower and middle crypt segments resulting in an increase of DBA- and SBA-binding sites in the goblet cell population. Appendicitis causes no detectable alteration of neoglycoprotein binding. In contrast, tumorigenesis of colonic adenoma is characterized by increases in lectin-reactive galactose (Gal; Gal-β1,3-GalNAc), fucose and N-acetylglucosamine moieties and by enhanced presentation of respective carbohydrate ligand-binding capacity. This work reveals that endogenous lectins and neoglycoproteins are valuable glycohistochemical tools supplementing the well-known analytic capacities of plant lectins in the fields of gastrointestinal anatomy and gastroenteropathology.

Abbreviations used in this paper:
Con A = Concanavalin A; DBA = *Dolichos biflorus* agglutinin; Fuc = fucose; Gal = galactose; GalNAc = N-acetylgalactosamine; GALT = gut-associated lymphoid tissue; Glc = glucose; GlcNAc = N-acetylglucosamine; HPA = *Helix pomatia* agglutinin; Lac = lactose; Mal = maltose; Man = mannose; RCA-I = *Ricinus communis* agglutinin-I; Rham = rhamnose; SBA = soybean agglutinin; UEA-I = *Ulex europaeus* agglutinin; VAA = *Viscum album* agglutinin.

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Introduction

The primary function of the large intestine is to absorb the fluid remaining in the luminal contents during digestion. As the fecal mass develops in the colon, secretions from the large population of goblet cells in the mucosa provide the lubrication necessary to prevent mucosal damage and infection as the feces pass through the colon. The gut-associated lymphoid tissue (GALT) of the large intestine cooperates with the mucosal epithelium in the defense against potentially pathogenic microorganisms in the gut lumen [Filipe, 1979; Podolsky, 1989].

Glycoproteins are functionally involved in these 'non-specific' and immunological defense mechanisms, since they are important constituents of the mucin produced by intestinal goblet cells and also take part in the binding and uptake of intraluminal antigenic material and potentially pathogenic microorganisms [Neutra et al., 1987]. Their glycan composition is not a constant feature and is subject to modulation. Recent evidence suggests that glycomoiety-binding proteins (lectins) used as histochemical tools reveal quantitative variations in the expression of binding sites in normal tissue of the large intestine in relation to the status of differentiation and activation of epithelial and nonepithelial cell types (macrophages, dendritic cells, lymphoid cells) involved in defense mechanisms [Brinck et al., 1995, 1996]. In addition, disease-associated alterations of lectin-reactive carbohydrate epitopes and glycoligand-binding sites of some of these cell types may occur during inflammatory reactions and neoplastic transformation.

To provide a further example of the illustration of the technical feasibility to closely monitor glycohistochemical changes and of the ensuing results of the application of exogenous and endogenous lectins and neoglycoproteins, we will present and discuss the detection of lectin-reactive carbohydrate epitopes and glycoligand-binding sites in different segments of the normal human large intestine and in relation to inflammatory and neoplastic disease. Glycohistochemical findings will be summarized at first for the proximal large intestine, which includes the vermiform appendix, cecum and the proximal part of the colon and then compared to the findings in the normal distal large intestine (colon sigmoideum and rectum). Inflammation-related alterations are exemplified with respect to acute phlegmonous appendicitis. Histochemical findings in neoplastic disease focus on adenoma of the distal colon to show early alterations within the adenoma-carcinoma sequence.

Table 1. Glycohistochemical tools applied in histochemical analyses of the large intestine [Brinck et al., 1995, 1996]

Lectins	Source of lectin	Specificity of lectins	Specificity of corresponding carbohydrate ligand-exposing neoglycoproteins
Con A	plant	α-*D*-Man α-*D*-Glc	α-*D*-Man Mal
UEA-I	plant	α-*L*-Fuc	α-*L*-Fuc
DBA	plant	α-*D*-GalNAc	–
SBA	plant	*D*-GalNAc	β-*D*-GalNAc
HPA	invertebrate	*D*-GalNAc	β-*D*-GalNAc
RCA-I	plant	β-*D*-Gal	Lac
VAA	plant	*D*-Gal	Lac
Galectin-1	mammalian	*D*-Gal	Lac
–	–	–	α-*L*-Rham
–	–	–	β-*D*-GlcNAc

Man = Mannose; Glc = glucose; Fuc = fucose; GalNAc = N-acetylgalactosamine; Gal = galactose; Mal = maltose; Lac = lactose; Rham = rhamnose; GlcNAc = N-acetylglucosamine.

Theoretical Basis for Selection of Glycohistochemical Tools

The glycan part of glycoconjugates has intrigued researchers in various fields for decades [Sharon, 1998]. Traditionally, plant and invertebrate lectins are used to determine whether distinct lectin-reactive carbohydrate epitopes are present [Danguy et al., 1994]. The chapters of Danguy et al. [1998], Mann and Waterman [1998], Plendl and Sinowatz [1998] and Zschäbitz [1998] further illustrate this application. Notably, the chapter of Rüdiger [1998] answers the question concerning in vivo functions of plant lectins which has been elusive for decades despite the popularity of the agglutins as laboratory instruments. Biochemical work over the last two decades has proven that lectins are also a functionally crucial part of mammalian cells [Gabius, 1987, 1997a, b; for a collection of recent reviews, see Gabius and Gabius, 1997; Kaltner and Stierstorfer, 1998; Zanetta, 1998]. To go beyond the mentioned monitoring with tools of non-mammalian origin, it is reasonable to extend such a panel by employing lectins isolated from mammalian tissue [Gabius et al., 1993]. To document this application, a mammalian galectin (galectin-1) was included in a selection of exogenous agglutinins with specificities to common constituents of cellular glycoconjugates. Compared to the direct analysis of glycoconjugate structure, outlined by Geyer and Geyer [1998] in this issue, lectin histo-

Table 2. Binding of lectins to epithelium of normal proximal large intestine

Location of lectin binding	Con A	UEA-I	DBA	SBA	HPA	VAA	RCA-I	Galectin-1
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
Pararetronuclear 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
Pararetronuclear 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

The percentage of positive structures (cellular subsites, cells, mucus) is grouped into the categories: – = 0%; (+) = 0–20%: + = 20–40%; ++ = 40–60%; +++ = 60–100%. The intensity of staining is grouped into the categories: 0 = no staining; 1 = weak, but significant staining; 2 = medium staining; 3 = strong staining; 4 = very strong staining [from Brinck et al., 1995, with modifications].

chemistry provides an insight into the presence of distinct glycan chain constituents in situ. Interestingly, this lectin is involved in growth-controlling mechanisms in cultured human neuroblastoma cells [Kopitz et al., 1998], providing an example of lectin-dependent, physiologically relevant signaling [Villalobo and Gabius, 1998].

As there is a growing notion to accept the idea that glycans are excellent information-storing elements [Laine, 1997], their ligand potential can be exploited for the detection of receptor sites, prompting the synthesis of neoglycoconjugates. Consequently, carbohydrate structures were chemically conjugated to a histochemically inert, labeled carrier, thus establishing a neoglycoconjugate and thereby making it possible to conveniently monitor the potential ligand properties of such epitopes [Gabius, 1988; Lee and Lee, 1994; Bovin and Gabius, 1995; Danguy et al., 1998]. Combined chemoenzymatic synthesis has recently even gained access to neoglycoconjugates with complex N-glycans as ligand part [André et al., 1997]. These tools were used to determine the expression of accessible carbohydrate-binding sites which were not negatively affected by the processing of tissue specimen. For example, in breast and lung cancer these tools have described tumor type-associated differences in the capacity to bind sugar ligands based on lectin expression [Gabius et al., 1986, 1988; Kayser et al., 1989; Kayser and Gabius, 1997].

Owing to the documented presence of lectins in colon cancer, initially demonstrated by affinity chromatography [Gabius et al., 1985], and the modulation of their expression by chemical agents inducing differentiation in culture [Gabius et al., 1990], it is of interest to perform a comparative analysis with lectins and neoglycoproteins to assess both sides of a potential recognition system. The types of carbohydrate ligands were deliberately chosen to be complementary to the specificities of the applied agglutinins to allow a comparison (table 1).

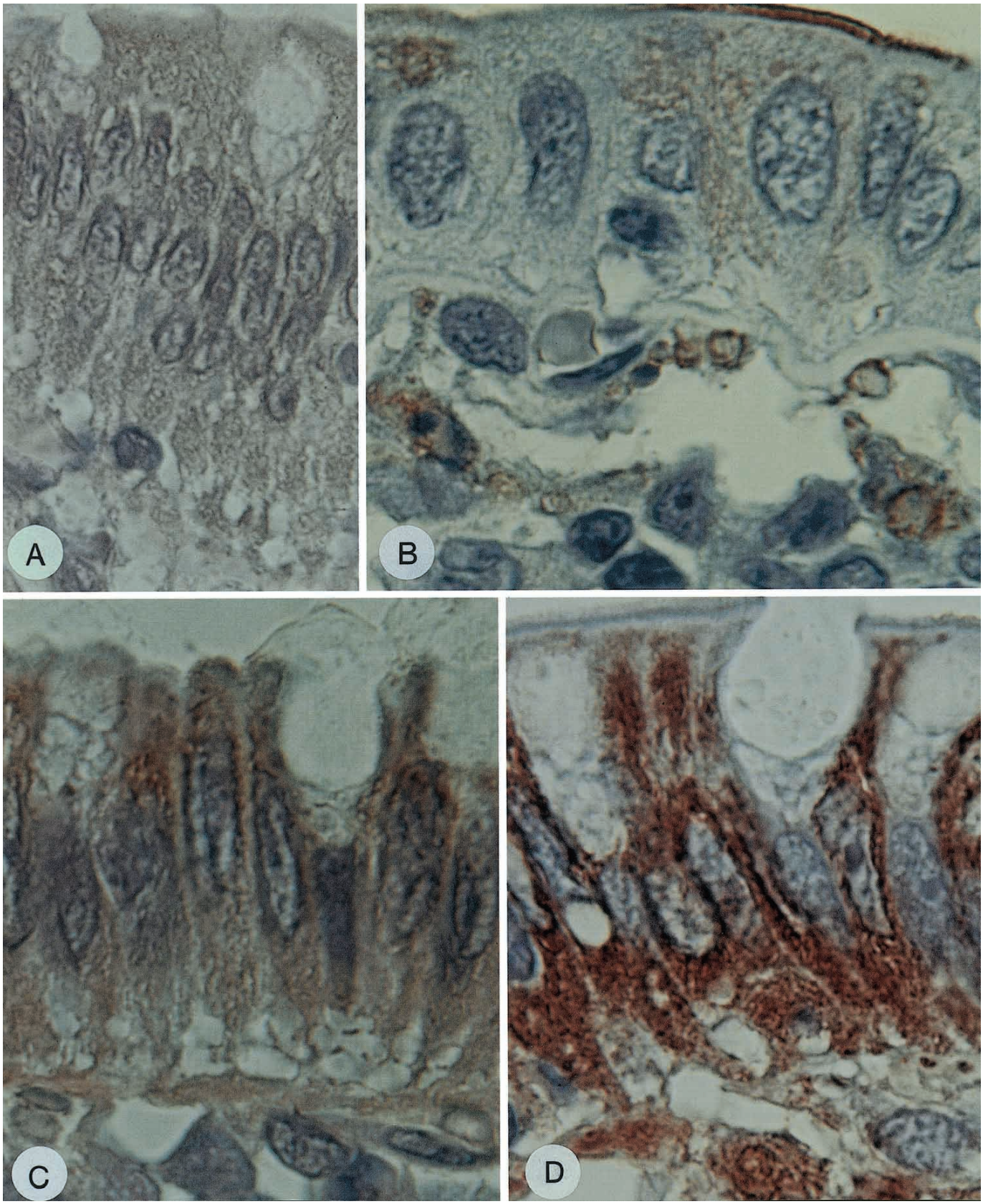
Normal Large Intestine

Glycoconjugate Expression in Different Cellular Constituents of Enterocytes (table 2)

In the epithelium of normal human proximal large intestine, cellular components of enterocytes differed in their capacity to present lectin-reactive epitopes. The apical surface of enterocytes in paraffin-embedded sections presented binding sites for *Ricinus communis* agglutinin-I (RCA-I)

Fig. 1. Light micrographs of normal human appendiceal surface epithelium (**A**–**G**) after stepwise application of biotinylated lectins, namely the galactoside-specific lectin from mistletoe (VAA; **B**), the mammalian β-galactoside-specific lectin galectin-1 (**C**), Con A (**D**), SBA (**E**), DBA (**F**) and galectin-1 in the presence of 0.2 *M* lactose for demonstration of inhibition (**G**), as well as of biotinylated lactosylated albumin (**A**), ABC reagents, the chromogen 3-amino-9-ethylcarbazole and hematoxylin counterstaining. ×2,500.

Fig. 2. Light micrographs of normal human appendiceal mucosa after stepwise application of biotinylated lectins, namely SBA (**A**) and HPA (**B**), ABC reagents, the chromogen 3-amino-9-ethylcarbazole and hematoxylin counterstaining. ×330.



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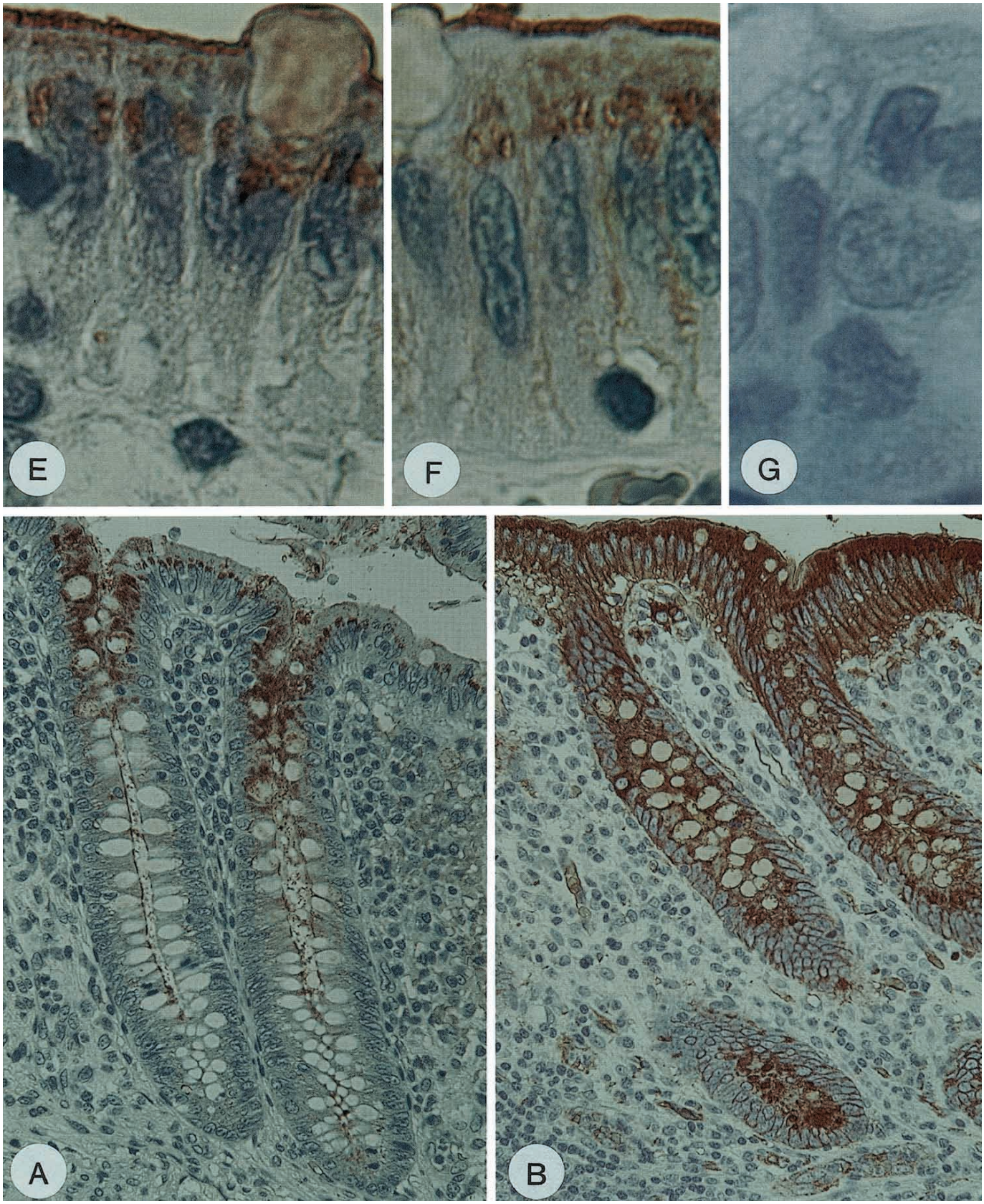

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on all cells, binding sites for *Ulex europaeus* agglutinin (UEA-I), *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), *Helix pomatia* agglutinin (HPA) and *Viscum album* agglutinin (VAA) heterogeneously and no binding sites for concanavalin A (Con A) and galectin-1. This is illustrated exemplarily for VAA, Con A and galectin-1 in figure 1B–D. Presence of SBA- and VAA-binding sites was restricted to the supranuclear and subapical cell regions (fig. 1E). UEA-I-, DBA-, HPA- and RCA-I-binding sites were located both in the supranuclear and subapical cell regions and, quantitatively different, in the basal cell region. Reactive glycoconjugates for all these lectins (SBA, VAA, RCA-I, UEA-I, DBA, HPA) were present focaly in the cells, thus suggesting binding primarily to organelles, most probably of the Golgi apparatus and the smooth endoplasmic reticulum in the subapical cell region [Lee, 1987]. This is demonstrated exemplarily for the agglutinins SBA and DPA in figure 1E and F. Con A and galectin-1 were bound to glycoligands in the cytoplasm of the supranuclear, subapical and basal cell regions (fig. 1C, D). The preference of Con A for cytoplasmic staining was attributable to the presence of oligomannose-like structures in the rough endoplasmic reticulum [Laurila et al., 1978; Fuhrman and Bereiter-Hahn, 1984].

Intracellular Distribution of Carbohydrate Ligand-Binding Sites within Enterocytes (table 3)

The epithelium of the intestinal mucosa not only presented lectin-binding sites, but also harbored the capacity to bind carbohydrate structures, as shown by labeled glycoligand-exposing neoglycoproteins (fig. 1A). Intracellular distribution of carbohydrate ligand-binding sites within enterocytes of appendiceal and colonic mucosa resembled the binding pattern of the mammalian galectin-1 more closely than that of any other above-mentioned lectins. This similarity of cellular binding patterns is illustrated for lactose-binding sites and the mammalian galectin (fig. 1A, C). As the presence of β-galactoside-binding sites intimates a potential interaction between the two types of epitopes, localized by an endogenous lectin and a lectin-detecting marker, functional implications for carbohydrate-lectin interactions in situ are encouraged. Binding of neoglycoproteins excluded the apical cell surface with the striated border, and the secretory vesicles of goblet cells. This appearance can be interpreted to reflect a masking of specific receptors at certain locations by an abundant presence of glycoconjugates. The potential of mucins to associate strongly with endogenous lectins has for example been underscored by the demonstration that galectins can bind colonic mucins [Bresalier et al., 1996; Wasano and Hirakawa, 1997].

Indications for Maturation-Associated Differences in Glycohistochemical Properties of Epithelial Cells

Remarkable quantitative differences were found for lectin binding between the enterocytes of crypts and of the surface epithelium. Enterocytes of the surface epithelium are derived from the crypt epithelium [Wolf and Bye, 1984] and establish a functionally specialized cover called the follicle-associated epithelium [Bockman, 1983]. Therefore, it is reasonable to suggest that these differences are related to the status of differentiation. Apparently, these differences mainly affected the presentation of binding sites of SBA (fig. 2A) and VAA, which appeared more frequently in enterocytes of the follicle-associated epithelium than in enterocytes of the crypt epithelium, and of HPA which did not bind to the basolateral membranes of the follicle-associated epithelium.

Maturation-associated differences of expression of lectin-reactive carbohydrate epitopes were also found within the goblet cell population. Within this cell type, the presence of N-acetylgalactosamine (GalNAc) residues detected by DBA or SBA was restricted to the upper third of the crypts and the surface epithelium (fig. 2A). The intensity of binding of carrier-immobilized carbohydrate ligands such as β-galactose, β-GalNAc, β-N-acetylglucosamine and α-mannose to epithelial cells of normal mucosa of the large intestine is apparently correlated with the status of maturation of the cells. There was an increased staining intensity extending from the bottom of the crypts to the surface epithelium. Such a pattern of labeling was also found for SBA (fig. 2A) and RCA-I (fig. 4A) in the proximal large intestine as well as for Con A and galectin-1 in enterocytes of the distal large intestine, making it possible to assume that the phenomenological result might have a physiological basis [Brinck et al., 1995].

Heterogeneity of Glycohistochemical Properties of Enterocytes and Goblet Cells

Heterogeneity of epithelial cells within single crypts of the colonic mucosa with respect to the expression of plant and invertebrate lectin-reactive carbohydrate epitopes was obvious and contrasted with a rather homogeneous distribution of accessible glycoligand-binding sites. Goblet cell mucus displayed heterogeneous distributions of lectin-binding sites: binding sites for Con A were not detected in goblet cells, for DBA, SBA, VAA and galectin-1 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.

The affinity of the apical surface of enterocytes including M cells differed notably among the lectins studies, as indicated by different staining intensities, heterogeneity of

Table 3. Binding of carrier-immobilized carbohydrate ligands to epithelium of the normal large intestine and to Peyer's patches of normal vermiform appendix

Site of neoglycoprotein binding	Lac	β-GalNAc	β-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%; +++ = 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 [from Brinck et al., 1996, with modifications].

binding to enterocytes or lack of apical surface binding. The apical surface of enterocytes is formed by microvilli with mucus intimately associated with it [Forstner et al., 1973; Etzler, 1979]. Since lectin-binding sites at the apical surface of intestinal epithelial cells can be used as adhesion sites by bacteria, protozoa, or viruses, lectin-binding sites in this location may play a role in intestinal infection [Gitler et al., 1985; Sharon, 1987; Doyle and Slifkin, 1994]. Galactose residues visualized on the apical surface of M cells and other enterocytes might for example provide suitable adhesion sites for *Entamoeba histolytica* with specificity to its surface lectins [Petri et al., 1987]. In this respect heterogeneity of the glycohistochemical features of enterocytes may be of interest. Based on the observed correlation of the presence of lectin-binding sites of DBA, SBA and VAA at the apical surface of enterocytes with the presence of equivalent 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 in the synthetic machinery of enterocytes rather than to accidental local variations in mucin binding in the tissue specimen. As reviewed by Brockhausen et al. [1998] and Hakomori [1998] in this issue, the cascade of glycosylation steps can be subject to alterations correlating for example with disease.

Glycohistochemical Subtyping of Macrophages and Dendritic Cells in the GALT (tables 3, 4)

In Peyer's plaques of normal vermiform appendix the ligand density for lectins can differ for cells with respect to cell type and location of cells. A panel of lectins (UEA-I, DBA, SBA, HPA, RCA-I, galectin-1) is useful for distinguishing three subtypes of macrophages in the GALT of the human vermiform appendix on the basis of quantitative differences in glycosylation. They comprise cells near the

Table 4. Binding of lectins to subregions of appendiceal Peyer's patches

Location of lectin binding	Con A	UEA-I	DBA	SBA	HPA	VAA	RCA-I	Galectin-1
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 (outer 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

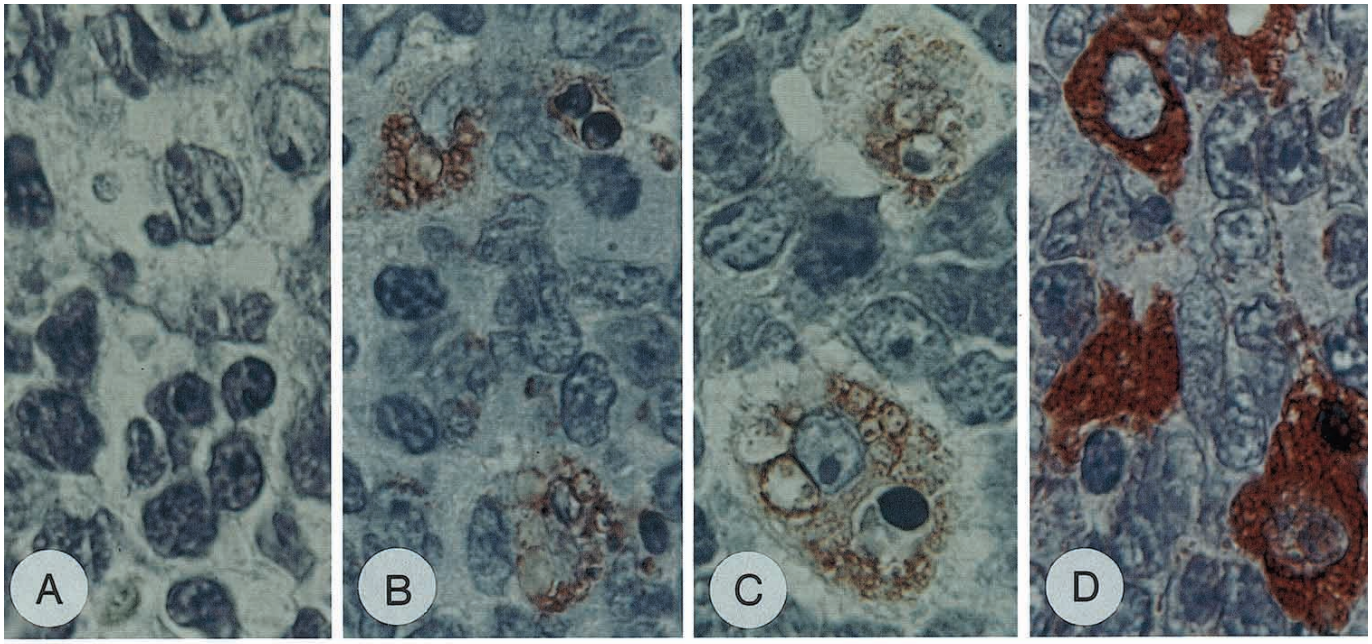
The percentage of positive cells is grouped into the categories: – = 0%; (+) = 0–20%: + = 20–40%; ++ = 40–60%; +++ = 60–100%. The intensity of staining is grouped into the categories: 0 = no staining; 1 = weak, but significant staining; 2 = medium staining; 3 = strong staining; 4 = very strong staining [from Brinck et al., 1996, with modifications].

lumen, that is 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 expressed the broadest spectrum of lectin-reactive carbohydrate structures. UEA-I and DBA were never bound to any of the tingible body macrophages of the germinal centers or the T region. The differential expression of lectin-reactive carbohydrate epitopes in tingible body macrophages of germinal centers is illustrated for the binding sites of the GalNAc-specific lectins DBA, SBA and HPA as well as the mannose- and glucose-specific lectin Con A in figure 3. UEA-I and DBA appeared to bind preferentially to lysosomal structures. Since it is common knowledge 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 in lectin binding may be attributable to a degradation of phagocytized material in deeper portions of Peyer's plaques and/or to altered (physicochemical) 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 ephithelium itself. 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 likely, but not proven beyond doubt that this observed property is associated with macrophage stimulation in this special environment, for example anti-

Fig. 3. Light micrographs of normal human appendiceal germinal center after stepwise application of biotinylated lectins, namely DBA (**A**), SBA (**B**), HPA (**C**) and Con A (**D**), ABC reagents, the chromogen 3-amino-9-ethylcarbazole and hematoxylin counterstaining. ×380.

Fig. 4. Light micrographs of normal human appendiceal mucosa after stepwise application of biotinylated lectins, namely RCA-I (**A**) and the mammalian β-galactoside-specific lectin galectin-1 (**B**), ABC reagents the chromogen 3-amino-9-ethylcarbazole and hematoxylin counterstaining. ×330.



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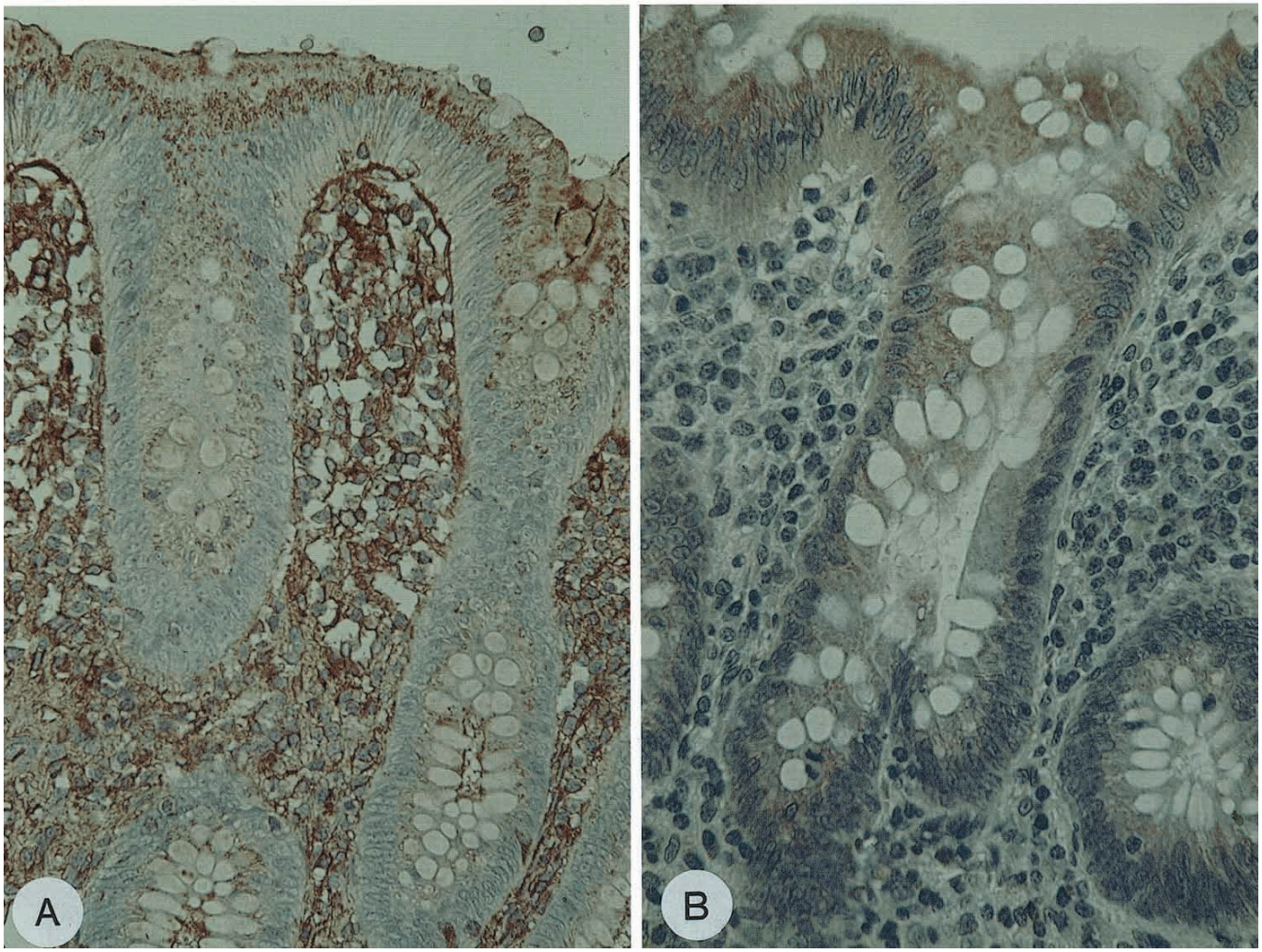

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(For legends, see p. 226.)

gen 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), for example 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, 1987, 1997a; Grillon et al., 1990; Abramenko et al., 1992; Sharma et al., 1992] which may be especially relevant at the conditions of activation. Notably, galectin ligands can under such conditions be downregulated, ascertaining the possibility for regulation on the level of binding partners for distinct tissue lectins [Smetana et al., 1998].

Marked differences were observed between the lectin-binding characteristics of dendritic cells in germinal centers and in the T region. Binding of lectins to interdigitating cells in the T region was 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 did not share lectin-binding characteristics of macrophages and of interdigitating cells is consistent with the notion that they have a different cellular origin, perhaps originating from perivascular tissue [Beranek and Masseyeff, 1986].

Glycohistochemistry of the GALT (tables 3, 4)

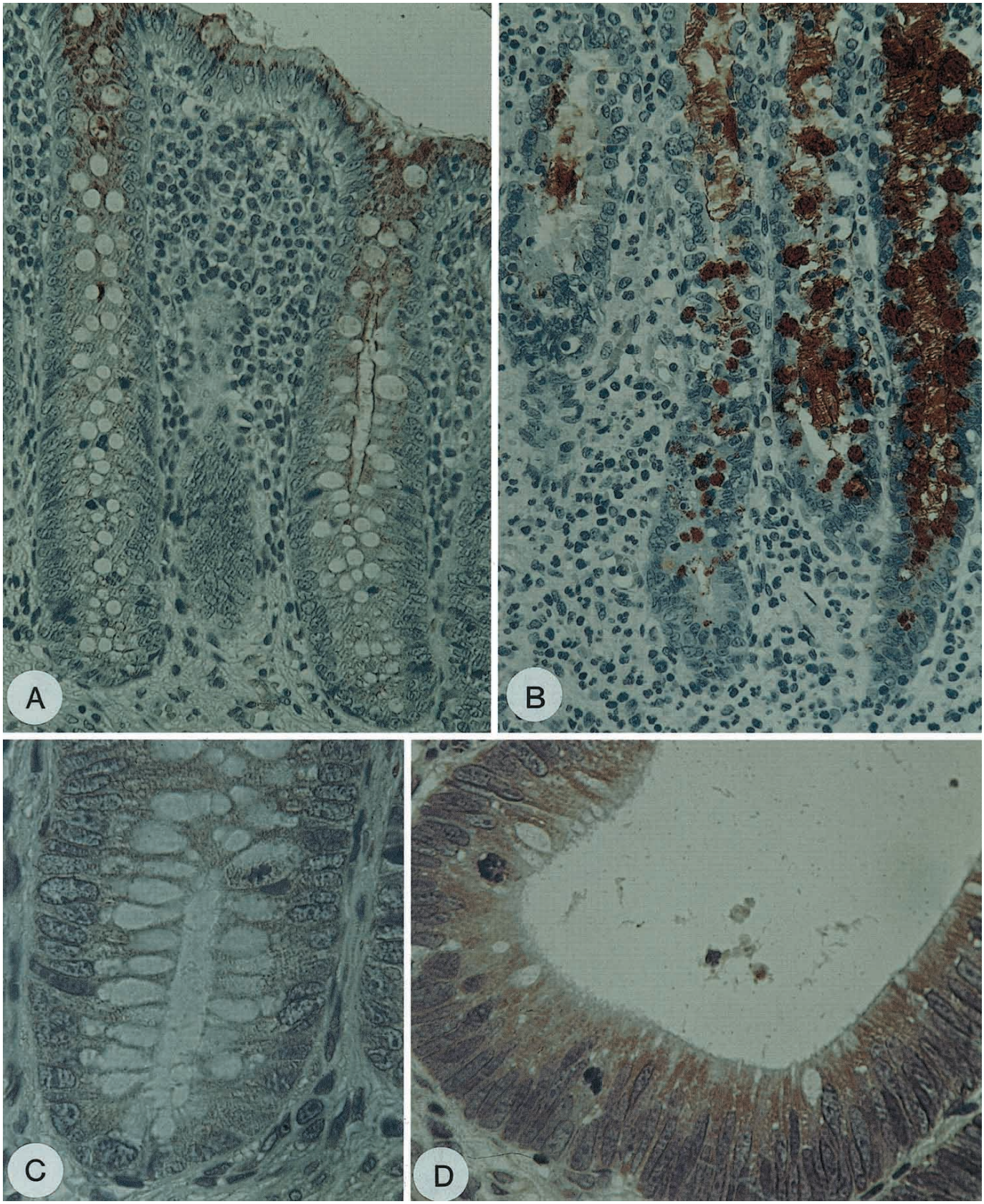
Lymphocytes in all anatomical subsites of the GALT, centrocytes, centroblasts and plasma cells all had common binding sites for Con A and RCA-I. This is illustrated exemplarily for RCA-reactive carbohydrate epitopes in figure 4A. Notably, a small number of lymphocytes, mostly in the T region but also in B-cell-rich areas, expressed intranuclear binding sites for fucose and mannose residues, strongly suggesting a role of nuclear lectins and glycoproteins, as discussed by Hubert et al. [1989]. Intraepithelial lymphocytes and lymphatic cells of the T region differed from lymphocytes in other regions by a more frequent expression of VAA-binding sites.

Dissimilarities in the Fine Structural Ligand Recognition of Lectins with Nominal Specificity to the Same Monosaccharide in Enterocytes, Goblet Cells and Macrophages

Historically, lectins have been grouped according to their monosaccharide specificity. This classification system, for example based on inhibition of hemagglutination, should not delude one into concluding that the actual binding partners of lectins of identical monosaccharide specificity are also identical. In any case, the subtleties of ligand binding must be thoroughly probed to accurately determine the fine specificity. To address the complexities presented by this task, either a battery of oligosaccharides is tested or the structure of the recognition structure is analyzed by techniques, outlined by Geyer and Geyer [1998] in this issue. To underscore the fact that this problem should not at all be viewed as incidental to histochemical studies when aspiring to document an analysis of glycan expression, it is pointed out that plant and animal lectins with reactivity to galactose differ notably with respect to the fine specificity regarding sequence extensions beyond the terminal monosaccharide [Lee et al., 1992, 1994; Galanina et al., 1997; Kaltner et al., 1997]. As proven recently by von der Lieth et al. [1998], disaccharides may even undergo differential conformer selection, reflecting disparities in the architecture of the binding sites. Overall, the shared specificity to galactose does not guarantee a comparable profile of binding to subpopulations of cellular galactose-containing glycoconjugates. This conclusion emphasizes the need to employ the tissue lectin for any functional correlations. The differential fine specificity of the galactoside-specific lectins VAA, RCA-I and galectin-1 for cellular constituents in glycohistochemical analysis is illustrated in figures 1B, C and 4A, B. With respect to the subcellular staining pattern, it is interesting that only the mammalian lectin exhibited nuclear staining. Such a result has already been detected in the initial immunohistochemical study with respect to tumor pathology [Gabius et al., 1986]. It is relevant to mention that galectin-1 has a role in pre-mRNA splicing [Vyakarnam et al., 1997]. As a consequence of different receptor properties, RCA-I and VAA stained macrophages and lymphocytes fairly well in contrast to the tissue lectin.

Corroborating the observations 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, goblet cells, macrophages and plasma cells, reflecting measurable levels of dissimilarities in the fine-structural ligand recognition. This is quite well illustrated for epithelial and endothelial cells of the intestinal mucosa as well as for macrophages of

Fig. 5. Light micrographs of normal human appendiceal crypts (**A**, **C**) and appendiceal crypts in acute appendicitis (**B**) as well as of adenoma of the large intestine (**D**) after stepwise application of biotinylated SBA (**A**, **B**) and lactosylated albumin (**C**, **D**), ABC reagents, the chromogen 3-amino-9-ethylcarbazole and hematoxylin counterstainin. (**A**, **B** ×330. **C** ×800. **D** ×500.



the germinal centers which were labeled by HPA and SBA, whereas DBA did not bind to these cells (fig. 2A, B, 3A–C).

In this context, one should bear in mind that even closely related lectins of the same monosaccharide specificity from the organism may exhibit differential binding capacity to homologous lymphocyte populations, for example the two avian galectins [Schneller et al., 1995]. The spatial organization of ligands may influence their reactivities besides their sequences and conformations, as expertly discussed for selectins [Varki, 1994]. To be able to dock on complementary sites, the presentation of binding pockets, as provided by crystallographic analysis, will have an impact on the level of affinity in situ. It is the indisputable strength of tailor-made neoglycoconjugates to correlate the spatial factor of optimal geometric ligand arrangement with affinity [Lee and Lee, 1994, 1997; André et al., 1997].

Comparative Glycohistochemical Analysis of the Proximal and Distal Large Intestine

A comparative study of lectin binding to goblet cell mucin in another region of the large intestine, namely the rectosigmoid, demonstrated that DBA, SBA and galectin-1 primarily bound to the distal colon, while UEA-I and VAA labeling was selectively found in goblet cell mucin of the proximal large intestine. These spatial differences must be taken into consideration in glycohistochemical studies of the diseased colon, in which altered tissue will be compared with control specimens of the mucosa from the same intestinal region of normal individuals.

Glycohistochemical Alterations in Acute Inflammation Demonstrated in Phlegmonous 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 the normal appendix (fig. 5A, B). 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.

Similar changes of glycosylation of goblet cell mucus (increase of extent of DBA and SBA binding, but not of HPA binding to goblet cell mucus) had 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 in inflammatory bowel disease is not necessarily related to malignant change, as has been controversely discussed for inflammatory bowel disease [Ahnen et al., 1987]. Although the precise functional implications of the observed alterations are at present not obvious, as in a general context further discussed by Brockhausen et al. [1998] and Hakomori [1998] in this issue, such changes clearly reflect a potentially pertinent impact of the regulation of glycosylation, warranting further studies.

Glycohistochemical Alterations in Colonic Adenoma

Numerous studies have described 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; Fucci et al., 1993; Jass et al., 1993]. The most frequently detected changes of lectin binding in the adenoma were an increase in the receptivity 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], UEA-I [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]. As discussed in the preceding paragraph, the applied tools contribute to the structural analysis of glycans. However, a functional correlation in terms of recognitive interplay can only be inferred by the detection of suitable receptor sites and by the demonstration of ligand properties to endogenous lectins (table 5).

Having synthesized markers to measure the ligand properties of carbohydrate moieties and having purified endogenous lectins, it is possible to compare glycohistochemically accessible carbohydrate moieties and equivalent receptors in tissue material with defined morphological alterations. The binding of neoglycoconjugates presenting blood group trisaccharides has already been shown to be of clinical significance. It correlates for A/H epitopes with morphometric features in lung and prostate cancer and with survival in patients with lung cancer [Kayser et al., 1994, 1995]. Focusing on colon cancer, initial studies with primary and metastatic lesions caution against the view of a simple picture for metastasis formation [Gabius et al.,

Table 5. Binding of carrier-immobilized carbohydrate ligands to mucosa and adenoma of the large intestine

Site of neoglycoprotein binding	Lac	β-GalNAc	β-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 [from Brinck et al., 1996, with modifications].

1991; Irimura et al., 1991; Schoeppner et al., 1995]. With the manifestation of an adenoma increases in presentation of lectin-reactive Gal (Galβ-1,3-GalNAc), fucose and N-acetylglucosamine moieties appear to be associated with enhanced presentation of carbohydrate ligand-binding capacity. In contrast to this quantitative aspect, the qualitative subcellular binding pattern of neoglycoproteins to adenoma cells continues to closely resemble that of epithelial cells of normal mucosa (fig. 5C, D). The apical surface and mucus of secretory vesicles are apparently free of detectable binding sites. As already mentioned, presence of high-affinity ligands can render endogenous galectins inaccessible to neoglycoconjugates, among them glycan chains of laminin, carcinoembryonic antigen and lysosome-associated membrane glycoproteins 1 and 2 [Ohannesian et al., 1995; Bresalier et al., 1996]. In summary, the measured quantitative increase intimates a potential role in the progression from adenoma to carcinoma. Similar to the upregulation of neoglycoprotein-binding sites in chorionepithelioma cells, reported previously [Gabius et al., 1989], the relevance of these alterations for cell sociology warrants further scrutiny.

Conclusion

Application of a panel of plant/invertebrate and endogenous mammalian lectins with similar nominal monosaccharide specificity as well as of neoglycoproteins with histochemically crucial ligand structures probes complementary aspects of protein-carbohydrate recognition. Monitoring of normal, inflamed and neoplastically transformed tissue of the large intestine allows a thorough comparison between the binding patterns of exogenous and endogenous lectins as well as of carrier-immobilized glycoligands to gain functionally valid insights such as the colocalization of lectin-reactive carbohydrate epitopes and glycoligand-binding sites. The endogenous lectin galectin-1 has an apparently very similar pattern of ligand localization as the carrier-immobilized glycoligand lactose. Its binding profile differed from plant and invertebrate lectins with identical nominal monosaccharide specificity.

Expression of lectin-reactive carbohydrate epitopes and glycoligand-binding sites appears to be related to the maturational status of the epithelial cells and allows subtyping of macrophages and dendritic cells with possible functional implications. Glycosylation of goblet cell mucus is specifically altered in acute inflammation, as demonstrated in phlegmonous appendicitis. Increases in the extent of presentation of glycoligand-binding sites and lectin-reactive carbohydrate epitopes in colonic adenoma accompanies the transition from adenoma to carcinoma. Conceptually, these results clearly illustrate the power of combined studies exploiting the target specificities of endogenous lectins and carbohydrate ligands to infer physiological protein-carbohydrate recognition in situ.

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