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Expression of α2,6-linked Sialic Acid Residues in Neoplastic But Not in Normal Human Colonic Mucosa

A Lectin-gold Cytochemical Study with Sambucus nigra *and* Maackia amurensis *Lectins*

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Increased sialylation of cell surface glycoconjugates has been demonstrated in malignant tumors and shown to be correlated with the invasive and metastatic growth of colon carcinoma cells. The authors have applied the* Maackia amurensis *lectin, which interacts with α2,3-linked sialic acid, and the* Sambucus nigra *I lectin specific for α2,6-linked sialic acid. In human colon, α2,3-linked sialic acid was detectable in normal and transitional mucosa as well as in adenomas with different degrees of dysplasia and in carcinoma. In contrast, α2,6-linked sialic acid as visualized with* Sambucus nigra *I lectin was found only in severe dysplasia and carcinoma. Thus expression of binding sites for* Sambucus nigra *I lectin was associated with the occurrence of histologic features of malignancy. It is concluded that malignant transformation in human colonic epithelium is accompanied by the* de novo *expression of an α2,6 sialyltransferase. These findings provide the basis for more detailed studies of the possible role of cell surface glycoconjugates bearing α2,6-linked sialic acid in growth behavior of human colonic epithelial cells. (Am J Pathol 1991, 139:1435–1448)

Intestinal cells synthesize various types of secretory and membrane glycoproteins with oligosaccharide side chains that exhibit a varying composition, depending on the state of cellular differentiation and the location of the cells in the gastrointestinal tract.[1] Changes in intestinal glycosylation reactions related to cancer are well documented.[2]

Sialic acids occupy the nonreducing terminus of oligosaccharides and are found in a variety of different linkages to other sugars,[3] which are established by the action of a large family of specific sialyltransferases.[4,5] It is well documented that sialylated oligosaccharides are involved in a variety of cellular recognition phenomena such as homing of peripheral lymphocytes to peripheral lymph nodes,[6–8] virus binding,[9,10] organ induction and differentiation,[11–14] and cellular invasiveness.[15–18]

The importance of cell surface sialic acid in metastasis of murine colonic carcinoma cells has been demonstrated.[19,20] Consistent with these observations, a higher content of sialyl-dimeric Lewis^x antigen was found in liver metastases of human colorectal carcinomas as compared with the primary carcinoma and normal colonic mucosa.[21] Investigations on human colon carcinoma cells that differ in metastatic capacity showed a direct correlation between the extent of cell surface sialylation and their *in vivo* tumorigenicity.[18] Furthermore binding to collagen IV was dependent on the presence of cell surface sialic acid and correlated with the *in vivo* aggressiveness of the colon carcinoma cell sublines. Other studies have investigated sialylated carbohydrate antigens carrying sialic acid in an α2,3-linkage. Monoclonal antibody CA 19-9[22] and CA 50[23] are reactive with the carbohydrate sequence sialic acid α2,3 galactose β1,3 (fucose α1,4) *N*-acetylglucosamine[24] and sialic acid α2,3 galactose β1,3 *N*-acetylglucosamine,[25] respectively. Both antibodies showed inconsistent immunostaining not only in human colon carcinoma but also in the normal and transitional mucosa and adenoma.[26–28] Sialylated

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α2,3 lacto-*N*-tetraose recognized by monoclonal antibody K4[29,30] was not detectable in normal colonic mucosa and only occasionally was found in colon adenoma and carcinoma.[31] Monoclonal antibody NUH2, obtained after immunization of mice with the disialoganglioside fraction of human adenocarcinoma, was shown to be reactive with disialogangliosides with binary α2,3 sialosyllactosamine.[32] This carbohydrate antigen present on a series of gangliosides was undetectable in proximal and distal regions of normal human colonic mucosa, but was present in colon adenocarcinoma. Monoclonal antibody TKH2 interacts with sialic acid α2,6 *N*-acetylgalactosamine α1-*O*-Ser/Thr, which is the sialosyl-T_n epitope.[33] Positive immunostaining with TKH2 was observed in colon carcinoma.[34–36] In one study, correlation between the immunostaining and the degree of tumor differentiation, as well as Dukes' stage and location, was found.[35] Contradictory data about TKH2 staining of normal colonic mucosa were reported that were found either to be negative[34] or variably positive.[35]

During the past few years, lectins that discriminate between various types of sialylated sequences have been reported. The lectin from elderberry (*Sambucus nigra* L.) bark (SNA I) has been shown to bind with high affinity to glycoconjugates containing the terminal sequence sialic acid α2,6 galactose/*N*-acetylgalactosamine.[37] The leukoagglutinin from *Maackia amurensis* (MAL) binds with high affinity to sialic acid α2,3 galactose β1,4 *N*-acetylglucosamine but not to the 2,6-linked isomers[38,39] and, therefore, is a most interesting complementary probe to the SNA. Both sialic acid–specific lectins bound to particles of colloidal gold have been used for the light and electron microscopic detection of their respective binding sites in tissue sections.[40,41] The exclusive reactivity of both lectins has been vigorously established by *in vitro* biochemical assays with a large series of substrates[37–39] and confirmed by a panel of histochemical control reactions.[40,41]

In the present study, we have performed a detailed lectin cytochemical analysis of the occurrence and distribution of α2,6- and α2,3-linked sialic acid residues in the mucosa of human colon. Several novel findings were made demonstrating that α2,6-linked sialic acid residues, although not detectable in normal and transitional mucosa, are expressed in severe dysplasia and colon carcinoma.

Materials and Methods

Reagents

Affinity-purified MAL was a gift of Dr. W. Peumans (Katholieke Universiteit Leuven, Belgium). Digoxigenin (DIG)-labeled SNA I and sheep anti-digoxigenin antibody (IgG fraction) were obtained from Boehringer Mannheim GmbH (Mannheim, Federal Republic of Germany). Tetrachloroauric acid, trisodium citrate, hydroquinone, and paraformaldehyde were from Merck (Darmstadt, Federal Republic of Germany); silver acetate, Carbowax 20M, and glutaraldehyde (vacuum distilled) from Fluka (Buchs, Switzerland); 3′sialyllactose (lot No. 6112-290) and 6′sialyllactose (lot No. 1137-150) from BioCarb Chemicals (Lund, Sweden); and tannic acid (EM grade) from Mallinckrodt (St. Louis, MO). All other reagents were of the highest available purity.

Particles of colloidal gold with a diameter of 8 and 10 nm, respectively, were prepared by reducing tetrachloroauric acid with tannic acid and trisodium citrate.[42] Directly gold-labeled MAL was prepared as described in detail previously.[41] In brief, the pH of the colloidal gold was adjusted to 6.0. Leukoagglutinin from *Maackia amurensis* (250 μg) was dissolved in 10-fold diluted phosphate-buffered saline (PBS), and 10 ml of the colloidal gold was added. Then 1 ml 10% bovine serum albumin (BSA) and 1 ml 10-fold concentrated PBS were added successively. The crude MAL-gold complexes were centrifuged at 105,000g_{rmax} for 45 minutes at 4°C, and the sedimented MAL–gold complexes were resuspended with PBS containing 2 mg/ml Carbowax 20 mol/l (molar).

The anti-DIG antibodies were complexed to particles of colloidal gold as described in detail previously.[43] Briefly, 20 μg or 40 μg of IgG in 1 ml PBS (pH 8.5) was added to 1 ml of colloidal gold. This was followed by the sequential addition of 200 μl 10% BSA in PBS (pH 8.5) and 100 μl 10-fold concentrated PBS (pH 8.5). The crude IgG–gold complexes were concentrated by ultracentrifugation in a fixed angle rotor at 105,000g_{rmax} for 45 minutes at 4°C. After careful removal of the supernatant, the sedimented IgG–gold complexes were resuspended in PBS (pH 8.5) containing 1% BSA and were stored at 4°C.

Tissues

The investigations were carried out as a retrospective study on materials from the files of the Institute of Pathology, University of Zürich. The tissues were obtained at surgical intervention and had been fixed in 4% phosphate-buffered formaldehyde and embedded in paraffin according to a standard protocol for histopathologic diagnosis. In the present study, paraffin blocks from a total of 43 colon adenocarcinomas (21 cases of right colon, 22 cases of left colon), including the normal appearing mucosa from the resection margin of 28 specimens (16 of right colon, 12 of left colon), were investigated. The transitional mucosa of a total of 37 cases (17 of right colon, 20 of left colon) was studied as well. The transitional mucosa

was defined as the 2-cm-wide zone encircling the carcinoma, exhibiting tall and often branched crypts and swollen goblet cells. In addition, paraffin blocks from 20 adenomas obtained at surgical polypectomy were investigated. From all blocks, consecutive serial sections were prepared. For histologic examination, sections were stained with hematoxylin and eosin (H&E).

Small pieces of tissue from normal colonic mucosa (from the resection margin of carcinoma-bearing patients or from biopsies from patients without carcinoma) and colon adenocarcinoma also were fixed in 3% formaldehyde (freshly prepared from paraformaldehyde) and 0.1% glutaraldehyde in PBS for 2 hours at room temperature. Afterwards the tissue pieces were briefly rinsed with PBS, placed in 50 mmol/l (millimolar) NH_4Cl in PBS for 1 hour to amidinate free aldehyde groups, and stored in PBS at 4°C. Dehydration with ethanol at progressively lowered temperature (down to −40°C), infiltration with Lowicryl K4M at −35°C to −40°C, and UV-light–induced polymerization at −40°C was carried out as described in detail elsewhere.[44] Semithin (1 μ) Lowicryl K4M sections were prepared and mounted on poly-*L*-lysine–coated glass slides.[45] Ultrathin sections were mounted on Parlodion/carbon-coated nickel grids.

Lectin Histochemistry

Paraffin sections (5 μ) were deparaffinized in two changes of xylene for 10 minutes each and rehydrated through a series of graded ethanol and finally brought into PBS. Semithin sections (1 μ) of Lowicryl K4M–embedded specimens were cut and placed on poly-*L*-lysine–coated glass slides.

Detection of α2,3-Linked Sialic Acid Residues with MAL–Gold Complexes

Sections were covered with MAL–gold complexes diluted in PBS containing 1% BSA and 0.01% Tween 20 and 0.01% Triton X-100 (Merck, Darmstadt, FRG) for 45 minutes in a moist chamber at room temperature. The MAL–gold was diluted to give an optical density $(OD)_{525\ nm} = 0.5$. After the lectin incubation, the sections were rinsed three times for 5 minutes each in PBS and fixed with 1% glutaraldehyde in PBS for 20 minutes. After rinses with PBS and distilled water, the sections were air dried and subjected to photochemical silver amplification (see below).

Detection of α2,6-Linked Sialic Acid Residues with SNA–Dig Conjugates

Sections were covered with SNA–DIG conjugate (25 μg/ml) in PBS containing 1% BSA, 0.01% Tween 20, and 0.01% Triton X-100 for 45 minutes at room temperature in a moist chamber. Afterwards sections were washed three times for 5 minutes each with PBS and covered with anti-DIG IgG–gold complexes for 45 minutes at room temperature in a moist chamber. The IgG-gold was diluted with PBS containing 1% BSA and 0.01% Tween 20 and 0.01% Triton X-100, to give an $OD_{525\ nm} = 0.05$. After three rinses of 5 minutes each with PBS, the sections were fixed with 1% glutaraldehyde in PBS for 20 minutes, rinsed with PBS and several changes of distilled water, and air dried.

Photochemical Silver Amplification

The technique for photochemical silver amplification using silver acetate[46] was performed as described previously.[44,47] Sections were placed in 0.25 mol/l citrate buffer (pH 3.8) containing hydroquinone (0.96 g/100 ml) for 5 minutes at room temperature. Next the sections were transferred into a solution of 0.25 mol/l citrate buffer (pH 3.8) containing hydroquinone (0.96 g/100 ml) and silver acetate (200 mg/100 ml). After 18 minutes, the sections were rinsed quickly with distilled water and placed in Superfix (Agfa, Leverkusen, FRG) for 5 minutes, rinsed with distilled water, counterstained with nuclear fast red, dehydrated, and mounted.

Cytochemical Controls

Before incubation of sections, MAL–gold complexes were preincubated for 20 minutes at room temperature with 3′sialyllactose (0.1 mmol/l, 1 mmol/l, 5 mmol/l, 10 mmol/l, 20 mmol/l) and the SNA-DIG conjugates with 6′sialyllactose (0.01 mmol/l, 0.1 mmol/l, 0.5 mmol/l). In other staining protocols, the SNA-DIG conjugate was omitted and the sections were incubated only with anti-DIG IgG–gold complexes ($OD_{525\ nm} = 0.05$) followed by photochemical silver amplification. Sections were pretreated with sodium metaperiodate (2.5 mmol/l in PBS for 45 minutes on ice) to oxidize sialic acid residues.

Results

Normal Mucosa

In the normal mucosa of right and left colon, positive staining for MAL was observed. The luminal surface of the absorptive enterocytes was intensely stained along the entire length of the crypts and in the surface epithelium of both right and left colon (Figures 1a and 2c). The

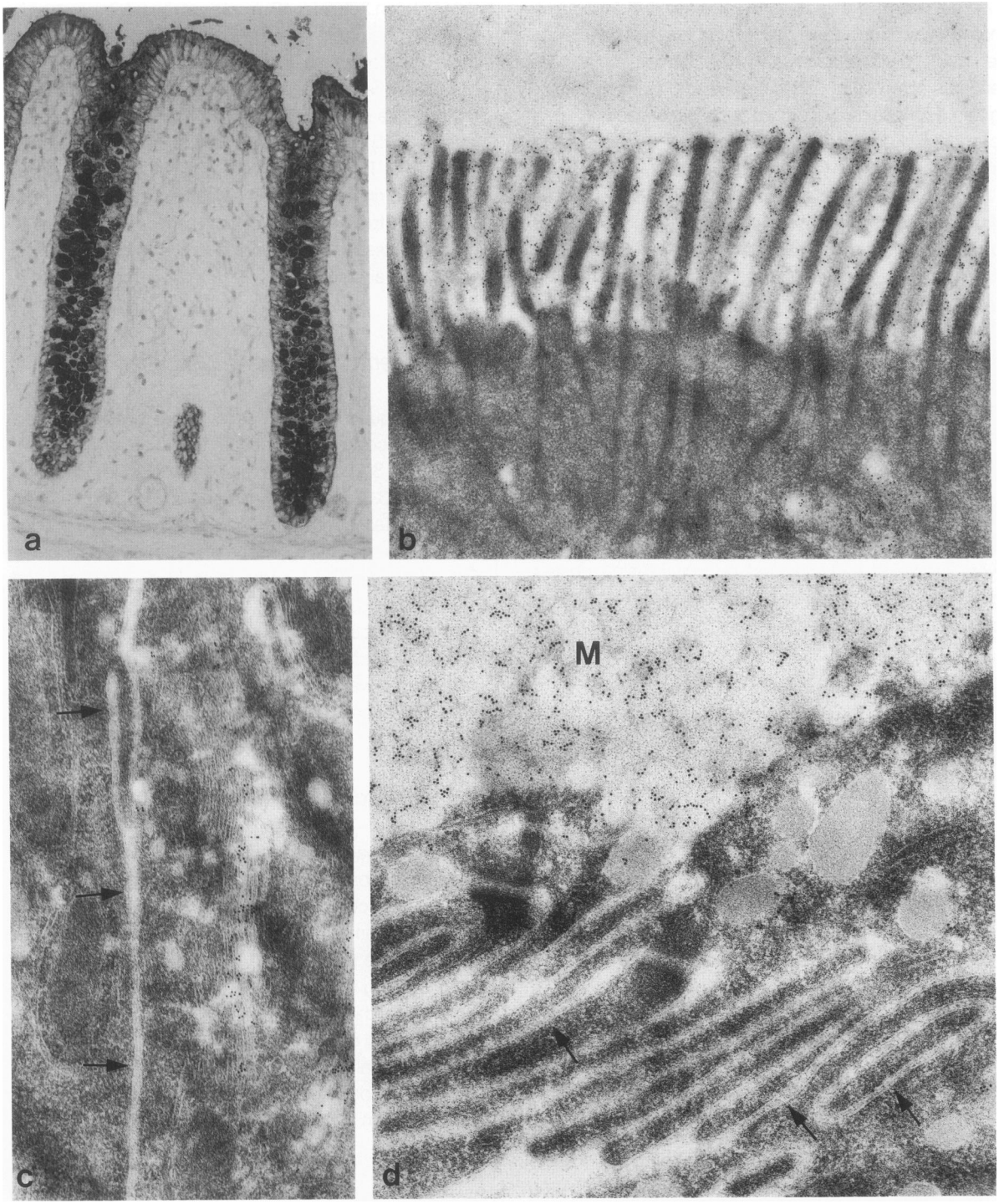

Figure 1. *Demonstration of α 2,3-linked sialic acid in normal human colon; Maackia amurensis lectin-gold technique.* **a:** *In a full-thickness paraffin section, positive staining of mucus of goblet cells as well as apical surface and Golgi apparatus of absorptive enterocytes can be seen, ×450.* **b, c, d:** *In ultrathin Lowicryl K4M sections, gold-particle labeling is present over the brush border, vesicular structures (arrowheads), the Golgi apparatus and goblet cell mucus (M), but absent along the lateral plasma membrane (arrows)* **b,** *×34,000;* **c,** *×41,000;* **d,** *×55,000.*

restriction of MAL labeling to the apical plasma membrane domain of absorptive enterocytes was unequivocally demonstrated by electron microscopy (Figure 1b–d). Intracellular staining was associated with endosomal elements, lysosomal bodies, and the Golgi apparatus (Figure 1b, c). The mucus of the goblet cells present along the crypts and in the surface epithelium was MAL positive (Figure 1a, d).

In the normal mucosa of both right and left colon, no staining with SNA could be detected by light microscopy

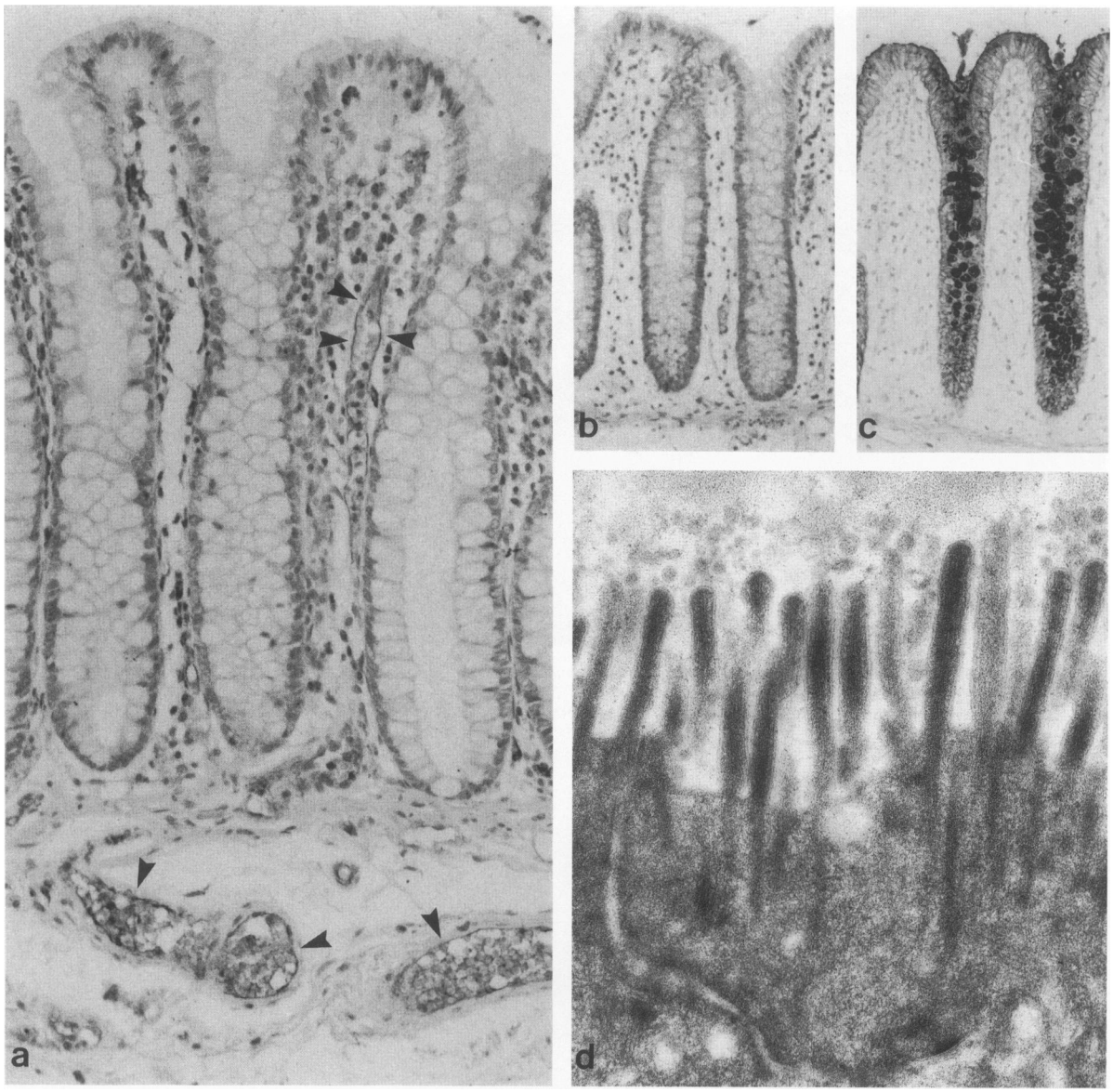

Figure 2. *Demonstration of α 2,6-linked sialic acid in normal human colon; Sambucus nigra I lectin-gold technique.* **a**: *No lectin staining is detectable in the absorptive and goblet cells along the crypts and in the surface epithelium. Note the positive reaction of red blood cells and endothelia of blood vessels (arrowheads) and cells of the stroma, (×750)* **b, c**: *Full-thickness adjacent serial paraffin sections stained with Sambucus nigra I lectin* **(b)** *and Maackia amurensis lectin* **(c)** *(b, c, ×400).* **d**: *Lowicryl K4M ultrathin section incubated with Sambucus nigra I lectin. No gold particle labeling can be detected over the brush border, lateral plasma membrane and cytoplasm of absorptive enterocytes, (×41,000).*

in paraffin sections (Figure 2a, b) nor in Lowicryl K4M semithin sections or by electron microscopy in ultrathin Lowicryl K4M sections (Figure 2d). Capillaries and red blood cells contained therein as well as lymphocytes in the stroma were stained by SNA (Figure 2a), however.

Adenoma with Different Degrees of Dysplasia

The grading of the dysplasia in adenoma was performed according to Morson et al[48] on H&E-stained sections (Figure 3a).

The investigation of the normal-appearing mucosa adjacent to the adenomas and mild dysplasia of adenoma disclosed a staining pattern for MAL and SNA as already described for the normal mucosa (Figure 3b, c).

The intensity of intracellular and apical cell surface staining by MAL progressively decreased with the degree of dysplasia (Figure 3b). The intensity of staining of the goblet cell mucus also decreased in regions with mild dysplasia. When the SNA was applied to stain consecutive serial sections, all cells of the regions exhibiting severe dysplasia were positive at the apical surface (Figure 3c). Ten of the 20 investigated adenomas showed severe

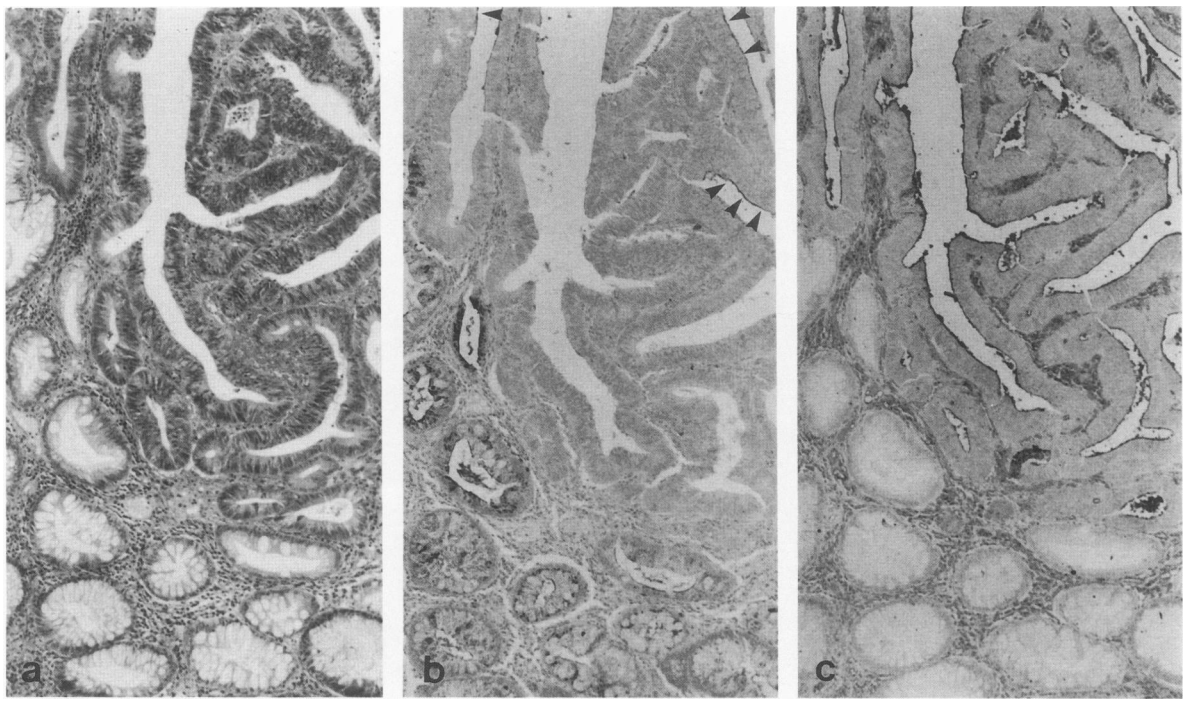

Figure 3. *Adenoma with severe dysplasia.* **a**: *H&E staining.* **b**: *Normal-appearing glands exhibit staining with Maackia amurensis lectin. Regions with severe dysplasia show restricted staining (arrowheads).* **c**: *Normal appearing glands are not stained with Sambucus nigra I lectin whereas intense staining can be seen in severe dysplasia. (***a–c***, ×150).*

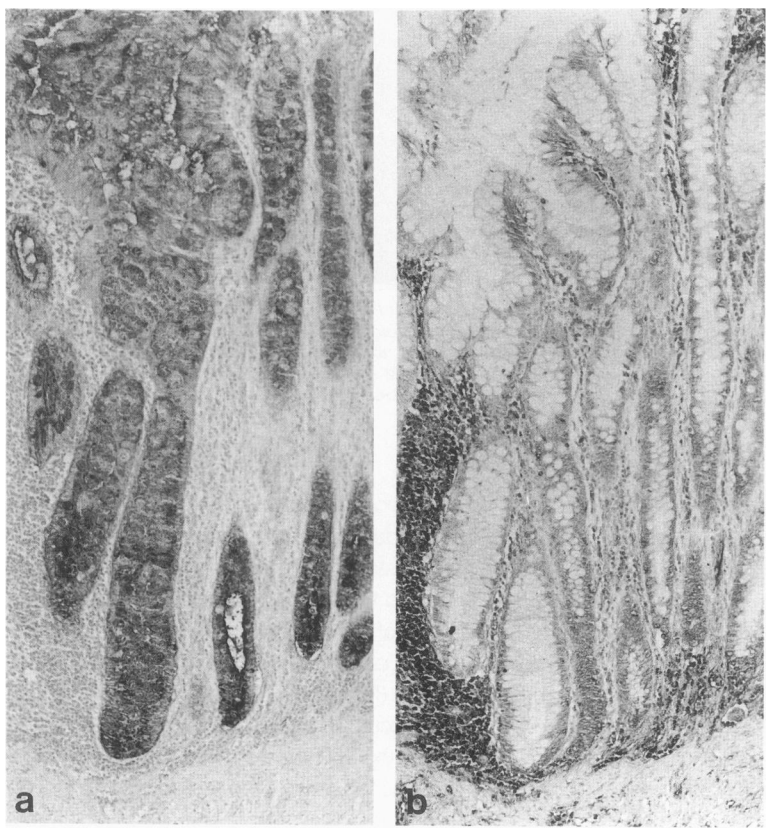

Figure 4. *Serial paraffin sections of transitional mucosa which exhibits staining with Maackia amurensis lectin* **(a)** *but not with Sambucus nigra I lectin* **(b)**. *(***a, b,** *×460).*

dysplasia that exhibited positive staining by SNA. Goblet cell staining was not observed irrespective of the degree of dysplasia.

Transitional Mucosa

The staining by MAL in the transitional mucosa (Figure 4a) of both right and left colon showed a pattern corresponding to that observed in the normal colonic mucosa. In general, staining by SNA of absorptive and goblet cells was not observed (Figure 4b). Only in 1 of 18 cases (right colon) and 2 of 19 cases (left colon) was any reactivity observed. This SNA staining was of weak intensity, however, and restricted to the lateral cell surface of some absorptive enterocytes of the surface epithelium and could not be detected in the crypt cells. No staining of the goblet cell mucus was observed.

Carcinoma

The carcinomas of the left colon comprised 5 well-differentiated, 14 moderately differentiated, and 2 poorly differentiated adenocarcinomas, as well as 1 mucinous carcinoma. From the right colon, 1 well-differentiated, 13 moderately differentiated, and 2 poorly differentiated ad-

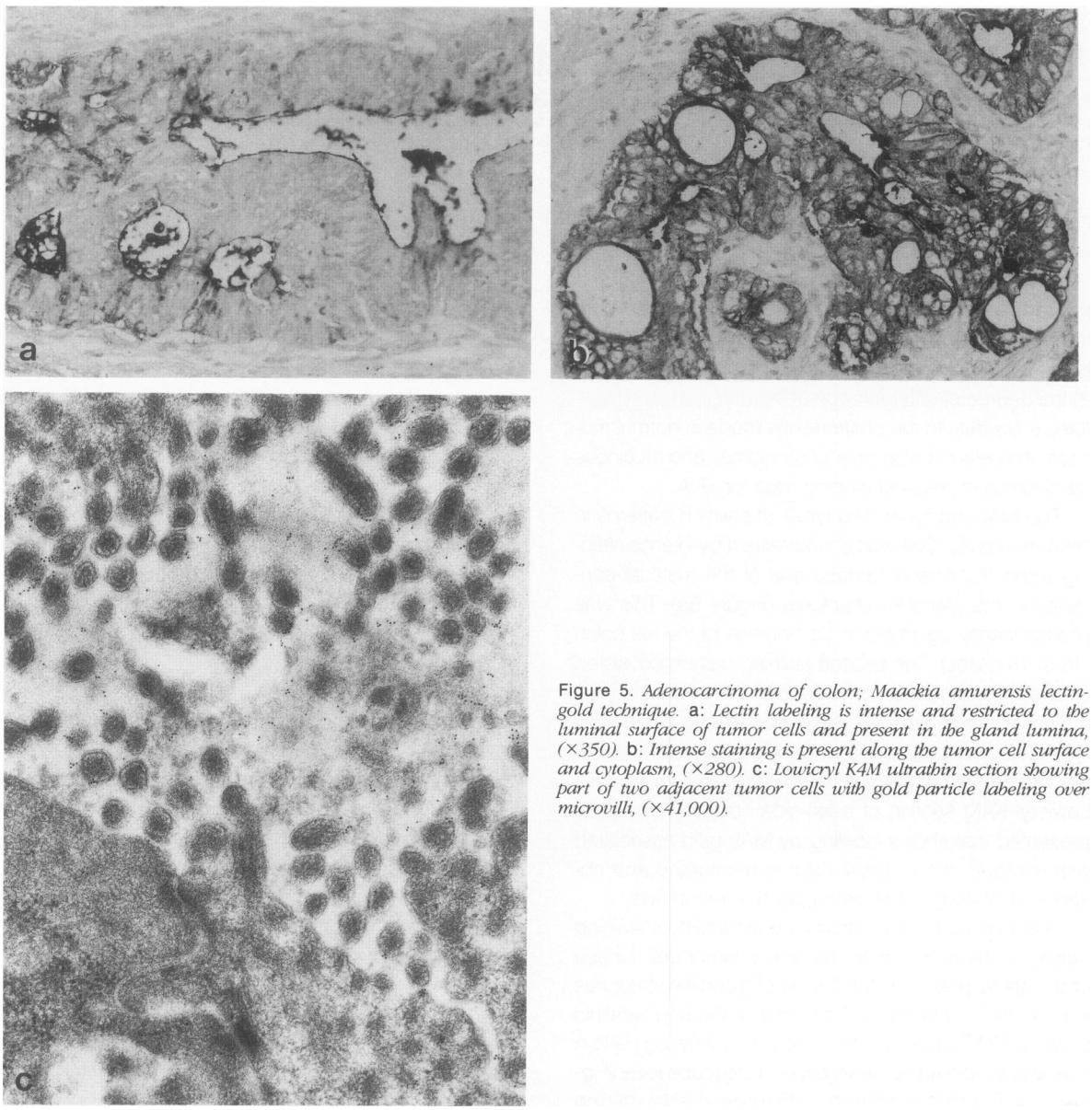

Figure 5. *Adenocarcinoma of colon; Maackia amurensis lectin-gold technique.* a: *Lectin labeling is intense and restricted to the luminal surface of tumor cells and present in the gland lumina, (×350).* b: *Intense staining is present along the tumor cell surface and cytoplasm, (×280).* c: *Lowicryl K4M ultrathin section showing part of two adjacent tumor cells with gold particle labeling over microvilli, (×41,000).*

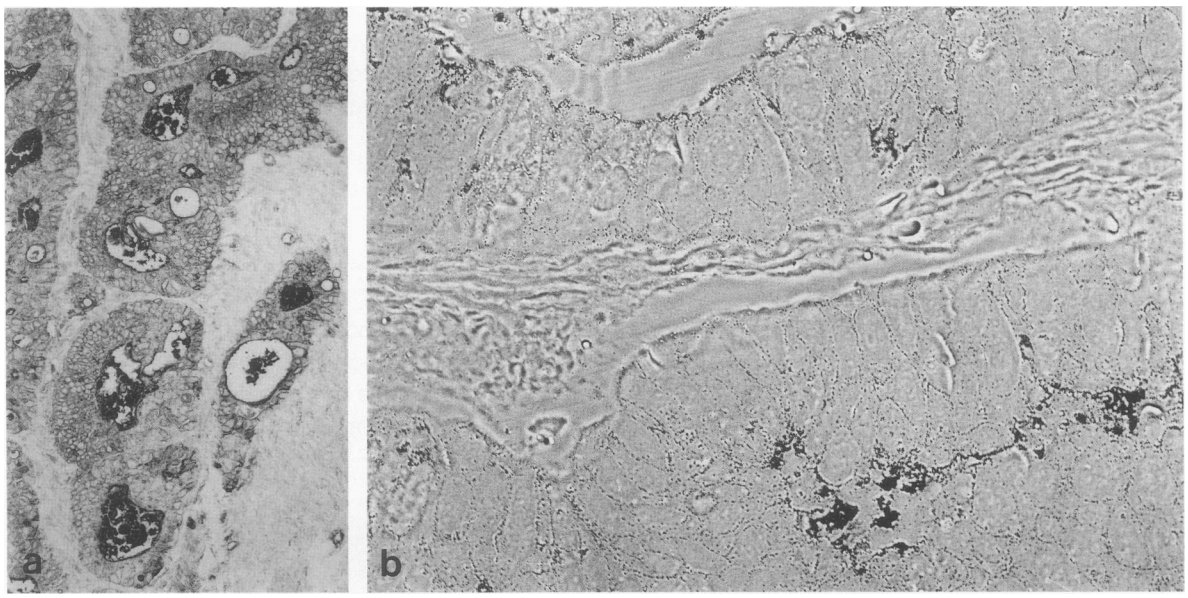

Figure 6. *Adenocarcinoma of colon; Sambucus nigra I lectin-gold technique.* **a:** *Positive staining indicating presence of α 2,6-linked sialic acid. Staining is more intense at the luminal surface and luminal content than basolateral tumor cell surface. (paraffin section, ×250).* **b:** *High resolution image of silver-intensified lectin-gold staining obtained on a Lowicryl K4M semithin section, (phase contrast, ×800).*

enocarcinomas as well as 5 cases of mucinous carcinoma were investigated.

The incubation of sections from carcinomas of the right and left colon resulted in a positive staining with both lectins (Figures 5–9). No correlation was observed between lectin staining patterns and 1) the histologic type or 2) the degree of differentiation of the carcinomas. Therefore, in contrast to the observations made in normal mucosa, the cells of colon adenocarcinomas and mucinous carcinomas expressed binding sites for SNA.

The MAL produced two types of staining patterns in the carcinomas. One was characterized by intense staining along the luminal surface and of the material contained in the glandlike structures (Figure 5a). This was predominantly observed in carcinomas of the left colon (12 of 18 cases). The second staining pattern consisted of labeling along the entire surface of the tumor cells (Figure 5b) and was predominant in carcinomas of the right colon (12 of 19 cases). Both staining patterns were usually found within all carcinomas. In Figure 5c, an ultrathin Lowicryl K4M section of a left-side adenocarcinoma is presented that shows labeling by MAL-gold associated with microvilli of the apical plasma membrane and absence of labeling in the lateral plasma membrane.

The labeling by SNA produced a consistent staining pattern in the carcinomas. The entire tumor cell surface and material present in the lumina of glandlike structures was labeled in paraffin sections (Figure 6a) and semithin Lowicryl K4M sections (Figure 6b). This labeling pattern was also observed at the electron microscope level (Figure 7). In Figure 8, the striking difference in SNA staining between part of an adenocarcinoma and the normal mucosa that had been invaded is shown.

When the extent of labeling by both MAL and SNA was evaluated in a given carcinoma, three cases of the right colon and two cases of the left colon showed labeling of all tumor structures by both lectins (Figure 9a, b). Three carcinomas of the right colon showed MAL staining over all structures and only regional SNA staining. One carcinoma of each side exhibited SNA staining of all structures but no MAL staining. As illustrated in Figure 9c and d, most carcinomas exhibited a variable and differential degree of staining, with some portions of the tumor positive for both MAL and SNA and others labeled only by SNA.

Cytochemical Controls

The lectin staining could be abolished by preincubation of MAL, with 5 mmol/l 3′sialyllactose, whereas 6′sialyllactose had no effect on the lectin staining. Preincubation of SNA–DIG conjugate with 0.1 mmol/l 6′sialyllactose resulted in abolition of staining; 3′sialyllactose had no effect. Incubation of tissue sections with anti-DIG IgG–gold complex alone resulted in no staining. The mild periodation of sections before lectin labeling reduced the labeling intensity significantly.

Discussion

The adenoma–carcinoma sequence of colorectal tumorigenesis is currently considered to be a multistep pro-

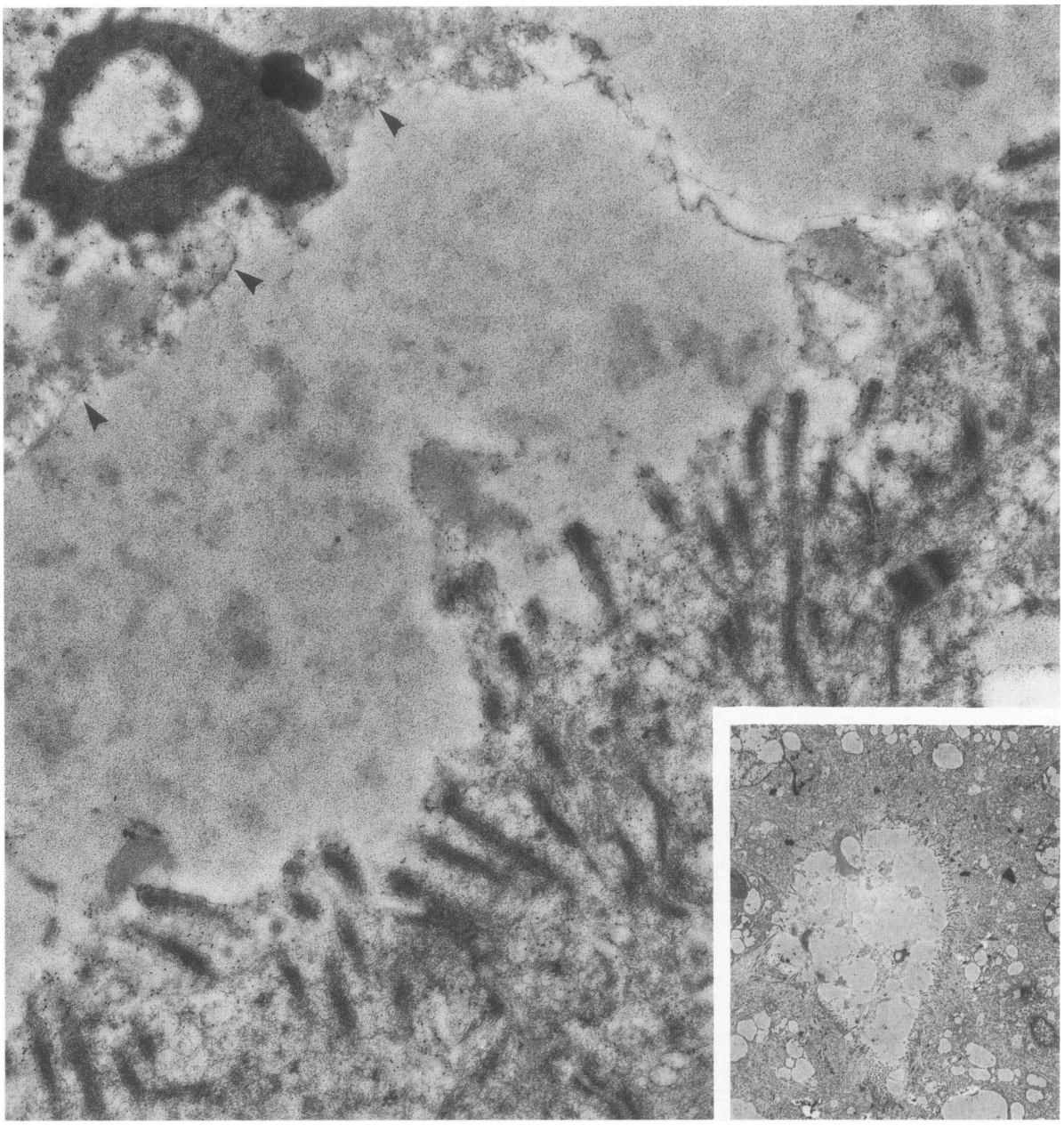

Figure 7. *Lowicryl K4M thin section from adenocarcinoma stained with Sambucus nigra I lectin-gold technique. Note presence of gold particle labeling over microvilli and cellular debris in the gland lumen (arrowheads). The inset shows part of the adenocarcinoma at low magnification. (×31,000; inset ×3,500)*

cess involving a variety of somatic alterations,[49] among them increased or aberrant expression of glycoconjugates.[2,15] In this respect, one of the more consistent alterations associated with neoplastic transformation and metastatic potential is increased β1,6 branching of asparagine-linked oligosaccharides.[50–52] Because most of these lactosamine antennae are substituted with sialic acid, the increased β1,6 branching may contribute to the carcinoma-associated increase in sialic acid.[18,21]

Earlier studies employing various lectins (Concanavalin A, wheat germ agglutinin, *Ricinus communis* agglutinin, etc.) demonstrated differences in lectin agglutinability between normal and transformed cells.[53,54] Subsequent electron microscopic investigations, however, showed the presence of cell surface binding sites for these lectins in both normal and transformed mesenchymal and epithelial cell lines.[55–62] Similarly we observed positive staining in both normal colonic mucosa and carcinoma employing the sialic acid–specific lectin from *Limax flavus* (unpublished), which interacts equally well with α2,3- and α2,6-linked sialic acid residues.[63] In the present study, we have applied two recently described

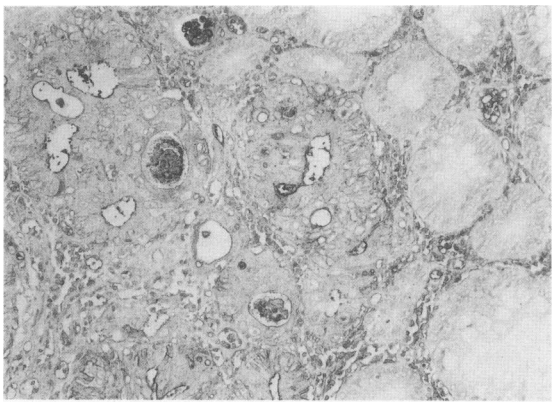

Figure 8. *Paraffin section from adenocarcinoma. Staining by Sambucus nigra I lectin can be observed in the carcinoma and is absent in adjacent normal mucosa, (×200).*

sialic acid–specific lectins for the histochemical detection and discrimination of α2,3- and α2,6-linked sialic acid residues, MAL[38,39] and SNA I,[37] respectively. The use of lectins with such a narrow specificity at the light and electron microscopic level has made several novel observations possible. Epithelial cells of the normal mucosa of right and left human colon as well as of mild dysplasia lacked cytochemically detectable α2,6-linked sialic acid residues but were positive for α2,3-linked sialic acid residues. In contrast, we found expression of α2,6-linked sialic acid residues in the epithelial cells of colon carcinoma and severe dysplasia. Staining was observed in colon carcinoma irrespective of the histologic type and degree of differentiation. The disaccharide recognized by SNA I bears resemblance to the sialylated Tn antigen, which consists of Neu5Acα2,6GalNAcα Ser/Thr. With the use of a monoclonal antibody specific for sialylated Tn antigen, we could exclude that SNA I staining is due to this antigen (Roth, Sata, Stamm and Heitz, manuscript in preparation). Thus, by using sialic acid–specific lectins discriminating between different linkages, a basic difference in sialylation of glycoconjugates could be shown between epithelial cells present in normal colonic mucosa and mild dysplasia and those of severe dysplasia and colon carcinoma. The expression of α2,6-linked sialic acid residues as detected with the SNA was positively correlated with the occurrence of established cytologic and histologic features of malignancy.

Our results demonstrate that the *de novo* expression of a specific terminal glycosylation sequence is associated with human colon carcinoma. This implies the expression of an α2,6 sialyltransferase, because the terminal glycosylation sequences produced by a cell are presumed to reflect the expression of the corresponding glycosyltransferases that synthesize them.[4,5,64] In this context, the recent isolation of a partial cDNA for human α2,6 sialyltransferase is noteworthy because S_1 nuclease analysis demonstrated expression of α2,6 sialyltransferase transcripts in a human colon adenocarcinoma cell line.[65] The concept of transcriptional regulation of cell-type–specific glycosylation[4,5,64] is strongly supported by experiments in which the cellular glycosylation machinery was altered by transfection of cells with DNA fragments or expression vectors containing cDNAs coding for late-acting glycosyltransferases.[66–69] Particularly relevant in the context of the present observations are our transfection experiments on Chinese hamster ovary cells.[69] Although wild-type Chinese hamster ovary cells produce only sialic acid linked α2,3 to galactose, clonal cell lines stably transfected and expressing activity for α2,6 sialyltransferase synthesize both oligosaccharides with sialic acid α2,3-linked and α2,6-linked to galactose.

Nakasaki et al[36] have investigated the distribution of immunostaining by several monoclonal antibodies directed against different tumor-associated carbohydrate epitopes. In serial sections of colon and gastric carcinomas, they found a mosaicism of antigen expression that correlated with the degree of differentiation. It was proposed that the different degree of glycosylation in spatially discrete tumor cell populations was a reflection of tumor cell differentiation and progression. The different staining patterns for α2,3- and α2,6-linked sialic acid residues in colon carcinoma observed in the present study were not correlated with spatially discrete cell populations. Further no correlation with the degree of tumor differentiation could be established. We consider that the observed staining patterns could be explained as follows: Carcinoma cells positive for both terminal sequences certainly express both α2,3- and α2,6 sialyltransferase. Differences in intensity of staining between single cells are most probably due to quantitative differences in the level of expression of the two sialyltransferases, which compete for a common acceptor substrate. The extreme situation in which carcinoma cells were positive for α2,6- and negative for α2,3-linked sialic acid residues may result either from such a competition or a lack of α2,3 sialyltransferase. The reasons for the observed differences should be clarified as soon as cDNAs for the two sialyltransferases become available. There are other aspects of the expression of α2,6-linked sialic acid residues that need to be clarified. The procedure of paraffin embedding results in the removal of certain glycolipids. Preliminary investigations on *N*-glycanase–pretreated paraffin sections of colon carcinoma, however, showed abolition of SNA staining (unpublished results). This suggests that the observed SNA binding occurs on sialylated asparagine-linked oligosaccharides of glycoproteins and apparently not on glycolipids. Currently we do not know if α2,6 sialylation in colon carcinoma is related to a single specific protein or not. It is also premature to predict whether or not the observed colon carcinoma-associated difference in these two relatively

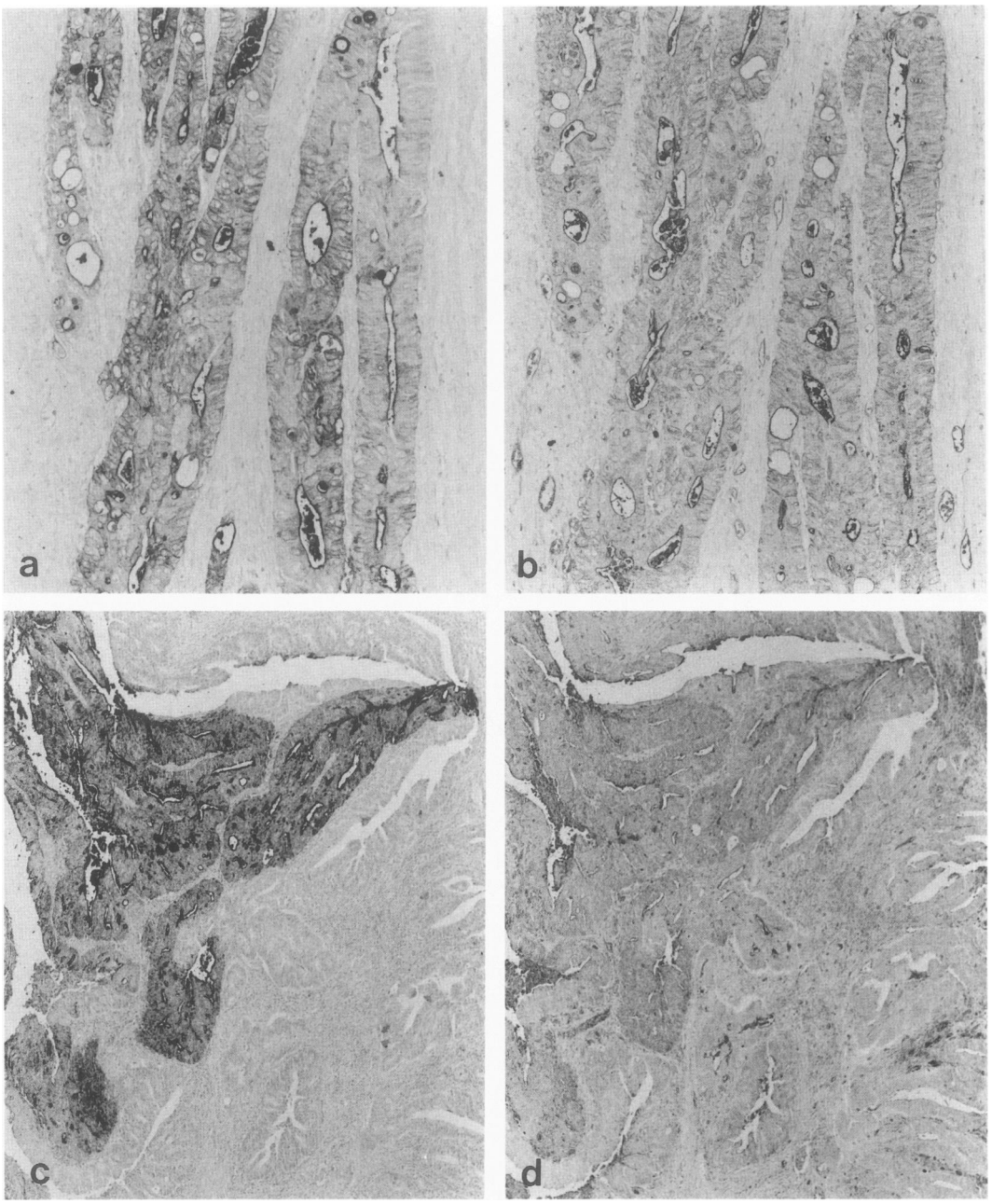

Figure 9. *Adjacent serial paraffin sections of adenocarcinomas.* **a, b**: *The carcinoma is stained by Maackia amurensis lectin* **(a)** *and Sambucus nigra I lectin* **(b)**, *(***a, b**, *×200).* **c, d**: *Only part of the carcinoma is positive with Maackia amurensis lectin* **(c)** *whereas all structures are positive with Sambucus nigra I lectin* **(d)**. *(***c, d**, *×40)*

common terminal glycosylation sequences is biologically significant. Further *in vitro* studies with experimentally manipulated cultures of human colon carcinoma cell lines may provide answers to these questions.

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