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Histochemical localization of sialylated glycoconjugates with *Tritrichomonas mobilensis* lectin (TLM)

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Summary. A new sialic acid-specific lectin from the colonic parasite of squirrel monkeys *Tritrichomonas mobilensis* (TML) was tested on human and mouse tissues for histochemical staining properties. There were no substantial differences in reactivity between frozen and formalin fixed paraffin sections. TML staining was blocked by preincubation with sialic acid or by sialidase digestion. TML/anti-TML antibody histochemistry was identical with the TML-gold technique. The staining pattern was not blood group dependent. TML stained strongly the luminal membranes of normal vascular endothelium as well as endothelial neoplasms. Lymphatic vessels and capillaries of kidney glomeruli and lung alveolar septi were negative or only slightly positive. In parenchymatous organs luminal membrane positivity was dominant, preferably of cells lining ducts. Weak fine-granular cytoplasmic and basolateral membrane staining was also observed. Umbrella cells in transitional epithelium and basal layers of squamous epithelia showed strong reactivity with cell membranes. Mucin in respiratory epithelium was positive whereas gastrointestinal mucins failed to stain uniformly. Erythrocytes and most white blood cell types showed distinct membrane positivity. Acetylation or alkaline O-deacetylation of tissue sections did not substantially change TML reactivity. Oxidation, however, completely blocked TML staining except for respiratory epithelium and colonic mucin.

Key words: Sialic (neuraminic) acid, *Tritrichomonas mobilensis* Lectin, Hemagglutinin, Histochemistry, Human, Mouse tissue

Introduction

Selective carbohydrate binding of lectins originating from plants and animals has been widely used for histochemical detection of glycoconjugates (Damjanov, 1987; Danguy et al., 1988, 1994; Walker, 1989; Spicer and Schulte, 1992). Sugar specificity and availability of the lectin are crucial determinants of use.

Lectin detection of sialic acid has usually involved wheat germ agglutinin (WGA), despite reactivity with both sialic acid (SA) and N-acetyl glucosamine, and galactose-specific peanut agglutinin (PNA) combined with sialidase treatment. Although a number of lectins have been reported to have SA specificity, only the *Limulus polyphemus* agglutinin (LPA) (Yamada and Shimizu, 1979; Muresan et al., 1982a,b), and the *Limax flavus agglutinin* (LFA) (Miller et al., 1982; Roth and Taatjes, 1985; Lucocq et al., 1986; Roth, 1993) have found application in histochemical visualization of sialoglycoconjugates. *Sambucus nigra* agglutinin (SNA) and *Maackia amurensis* leukoagglutinin (MAL) have added a new dimension to SA histochemistry by their ability to differentiate α2,6 and α2,3 SA linkages, respectively (Sata et al., 1989, 1991).

The recently described lectin from *Tritrichomonas mobilensis* (TML) is highly specific for SA (with or without O-acetylation). There is less reactivity with N-glycolyl SA, free or in both the α2,3 and α2,6 linkages (Babál et al., 1994). Basic histochemical studies with TML have produced useful results with minimal background staining (Babál et al., 1992, 1995). There was also complementary reactivity with the SA linkage specific lectins, SNA and MAL (Babál et al., 1993). The strict specificity for SA, ready availability and long-term stability, compared to LPA or LFA, make TML a prospective tool in sialic acid histochemistry. In order to employ this new histochemical tool its basic reactivity in a spectrum of tissues has been characterized.

Materials and methods

Tissue samples

Fresh samples of human tissues were obtained from surgically resected specimens. Formalin fixed and paraffin embedded specimens were obtained from

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archival material and from C57 BL/6 mice. A minimum of three (maximum seventy) different specimens of each tissue type were used.

Preparation of TML, anti-TML antibodies and TML-gold complex

Lectin from *T. mobilensis* was isolated and mucin-affinity purified as described elsewhere (Babál et al., 1994). Monoclonal antibodies (MoAb) were prepared using affinity purified TML for immunization of mice (Babál et al., 1994).

TML-gold complexes were prepared with a modification of the method used by Sata et al. (1989): 5 ml of gold colloid with 10 nm particles (Sigma, St. Louis, MO), 0.5 mg of TML in 2 ml PBS, 0.5 ml of 10% BSA in PBS, and 0.4 ml of 10 fold concentrated PBS were added successively and incubated 15 minutes at room temperature. The TML-gold complexes were then centrifuged at x20,000 g for 1 hour at 4 ºC, the sediment was resuspended in 3 ml of PBS containing 6 mg of carbovax PEG 8000 (Fisher Scientific Fair Lawn, NJ), filtered through 0.45 µm pore membrane and stored at 4 ºC.

Staining protocol

Cryostat sections and deparaffinized formalin fixed tissue slides were rehydrated in PBS. TML-gold complexes with final concentration of 5 µg TML/ml PBS were applied to slides for 60 minutes, after 2 washes staining was developed with Silver enhancing kit (Biocell Research Ltd., Philipsburg, NJ), counterstained with hematoxylin, cleared in xylene and mounted.

TML-anti-TML MoAb histochemistry

Sections were incubated 60 minutes with 2 µg TML/ml PBS; washed; exposed 10 minutes to 5% non-fat milk (to block nonspecific background staining of the MoAb) and 0.05% H_2O_2 in PBS; 2 washes; B-VI-10C4 anti-TML MoAb (ascites diluted 1:500 in PBS) 30 minutes; 2 washes; anti-mouse IgG biotinylated antibodies 20 minutes; 2 washes; ABC-Px complex (Vector Laboratories, Burlingame, CA) 15 minutes; 2 washes; aminoethyl carbazole (Biomeda corp., Foster City, CA). All washes were done in PBS for 5 minutes, incubations were at room temperature. Slides were counterstained with hematoxylin and mounted in glycerol-gelatine.

Tissue SA modifications

Acetylation of sialic acid was by overnight incubation of slides in 4% acetic acid in 70% ethanol; alkaline treatment consisted of 30 minute incubation in 1% KOH in 70% ethanol; oxidation was performed with 15 minute action of 1% aqueous solution of periodic acid (Schauer, 1982; Schulte et al., 1984; Danguy et al., 1994).

Controls

I) TML was 15 minutes preincubated with 12.5 mM sialic acid in PBS. II) Tissue sections were pre-treated with *Clostridium perfringens* neuraminidase Type VIII (Sigma, St. Louis, MO), 10 U/ml in 0.1M acetate buffer pH 5.5 for 12 hours at 37 ºC. III) As negative control, serial sections were incubated with PBS in place of TML. Positive staining of vascular endothelium constituted the internal control.

Results

No differences were observed between the TML-gold and the TML-anti-TML/avidin-biotin-peroxidase immunohistochemistry. Cryostat sections showed the same staining pattern as paraffin embedded tissues, however, morphological detail was better in the latter. Preincubation of TML with SA blocked completely the histochemical reaction, as did digestion of tissue sections with neuraminidase under conditions described above. Negative controls incubated with PBS instead of TML did not show any peroxidase reactivity. Lectin reactivity was not related to the blood group status of tissue samples. The staining pattern of tissues was constant and easily reproducible.

For most organs and tissues the strongest TML positivity was found in vascular endothelium. There was also luminal positivity in glandular organs and membrane positivity on cells of some epithelia. Results from human tissues are summarized in Table 1. Positivity was semiquantitatively evaluated as strong, moderate and weak. Positivity patterns in most mouse tissues are noted below in comparison with analogous human tissue staining.

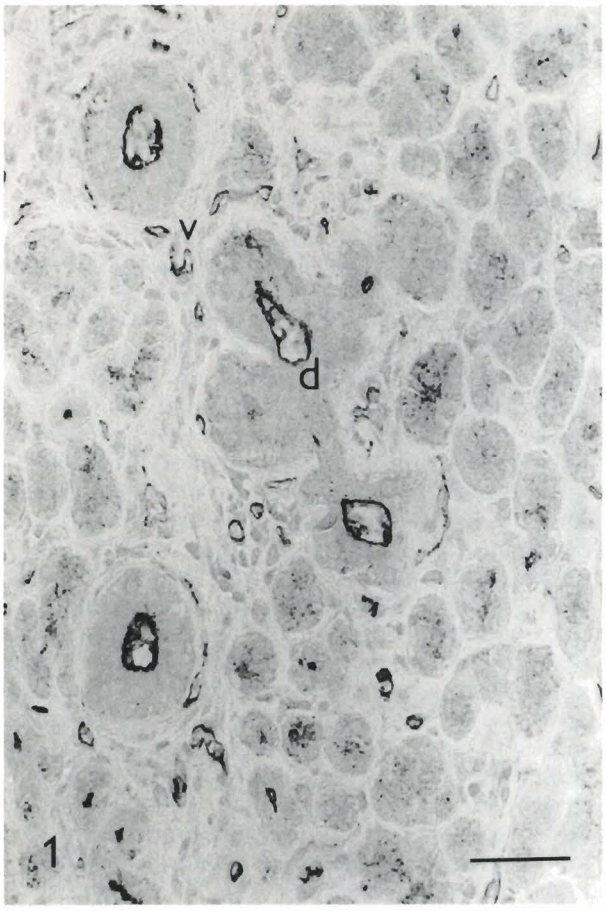
Lacrimal gland

Luminal surface positivity of ductal epithelium was strong in contrast to acinar cells which showed only

Fig. 1. Human parotid gland with predominant luminal membrane positivity in ducts (d) and blood vessels (v). TML-anti-TML, avidin-biotin complex-peroxidase. ABC-Px. Bar: 10µm.

Fig. 2. Human small intestinal mucosa. Luminal membrane staining of absorptive cells and goblet mucin positivity, especially in the deeper crypts. TML-anti-TML, ABC-Px. Bar: 100 µm.

Fig. 3. a. Human colon focal mucin goblet positivity. **b.** Mouse colonic mucosa with strong TML positivity in basal glands. TML-anti-TML, ABC-Px. Bar: 100 µm.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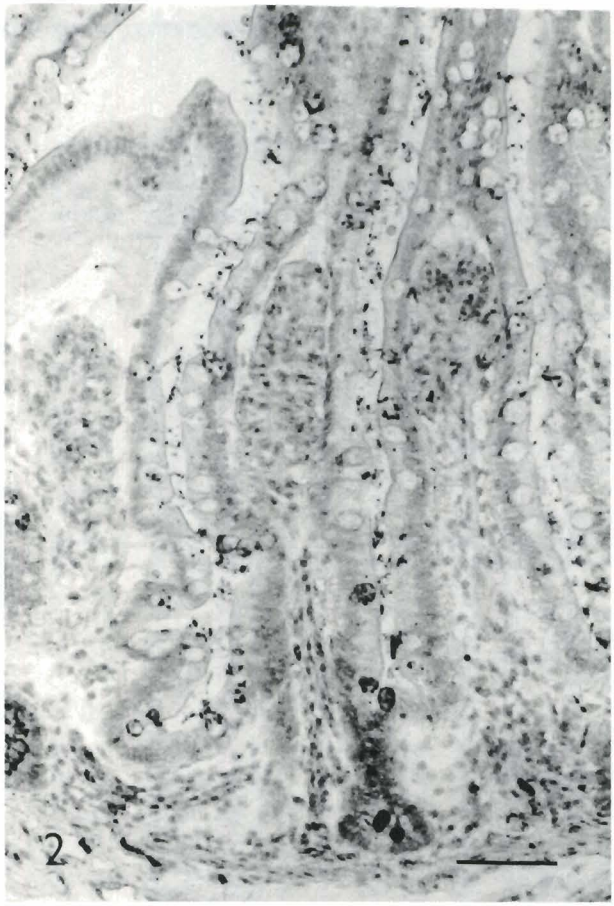
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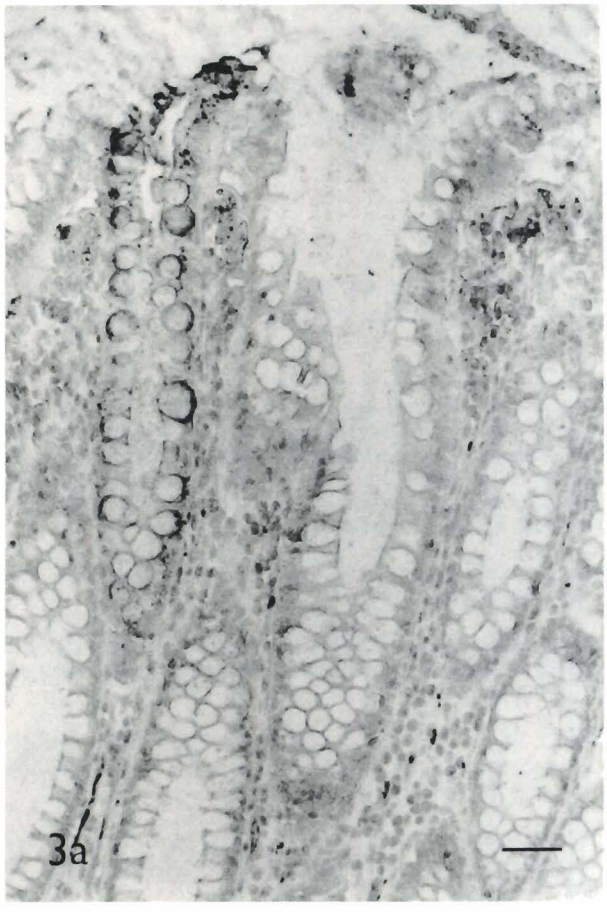
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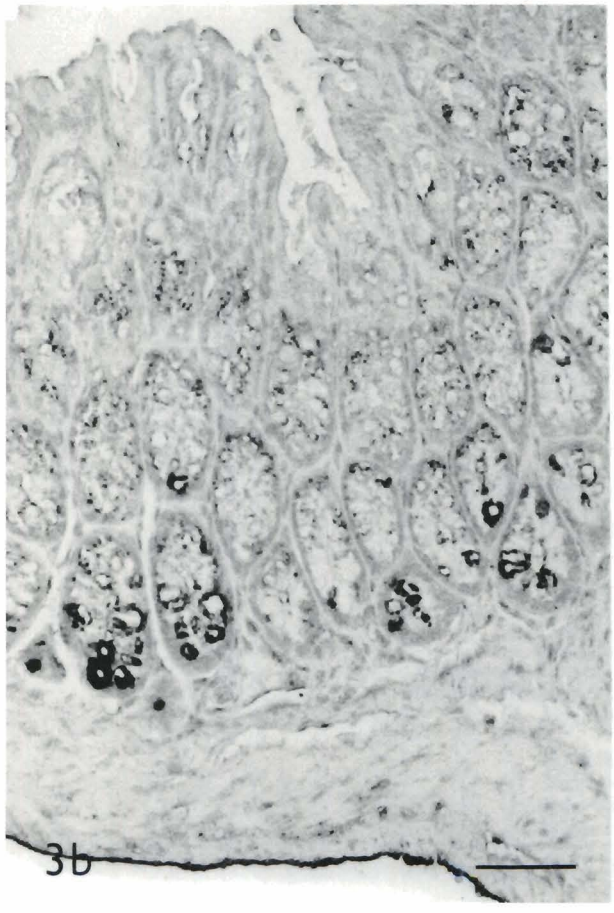
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Table 1. Histochemical reactivity of *T. mobilensis* lectin in cells of human tissues.

TISSUE/ STAINING	MEMBRANE	CYTOPLASM	MUCIN
Lacrimal gland	+++[d]	-	
Digestive tract			
Salivary gland	+++[d]/(+)[a]	+[a]	
Pancreas	++[d]/(+)[a]	+[a]	
Biliary tree	++	-	
Gallbladder	++	-	++
Liver	-	+	
Stomach	+	-	-
Duodenum/small intestine	+~++	-	+++
Brunner's gland	-	-	
Colon	+	(+)	+~+++
Respiratory tract			
Bronchi/bronchial glands	++~+++	-	++~+++
Type II pneumocytes	+++	++	
Kidney			
Proximal tubules	(+)	(+)	
Distal tubules	+++	-	
Bowman's capsule	++	-	
Podocytes	+++	-	
Genitals			
Prostate	+	+	
Paraurethral glands	+~++	-	
Endometrial glands	+++	-	
Fallopian tube	+++	-	
Endocervix	++	(+)	+++
Testes	-	-	
Ovaries			
Corpus luteum	-	+	
Follicular cells	++	-	
Zona pellucida			++
Skin associated glands			
Breast	+++	-	
Sweat glands (eccrine)	+++[d]/++[a]	-	
Sebaceous glands	-	(+)	
Endocrine cells			
Langerhans islets	-	+	
Parathyroid, adrenals	-	-	
Pituitary	-	(+)	
Thyroid gland	(+)	(+)	
Squamous epithelia	+~+++	-	
Transitional epithelium	++~+++	-	
Nervous system			
Neurons	-	(+)	
Glial cells	-	(+)	
Ependyma	+	-	
Schwann cells	-	(+)	
Melanocytes	-	(+)	
Fetal tissues			
Trophoblast	++~+++	+	
Amniotic epithelium	+~++	-	
Other cell types			
Fibroblasts	-~+	-	
Lypocytes	-~+	-	
Myocytes	-~+	-	
Osteoblasts	-	-	
Osteoclasts	-	-	
Chondrocytes	-	(+~++)	
Endothelium blood vessels	+++	(+)	
Endothelium lymph. vessels	-	-	
Macrophages	+~+++	-	
PMN	+~+++	-	
Lymphocytes	-~++	-	
Erythrocytes	++	-	
Megakaryocyte	(+)	-	
Thrombocyte	(+)	-	

[d]: duct; [a]: acini; (+): occasional positivity; +~++: weak to moderate positivity.

weak membrane positivity.

Digestive tract

Findings in the salivary gland ducts were similar to those observed in the lacrimal glands. There was weak fine granular cytoplasmic positivity of acinar cells with increased apical density (Fig. 1). There was also weak basolateral membrane staining of acinar and ductal cells. Although similar to salivary glands, the luminal reactivity in pancreatic ducts was only moderate. The biliary tree and the gallbladder showed moderate luminal membrane staining. Gallbladder mucin was moderately positive. Liver parenchyma cells showed diffuse weak cytoplasmic fine granular positivity. Stomach superficial columnar epithelium showed weak surface membrane SA expression, negativity of mucin and other glandular cells except for weak fine granular cytoplasmic positivity in parietal cells. Mouse gastric glands stained moderately on their luminal surface. The strongest positivity of human duodenum and small intestine was seen in goblet cells of the deep intestinal crypts (Fig. 2). Brunner's glands were negative. Mouse small intestine showed strong staining of both goblet mucin and the absorptive cell surfaces. In colonic mucosa, weak luminal and occasional basolateral membrane TML reactivity was expressed. The TML reactivity with goblet cell mucin was of variable intensity i.e. mostly positive but negative in some cases. Glands with both TML-positive or negative mucin goblets could be observed in the same specimen (Fig. 3a). In the mouse colon luminal mucosal surfaces and the mucin in the basal region of glands were strongly stained with TML (Fig. 3b).

Respiratory tract

Type II pneumocytes showed strong reactivity in human lungs (Fig. 4a). In mice the entire alveolar

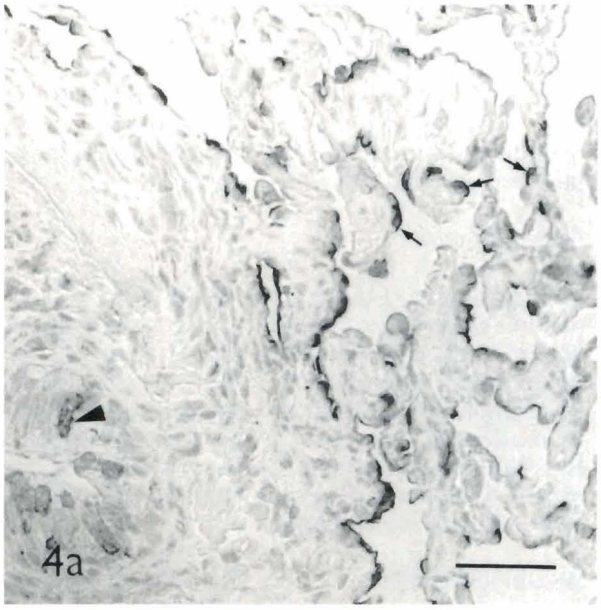
Fig. 4. a. Human lung with staining of goblets in bronchial epithelium (arrowhead), type II pneumocytes (arrow). **b.** Human kidney with strong TML reactivity on podocytes and Bowman's capsule epithelium. There is strong luminal positivity of vascular endothelium (arrowhead). a and b. Weak to no staining of alveolar wall and glomerular capillaries. TML-anti-TML, ABC-Px. Bars: 50 µm.

Fig. 5. Human Fallopian tube luminal positivity of epithelium. TML-anti-TML, ABC-Px. Bar: 100 µm.

Fig. 6. Human endometrium with TML reactivity of the luminal surface of glands. **a.** Proliferative phase. **b.** Late secretory phase. TML-anti-TML, ABC-Px. Bar: 50 µm.

Fig. 7. Human uterine cervix. Membrane TML positivity of cells in the basal layer, upper squamous epithelium and in vascular endothelium. TML-anti-TML, ABC-Px. Bar: 100 µm.

Fig. 8. Human placenta. Membrane staining of cyto- and syncytiotrophoblast and of blood vessel endothelium. TML-anti-TML, ABC-Px. Bar: 100 µm.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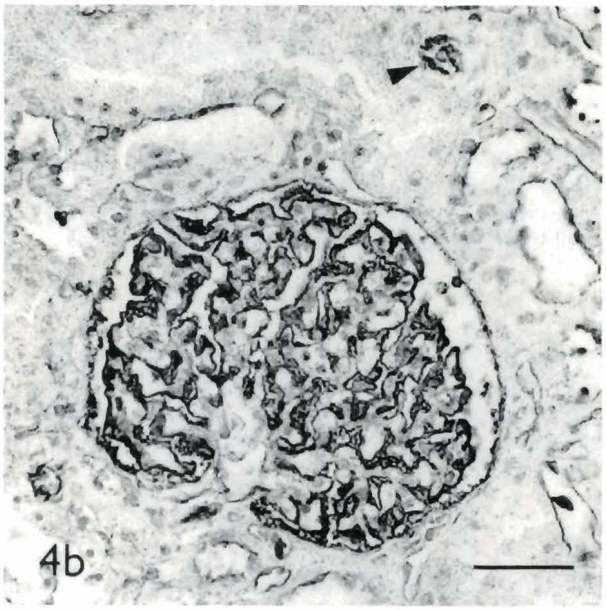
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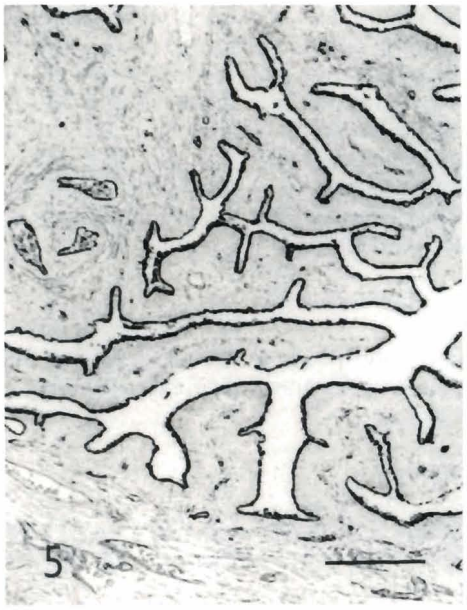
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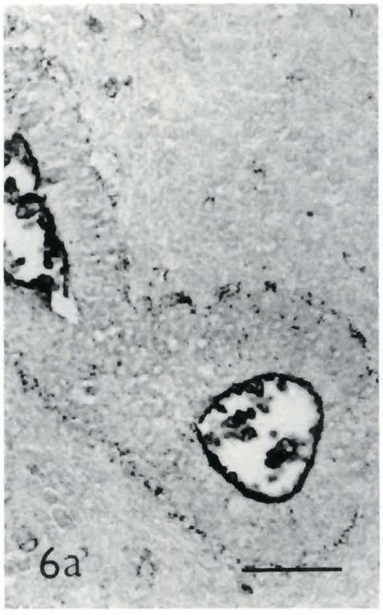
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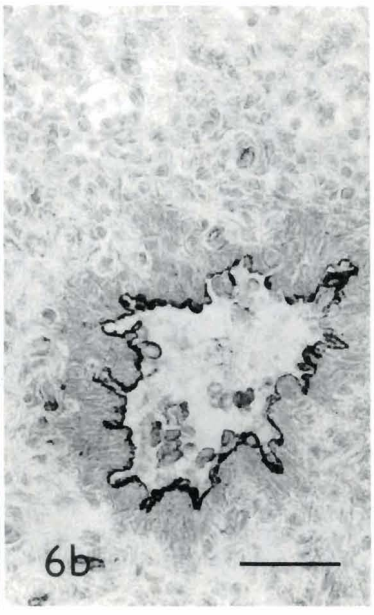
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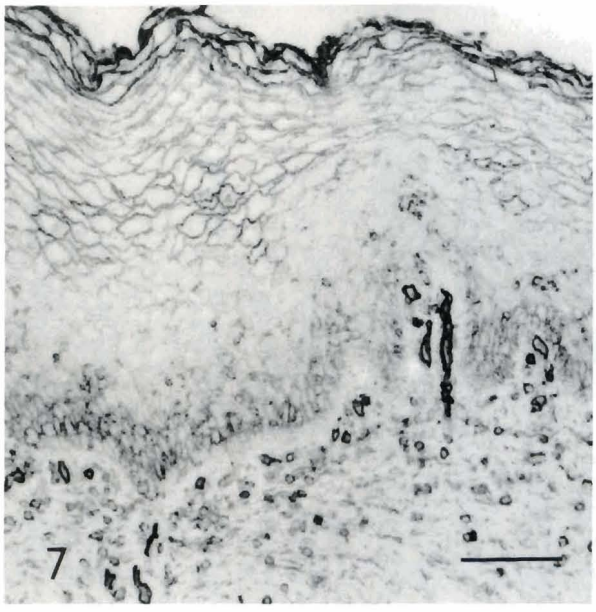
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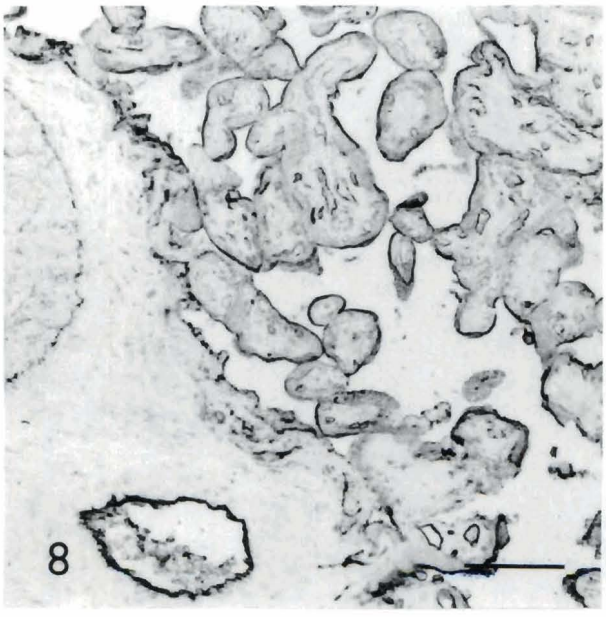
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surface was moderately positive, although type II pneumocyte staining was less than in human lungs. The bronchial mucosa and adjacent glands disclosed strong mucin and luminal cell membranes staining with TML.

Kidney

Distal kidney tubules, beginning, with descending limb of the loop of Henle, expressed moderate to strong luminal positivity. Bowman's capsule stained moderately and glomerular podocytes were strongly TML positive (Fig. 4b).

Genitals

There was weak, mostly luminal, expression of SA on prostatic acinar cells and in their secretion product. The cells of the paraurethral glands showed weak to moderate membrane staining. Luminal membranes of Fallopian tube epithelium (Fig. 5) and of endometrial glands showed strong luminal and weak basolateral membrane positivity with no staining changes during the menstrual cycle (Fig. 6). Moderate membrane TML-staining and strong mucin positivity were observed in the cervical mucosa. In testes there was no conspicuous TML reactivity in either germinal epithelium or interstitium. Corpus luteum cells of the ovary showed weak cytoplasmic positivity. The zona pellucida reacted moderately with TML as did surface membrane of follicular cells in primordial follicles. The latter disappeared in granulosa cells of the primary follicle.

Skin associated glands

Breast lobules and ducts showed strong luminal membrane TML reactivity. Sweat glands of the eccrine type disclosed moderate staining of the whole surface membrane of the cells and strong luminal membrane positivity of cells lining the spiral ducts.

Endocrine cells

Endocrine gland cells (Langerhans islets of pancreas, parathyroid gland, pituitary gland, adrenal cortex) in general showed no or only weak fine granular cytoplasmic staining with TML. Some follicles in the thyroid gland contained cytoplasmic granules with weak to moderate positivity at the luminal side. Weak basolateral membrane staining of some cells was observed. In most follicles the colloid was negative, in some showing weak staining.

Squamous epithelia

The most intense and consistent positivity was noted on surface membrane of epidermal basal cells. Weak membrane positivity was occasionally observed on cells of the stratum spinosum, less of the stratum lucidum in epidermis; more intensive staining of these two was in mucosal squamous epithelia (Fig. 7), especially in the presence of hyperplasia or inflammation. Epithelium of Hassal's bodies showed membrane positivity similar to stratum spinosum cells.

Transitional epithelium

A staining pattern was similar to squamous epithelia with umbrella cell membrane positivity especially strong on the luminal face.

Nervous system

Neurons and glial cells were negative or had only minimal TML cytoplasmic staining, as was the neuropil

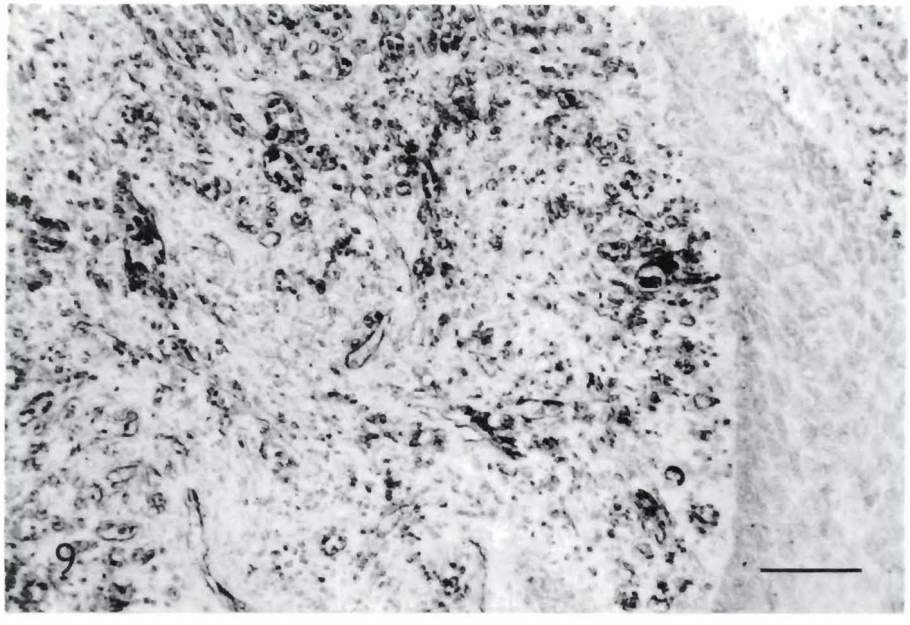
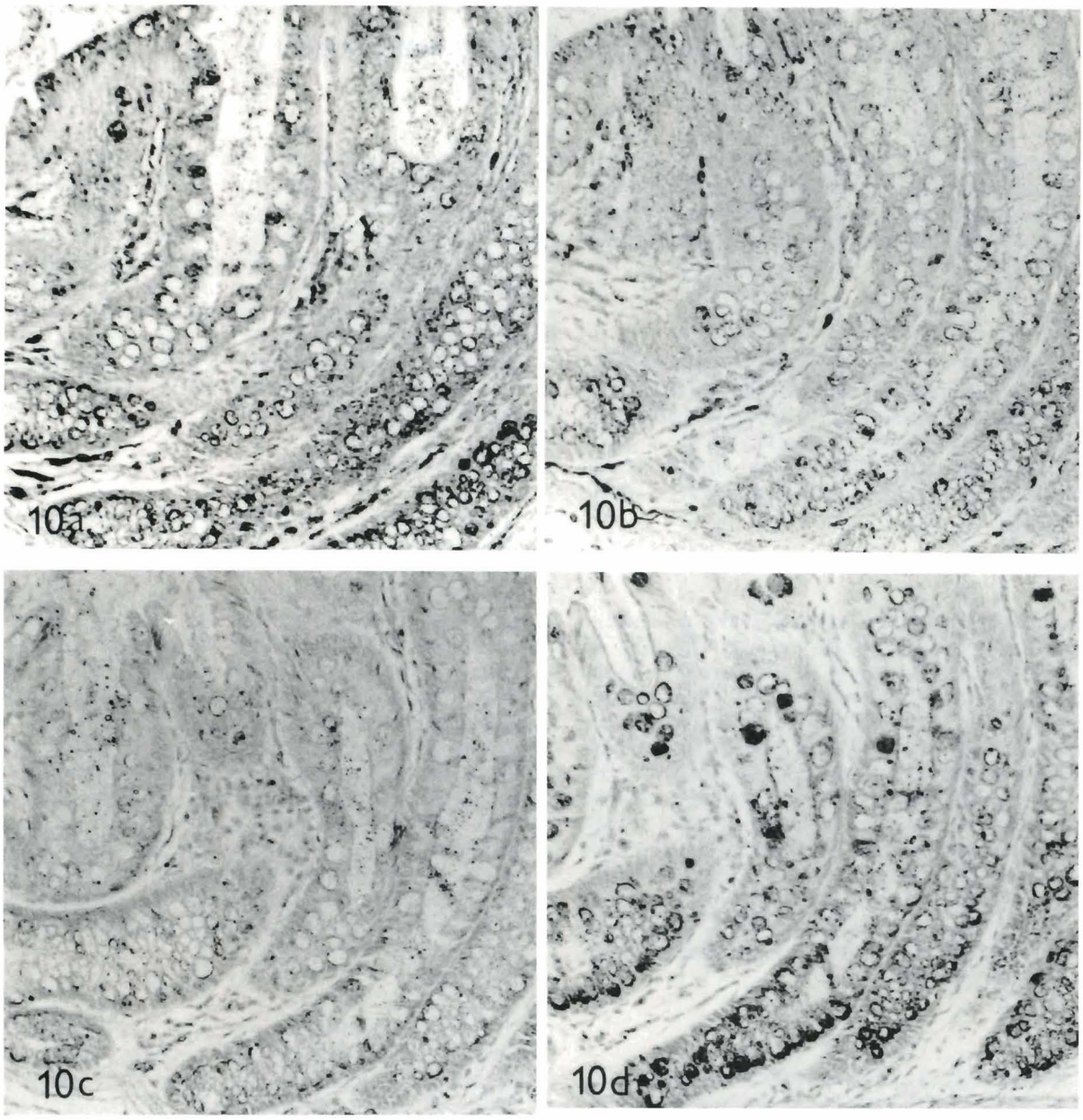


Fig. 9. Kaposi sarcoma (skin) with positive staining of vascular lumina. Basal cells in epidermis show weak membrane positivity. TML-gold. Bar: 50 µm.



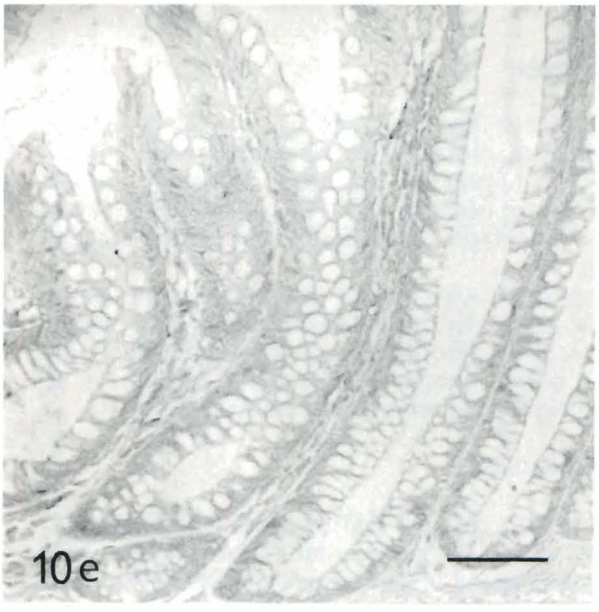


Fig. 10. Histochemical modifications of sialic acid (human colon). **a.** TML reactivity without treatment. **b.** After alkaline treatment. **c.** Acetylation. **d.** Periodic acid oxidation. **e.** Alkaline treatment with following oxidation. TML-anti-TML, ABC-Px. Bar: 50 µm.

of the white matter in CNS. Ependymal cells showed weak luminal positivity. Schwann cells in peripheral nerves were in most cases negative. Melanocytes were negative or expressed weak cytoplasmic reactivity with TML.

Fetal tissues

In the placenta, cyto- and syncytiotrophoblast showed weak cytoplasmic and moderate to strong surface membrane expression of SA. Fetal vasculature in the villi stained as well (Fig. 8). There was weak TML staining of amnionic epithelium luminal surface. In the umbilical cord only endothelial surfaces showed strong TML reactivity.

Vascular tree

Vascular endothelium showed strong luminal membrane expression of sialic acid in virtually all organs and tissues. Staining in veins was weaker compared to capillaries or arterial lining. Capillaries of lungs (Fig. 4a) and kidney glomeruli (Fig. 4b) were negative or showed only minimal luminal membrane positivity. Sinus lining cells in both spleen and bone marrow were mostly negative. Liver sinusoids showed moderate luminal membrane TML staining with maximum in the center of lobules. High endothelial cells of veins in lymph nodes showed weak membrane positivity. Lymphatic vessel endothelium in contrast to the above was consistently TML negative, unless associated with a pathological process (e.g. inflammation, tumor metastasis).

Mesothelium

Mesothelial cells of serous cavities were negative or showed weak luminal membrane and cytoplasmic positivity.

Other cell types

Fibroblasts, lipocytes and myocytes, smooth or cross-striated, showed inconsistent weak or no superficial membrane staining. Osteoblasts and osteoclasts were negative with TML, some chondrocytes contained cytoplasmic granules with weak to moderate positivity.

Lymphocytes disclosed inconsistent moderate membrane positivity; small and medium-size lymphocytes especially in the T zone in lymph nodes and in the spleen, scattered lymphocytes in other locations or in inflammatory infiltrates showed membrane positivity.

Neoplastic tissues

Generally, in dysplastic glandular epithelia, positivity was at the surface/luminal membrane. This polarity disappeared in malignancy and the staining was diminishing in poorly differentiated adenocarcinomas. In squamous dysplasia, hyperplasia and in inflammation, the membrane positivity of cells increased, was moderate to strong in well differentiated and decreased in poorly differentiated carcinomas. Neuroectodermal and mesenchymal tumors were mostly negative with TML. Both benign and malignant tumors originating from vascular endothelium showed strong to moderate luminal membrane reactivity e.g. Kaposi sarcoma (Fig. 9), with loss of the polar character of TML staining in endothelial sarcoma.

Tissue SA modifications

Alkaline treatment of tissue slices did not substantially change the lectin staining (Fig. 10b). Acetylation resulted in slight decrease of reactivity of vascular endothelia, and in substantial diminution of squamous basal cells membrane staining. Staining of mucins was slightly decreased (Fig. 10c). Periodic acid oxidation completely blocked almost all the natural TML reactivity except for persistent weak positivity of respiratory epithelium brush border and mucin. This oxidation produced strong staining of colonic mucin (Fig. 10d), increased granular positivity in chondrocytes and, weak to moderate positivity of collagen fibers and weak granular positivity in smooth muscle cell sarcoplasm. Described TML staining after oxidation could be blocked by alkaline pretreatment O-deacetylation (Fig. 10e). All of these new reactivities could be blocked with SA preincubation of TML.

Discussion

Several techniques are used for histochemical detection of sialylated glycoconjugates. Alcian blue at pH 2.5 histochemistry has been the most widely employed non-lectin technique (Spicer and Schulte, 1992). Lectin detection of sialic acid is however more sensitive. Despite of a number of studies on the SA specific lectins *Limulus polyphemus* agglutinin (LPA) (Yamada and Shimizu, 1979; Muresan et al., 1982a,b; Danguy et al., 1988) and *Limax flavus* agglutinin (LFA) (Schulte et al., 1984; Roth et al., 1985; Lucocq et al., 1986; Calderó et al., 1989; Kaneko et al., 1991), many authors continued to use the less specific wheat germ agglutinin (WGA) (Holthöfer et al., 1981; Damjanov, 1987; Danguy et al., 1988; Walker, 1989) or peanut agglutinin (PNA) reactivity after sialidase treatment (Holthöfer et al., 1981; Faraggiana et al., 1982; Damjanov, 1987; Danguy et al., 1988; Ackerman et al., 1991; Jass and Smith, 1992). The fact that LPA and LFA are frequently only cited but not used, may reflect their limited availability and relatively poor stability (Schulte et al., 1984). This latter limitation could explain the failure of LPA binding in kidney (Tomino et al., 1988) or in colon tissue (Babál et al., 1993).

Although there have been no comprehensive studies of histochemical reactivity of LPA or of LFA, collected

data on tissue staining in different species are in most cases similar to those obtained with TML. In general, the combination of positivities of α2,3 SA linkage-specific *Maackia amurensins* leukoagglutinin (MAL) and α2,6 linkage-specific *Sambucus nigra* agglutinin (SNA) (Shibuya et al., 1987; Sata et al., 1989; Knibbs et al., 1991; Babál et al., 1993, 1996; Danguy et al., 1994) corresponds to the TML staining pattern in tissues. The most striking contrast with the above mentioned SA specific lectins is the minimal to absent staining of connective tissue intercellular matrix with TML.

Salivary glands are rich in sialylated glycoproteins (Menghi and Materazzi, 1994). LPA staining localized only at the periphery of acini and ducts of both rat and rabbit parotid gland (Muresan and Muresan, 1987). This differs from the strong cytoplasmic SA expression (predominantly of ductuli and ducts) described in mouse and rat sublingual glands (Yamada and Shimizu, 1979). TML positivity in human parotid gland was similar to that reported with LPA (Yamada and Shimizu, 1979; Muresan et al., 1982a,b; Muresan and Muresan, 1987). The strong LPA cytoplasmic staining of ductal cells (Yamada and Shimizu, 1979), however, was not observed with TML. Salivary glands staining with TML and LFA (Schulte et al., 1984) was similar to that of exocrine pancreas and corresponded to the combination of MAL and SNA staining (Sata et al., 1989). TML positivity of gallbladder mucin was consistent with SA-findings of others (Madrid et al., 1994).

As has been the case with other histochemical studies of human gastric mucosa (Macartney, 1986; Madrid et al., 1990), SA was not detected with TML. Species differences were noted with luminal membrane TML positivity in mouse stomach and LPA staining of foveolar mucus in the rat (Yamada and Shimizu, 1979). Similar to TML reactivity in the human colon, LPA and LFA staining patterns of colon in different species (Taatjes and Roth, 1988; Calderó et al., 1989; Filipe et al., 1989; Ellinger and Pavelka, 1992) were inconsistent. Increasing LFA staining of rat and human goblet cells with migration to the lumen (Schulte et al., 1984) contrasted with overall strong TML staining of mucin in mouse colon. Local variation of SA expression was indicated by the finding of different areas or groups of glands with or without TML positivity.

SA has been documented in goblet cells and in the ciliary layer of the respiratory epithelium (Schulte et al., 1984; Plotkowski et al., 1990; Ackerman et al., 1991; Castells et al., 1991). The reactivity pattern of TML in respiratory epithelium represented a combination of the reported positivities of MAL and SNA (Baum and Paulson, 1990). The preferential localization of SA in the glycocalyx of type II pneumocytes (Faraggiana et al., 1986) was confirmed with the TML.

The strong reactivity of LPA and LFA in the thyroid gland and in Langerhans islet cells (Yamada and Shimizu, 1979; Muresan et al., 1982a; Schulte et al., 1984; Danguy et al., 1988) was not observed with TML. The pattern of TML reactivity in the kidney did not differ substantially from that of LPA and LFA (Holthöfer et al., 1988, 1990; Wagner and Roth, 1988; Roth, 1993; Babál et al., 1996). The presence of SA in glomerular basement membrane indicated by LFA (Holthöfer et al., 1988; Roth, 1993) or by phosphotungstic acid at low pH (Quatacker et al., 1987) could not be confirmed with TML.

TML membrane positivity of follicular cells and negativity of granulosa cells points at the significance of SA in the differentiation process of ovarial follicles. TML reactivity confirmed previous report of SA in the zona pellucida (Avilés et al., 1994).

The TML reactivity especially with the surface membrane of cells in the basal layer of squamous epithelium was comparable to that with LFA (Watt et al., 1989). The latter is known to be due to a 250 kDa sialoglycoprotein in keratinocytes (Keeble and Watt, 1990).

Vascular endothelial cells almost uniformly stained strongly with TML. This is similar to MAL and SNA (Sata et al., 1989) and LFA (Schulte et al., 1984; Tomino et al., 1988; Vorbrodt et al., 1988; Szumanska and Gadamski, 1992). The staining of endothelium with TML was so distinct and pronounced, even in endothelial cell malignancies, that it has been proposed as a useful marker of endothelial cells (Babál and Gardner, 1993). Neither TML (Babál et al., 1996) nor LFA (Holthöfer et al., 1990) stained the endothelium of glomerular capillaries. This fact, together with similar failure of TML to stain lung alveolar wall capillaries, indicates a distinctive feature of these endothelial cells and further points out the anatomic diversity of endothelium. The positivity of glomerular capillary wall described with WGA (Faraggiana et al., 1982; Liska et al., 1993) is attributed only partly to sialic acid (Holthöfer et al., 1981).

Our findings of membrane positivity of epithelial cells in many neoplasms, the decreasing positivity with loss of differentiation of tissue and the loss of staining polarity of neoplastic cells are similar to those described with LPA (Bratila and Muresan, 1989) and with PNA after sialidase treatment (Chew et al., 1991).

Staining of red blood cell membranes with TML corresponded to the lectin hemagglutinability (Babál et al., 1994). The irregular membrane positivity of both T and B lymphocytes indicates lack of specificity to lymphocyte subtype. Previous studies of lymphocyte surface SA with LFA (Simmons and Cattle, 1992), LPA (Powell et al., 1987) and the sialidase digestion (Collins et al., 1988) indicated that SA expression could be correlated to the activation of T and B lymphocytes and their adhesion properties.

SA modifications provided insight to the specific reactivity of TML. SA hydroxyl groups changes modify the reactivity with SA-specific lectins. Acetylation blocked the reactivity of LFA in all sites (Schulte et al., 1984). This step did not affect TML staining and confirmed good reactivity of TML with O-acetylated derivates of SA (Babál et al., 1994). O-acetylated groups

are not essential for the lectin binding as indicated O-deacetylation by alkaline treatment (Schauer, 1982) which did not change TML reactivity. This differs from LFA which showed increased binding after alkaline treatment (Schulte et al., 1984). Oxidation of SA hydroxyl groups to aldehydes (Schauer, 1982), as expected, completely blocked almost all TML reactivity. The increased staining of human colonic mucin could be explained by the presence of nonoxidizeble poly-hydroxyl side chains possessing O-acetylated groups. Possible changes in molecular configuration resulting form oxidation can not be excluded as a cause of increased TML reactivity in these special cases, either.

TML produced histochemical results comparable to those of LFA and LPA, lectins with similar binding properties. TML can be applied in concentration up to 50 times lower than LPA (Yamada and Shimizu, 1979) and 5-10 times lower than LFA (Schulte et al., 1984). The specificity limited to one sugar (the sialic acid), ready availability and stability of the protein make TML a useful tool for studies of sialylated glycoconjugates.

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