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Sialoglycoproteins and Penultimate Sugar Expression Pattern in Developing Murine Olfactory and Respiratory Mucosa

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Sialic acid residues are constant constituents of the glycoproteins of the airways in all species. Sialoglycoproteins are the main acidic glycoprotein and their functions are to mediate cell adherence, to control the viscoelasticity of mucus and to serve as receptor sites for the binding of exogenous macromolecules. The purpose of this study was to investigate the differences in the distribution of sialoglycoproteins as a terminal sugar and in the composition of the penultimate sugar according to aging in the murine nasal respiratory and olfactory mucosa. Nasal cavities of mice (BALB/c) were fixed by intracardiac perfusion with 2.0% glutaraldehyde and embedded in Epon 812. First, the serial sections were stained with Maackia amurensis agglutinin (MAA) and Sambucus nigra agglutinin (SNA). Then, the adjacent sections were stained with DBA and PNA before and after neuraminidase digestion in all experimental groups. Apical cell surfaces of olfactory mucosa and cilia on a few ciliated cells in the mucosa of the septum and nasal floor were labelled with MAA, but cell surfaces of respiratory mucosa, Bowman's glands and goblet cells were not labelled with MAA, irrespective of aging. Apical cell surfaces of both olfactory and respiratory mucosa and Bowman's glands were stained with SNA, however, goblet cells were not labelled with SNA. After neuraminidase digestion to remove terminal sialic acid residues of sialoglycoproteins, only cell surfaces of respiratory mucosa were labelled with PNA, but goblet cells, cell surfaces of olfactory mucosa and Bowman's glands were not labelled with PNA. Cell surfaces and Bowman's glands of olfactory mucosa were labelled with DBA after neuraminidase digestion, but cell surfaces of respiratory mucosa and goblet cells were not labelled with DBA. Our results indicate that there were different carbohydrate structures of sialoglycoconjugates in olfactory and respiratory mucosa, and it was not influenced by aging.

Key Words: Sialoglycoproteins, olfactory mucosa, respiratory mucosa

Airway hypersecretion is a frequent feature of several respiratory tract diseases including rhinitis, sinusitis, otitis media and bronchitis. Our understanding of mechanisms regulating different secretions is still very incomplete. Airway mucin is the main glycoprotein in airway secretions. Sialic acid residues are constant constituents of the glycoproteins of the airways of all species (Castells *et al.* 1990) and are usually present as non-reducing terminal carbohydrate moieties of glycoproteins, often linked to D-galactose or N-acetyl-D-galacosamine through α 2, 3-or α 2, 6-linkages (Shibuya *et al.* 1987a, b). The sialoglycoproteins have three important functions. First, they act as mediators of adherence by a negative charge. Second, they serve as

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cell surface receptors for bacteria and viruses. Human influenza A viruses bearing H_1 hemagglutinin had a tendency to bind to the terminal NeuAc α2, 3Gal sequence, while human H_3 hemagglutin in bound preferentially to NeuAc α2, 6Gal (Suzuki *et al.* 1987). Sendai virus can utilize the sialyloligosaccharides sequence NeuAc α2, 3Gal β1, 3GalNAc but not NeuAc α2, 6Gal β1, 4GlcNAc as a receptor determinant on host cells (Markwell and Paulson, 1980). The adherence of Escherichia coli and Salmonella typhi is mediated by a mannose (Bar-Shavit *et al.* 1977) and the adherence of Vibrio cholera is mediated by a fucose (Cuatrecasas, 1973). Also, Cholera and tetanus toxin bound gangliosides which have sialic acid residues (Ledeen and Mellanby, 1977). Third, sialoglycoproteins have the influence of viscoelastic properties. The viscoelastic properties of the secreted mucus possibly depend on the structure of the sialoglycoproteins, since the α2, 3 linkage is relatively rigid in comparison to the free rotation of the α2, 6 linkage (Montreuil. 1980). Neuraminidase treatment removes sialic acid and exposes the penultimate sugar, but there has been little information about the penultimate sugar and its role.

Lundh *et al.* reported that lectin-binding patterns showed marked differences between olfactory and respiratory mucosa in the murine nasal cavity (Lundh *et al.* 1989). Ueno *et al.* found that there are different carbohydrate structures in sialomucin between respiratory and olfactory mucosa(Ueno *et al.* 1994). However, changes in the terminal sugar structures by aging in the nasal cavity and penultimate sugars which are linked to sialic acid residues have not been elucidated.

Serous cells are known to appear in surface epithelium before, but not after birth. This suggested that trans-differentiation of serous to mucous cells may occur under various conditions. Furthermore, in our previous morphological study on human nasal glandular cells, an isolated mucous cell in serous acini was noted. And if trans-differentiation of serous to mucous cells does occur, their secretory components including carbohydrates could be changed by aging. The authors wanted to know whether sialic acid residues appear during the perinatal period because of the presence of airflow through the nasal cavity after birth and whether the changes in terminal sugar of sialoglycoproteins occur by aging. The purpose of this study was to investigate the differences in the distribution of sialoglycoproteins as a terminal sugar and the composition of the penultimate sugar according to aging in murine nasal respiratory and olfactory mucosa.

MATERIALS AND METHODS

Groups of five mice (BALB/c) each for gestational day 16/18, and postnasal day 1, day 3, day 7, day 14 and adult were used respectively. After intraperitoneal anesthesia with phenobarbital, the animals were fixed by intracardiac perfusion with 2.0% cold glutaraldehyde solution (0.1 M phosphate buffer, pH 7.4). En-bloc excision of the nasal cavity was performed. Following decalcification with 10% EDTA, the specimens were dehydrated in ethanol and embedded in Epon 812. Coronal sections were cut serially with a thickness of 2 μm using an ultramicrotome. We selected the sections which showed both ethmoturbinate and maxilloturbinate. Biotinylated MAA, PBA, DBA (Table 1) and Avidin-biotin peroxidase complex (ABC) kit were obtained from Vector Laboratories (Burlingame, CA, USA). Biotinylated SNA (Table 1) was obtained from E.Y. Lab. (San Mateo, CA, USA). Neuraminidase was type V from Clostridium Perfringens (Sigma, St Louis MO, USA).

Lectin staining in Epon-embedded tissue was carried out according to methods described by Lim *et al.* (1991). Semi-thin sections (2 μm) were etched in a 1:1 mixture of saturated sodium ethoxide and

Table 1. Lectins used in this study

Lectins	Source	Oligosaccharide specificity
MAA	Maackia amurensis	NeuAc (α 2,3) Gal
SNA	Sambucus nigra	NeuAc(α 2,6) Gal/GalNAc
PNA	Arachis hypogaea	Gal(β 1-3)GalNAc
DBA	Dolichos biflorus	GalNAc(γ 1,3)GalNAc

NeuAc: N-acetyl neuraminic acid
Gal: galactose
GalNAc: N-acetyl galactosamine
GlcNAc: N-acetyl glucosamine

pure ethanol for 20 minutes at room temperature and hydrated through graded ethanol. Then, the sections were covered with 3% H_2O_2 in methanol and liver acetone powder for 20 minutes respectively, followed by incubation with biotinylated lectins (1:200 or 1:500) for 24 hours at 4°C. Subsequently, the sections were rinsed with Tris buffered saline, and covered with avidin biotin peroxidase complex (ABC) solution for 30 minutes at 37°C. After washing the sections with Tris buffered saline, distilled water and acetate buffer, peroxidase was developed in acetate buffer containing 3-amino- 9-ethylcarbazol for 5 minutes. The sections were counterstained with Mayer's hematoxylin and mounted with glycerol. Sialic acid residues were removed through the digestion of sections with neuraminidase before lectin incubation. The slides were incubated for 30 minutes in a solution of 0.02 M acetate buffer (pH 5.2) containing 0.5 units neuraminidase per ml and 2% bovine serum albumin (Castells *et al.* 1990). Positive control was carried out with WGA (1:500), which reacts with β-GlcNAc and sialic acid and is a very common component in all the tissues. Negative control was carried out by pre-incubation of the lectins with their specific simple sugar.

RESULTS

Apical cell surfaces of olfactory mucosa and cilia on a few ciliated cells in the mucosa of the septum and nasal floor were labelled with MAA but cell surfaces of respiratory mucosa, Bowman's glands and goblet cells were not labelled with MAA irrespective of aging (Table 2, Fig. 1). Apical cell surfaces of both olfactory and respiratory mucosa and Bowman's glands were stained with SNA, however, goblet cells were not labelled with SNA (Table 3, Fig. 2). Neuraminidase digestion was performed in all experimental groups. None of the mucosa of the murine nasal cavity was labelled with

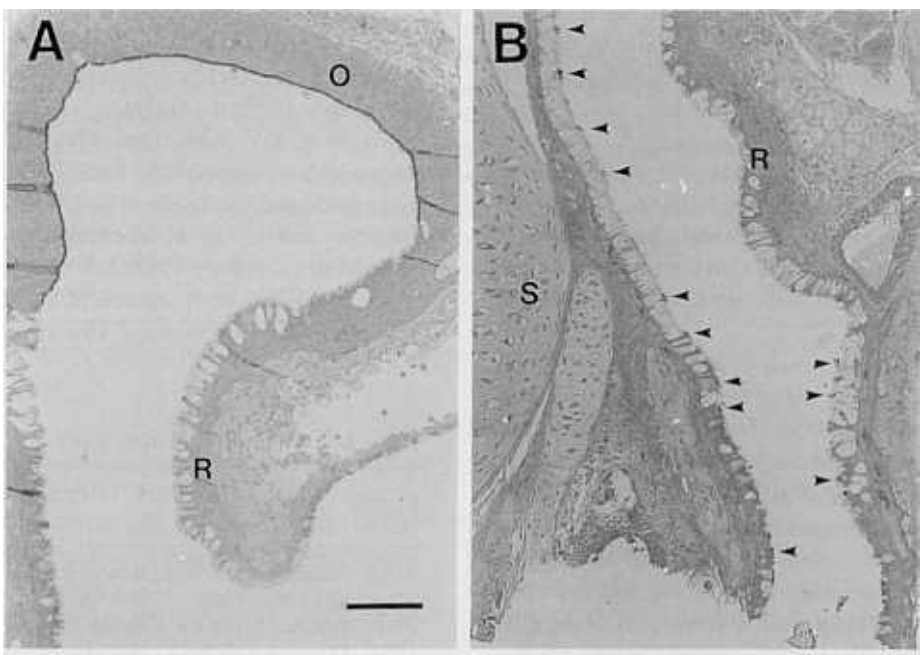

Fig. 1. MAA-binding patterns in the upper(A) and lower(B) part of nasal cavity in adult mice. Cell surfaces of olfactory mucosa were labelled with MAA, but cell surfaces of respiratory mucosa of nasal septum and nasoturbinate, goblet cells and Bowman's glands were not labelled with MAA regardless of aging. Cell surfaces on a minority of ciliated cells in the mucosa of the septum and nasal floor were stained(arrows). O: Olfactory mucosa, R: Respiratory mucosa, S: Septum, Bar=50 μm.

PNA or DBA before neuraminidase digestion in all experimental groups. After neuraminidase digestion to remove terminal sialic acid residues of sialoglycoproteins, only cell surfaces of respiratory mucosa were labelled with PNA, but goblet cells, cell surfaces of olfactory mucosa and Bowman's glands were not labelled with PNA (Table 4, Fig. 3). Cell surfaces and Bowman's glands of olfactory mucosa were labelled with DBA after neuraminidase digestion but cell surfaces of respiratory mucosa and goblet cells were not labelled with DBA (Table 4, Fig. 4).

Table 2. Distribution of MAA according to aging

Sites \ Age	G16	G18	P1	P3	P7	P14	adult
Olfactory							
cell surface	+	+	+	+	+	+	+
Bowman's gland	−	−	−	−	−	−	−
Respiratory							
cell surface*	−	−	−	−	−	−	−
goblet cell	−	−	−	−	−	−	−

G: gestational, P: postnatal
*: Cilia on a minority of ciliated cells in the mucosa of the septum and nasal floor was stained.

Table 3. Distribution of SNA according to aging

Sites \ Age	G16	G18	P1	P3	P7	P14	adult
Olfactory							
cell surface	+	+	+	+	+	+	+
Bowman's gland	+	+	+	+	+	+	+
Respiratory							
cell surface	+	+	+	+	+	+	+
goblet cell	−	−	−	−	−	−	−

G: gestational, P: postnatal

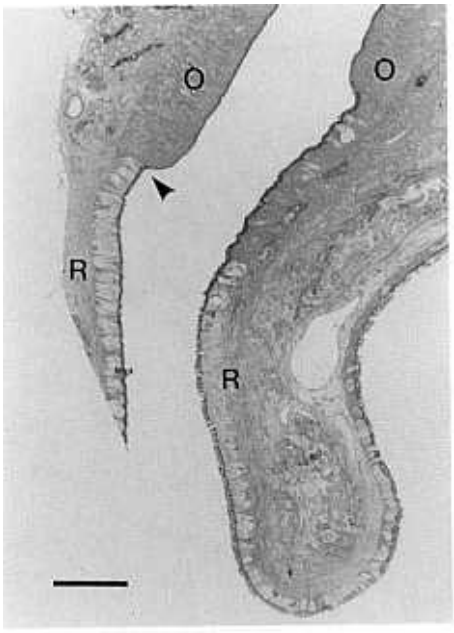

Fig. 2. SNA-binding pattern in adult mice. Arrow indicates the boundary between olfactory and respiratory epithelium of nasal septum and nasoturbinate. All cell surfaces of olfactory and respiratory mucosa were labelled with SNA but goblet cells were not labelled with SNA regardless of aging. O: Olfactory mucosa, R: Respiratory mucosa, Bar=50 μm.

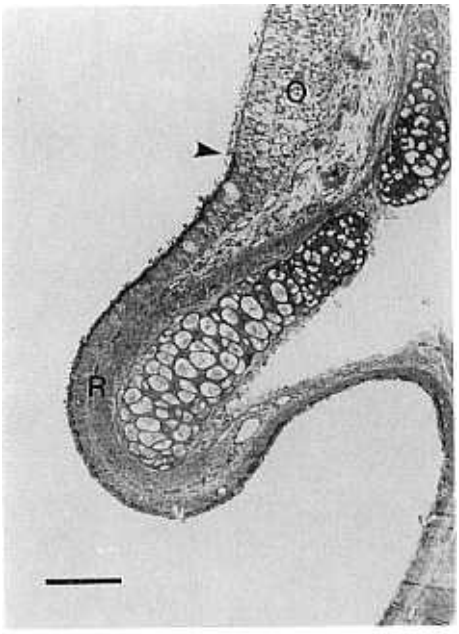

Fig. 3. PNA-binding pattern after neuraminidase treatment in adult mice. Arrow indicates the boundary between olfactory and respiratory epithelium. Only cell surfaces of respiratory mucosa of nasal septum and nasoturbinate. were labelled with PNA but goblet cells, cell surfaces of olfactory mucosa and Bowman's glands were not labelled with PNA. O: Olfactory mucosa, R: Respiratory mucosa, Bar=50 μm.

Table 4. Change of PNA and DBA binding patterns before and after neuraminidase treatment

Sites \ Lectins	PNA	DBA
Olfactory		
cell surface	−/−	−/+
Bowman's gland	−/−	−/+
Respiratory		
cell surface	−/+	−/−
goblet cell	−/−	−/−

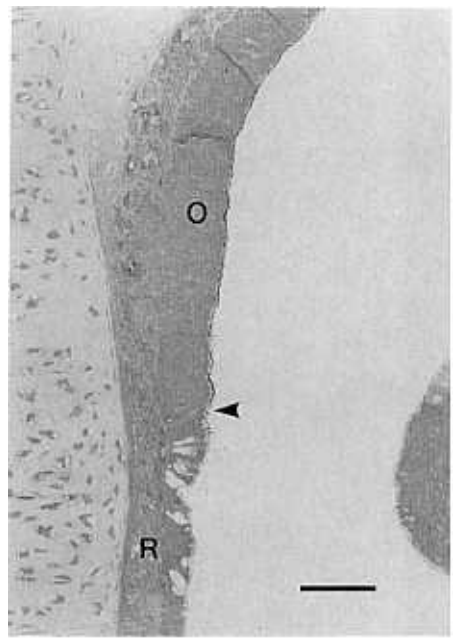

Fig. 4. DBA-binding pattern after neuraminidase treatment in adult mice. Arrow indicates the boundary between olfactory and respiratory epithelium. Cell surfaces of olfactory mucosa and Bowman's glands were weakly labelled with DBA but cell surfaces of respiratory mucosa of nasal septum and nasoturbinate and goblet cells were not labelled with DBA. O: Olfactory mucosa, R: Respiratory mucosa, Bar=50 μm.

DISCUSSION

Biochemical analysis of homogenates of tissues and glands, saliva and other epithelial secretions has revealed a mixture of 0- and N-glycosylproteins containing sialic acid via α2, 3 or α2, 6 linkage or another (Schulte and Spicer, 1985). However, it has been impossible to determine the precise cellular origin of these different types of sialoglycoproteins due to the cellular heterogeneity of tissues and organs, as well as the presence of plasma proteins, sloughed epithelial cells, and other contaminants in epithelial secretions.

Recently, MAA and SNA which are specific to NeuAc (α2, 3) Gal and NeuAc (α2, 6) Gal/GalNAc respectively were developed (Shibuya *et al.* 1987b; Taatjes *et al.* 1988; Wang and Cummings, 1988; Sata *et al.* 1989). The binding patterns of MAA and SNA, which can detect NeuNAc- α2, 3 or NeuNAc- α2, 6 linkage respectively, and the lectin-binding patterns of PNA and DBA before and after neuraminidase digestion, were studied.

Our results showed that only apical cell surfaces in the olfactory mucosa and on a few of the ciliated cells in the mucosa of the septum and nasal floor were labelled with MAA from the 16th gestational day irrespective of aging (Fig. 1). Apical cell surfaces in the respiratory epithelium and apical cell surfaces and Bowman's glands in olfactory epithelium were labelled with SNA from the 16th gestational day irrespective of aging (Fig. 2). Interestingly, none of the goblet cells were labelled with MAA or SNA. These results indicate that there are differences in terminal carbohydrate structures of sialoglycoproteins between the respiratory and olfactory mucosa and that they are consistent irrespective of aging. Although we wanted to know whether sialic acid residues appear during the perinatal period and whether the changes in terminal sugar of sialoglycoproteins occur by aging, there was no change in the binding patterns of MAA and SNA by aging in the perinatal period.

Ueno & Lim, and Lim *et al.* reported that goblet cells in chinchilla middle ear epithelium were labelled with SNA (Lim *et al.* 1991; Ueno and Lim, 1991). Ueno *et al.* reported that cell surfaces and goblet cells in murine tubotympanum epithelium were labelled with MAA, but not with SNA (Ueno *et al.* 1992). These differences in lectin-binding patterns of MAA and SNA indicate that they might depend on the species and site of the organ. However, it is not clear why goblet cells in surface epithelium were not stained with MAA or SNA

specific for sialomucin. An intriguing report by Ueno *et al.* proposed that MAA labelled the epithelial goblet cells, Bowman's glands, and all cell surfaces of both olfactory and respiratory epithelium, but by contrast, SNA labelled cell surfaces and Bowman's glands in the murine olfactory epithelium, which is at variance with our results (Ueno *et al.* 1994). The reason for this discrepancy is unclear, but a possible explanation exists. We used almost the same methods except for the fact that they used microwave irradiation to reduce the incubation time, which may have changed the composition of the carbohydrate moieties in sialoglycoproteins.

We also wanted to investigate which carbohydrate was linked to terminal sialic acid residues because sialic acid residues were often known to be linked to D-galactose or N-acetyl-D-galacosamine through α 2, 3- or α 2, 6- linkages (Shibuya *et al.* 1987a, b). We tried to investigate the binding patterns of PNA which reacts with Gal(β 1~3) GalNAc and DBA which reacts with GalNAc(γ 1, 3) GalNAc before and after neuraminidase digestion. We could not find any positive reaction of PNA and DBA in all nasal mucosa before neuraminidase digestion, which means they were masked with sialic acid residues. After neuraminidase digestion, however, apical cell surfaces of respiratory epithelium were labelled with PNA and apical cell surfaces of olfactory epithelium and Bowman's glands were labelled with DBA. Lundh *et al.* reported that only olfactory cilia in paraffin embedded mouse nasal mucosa were labelled with PNA and DBA (Lundh *et al.* 1989). Lee *et al.* reported that respiratory cilia and goblet cells in canine nasal mucosa were not labelled with PNA or DBA(Lee *et al.* 1991). Ueno *et al.* found that cell surfaces and goblet cells in murine tubotympanum epithelium were labelled with PNA only after neuraminidase digestion (Ueno *et al.* 1992). Yoon *et al.* reported that olfactory cilia of guinea pigs were labelled with PNA and respiratory cilia of guinea pigs with DBA (Yoon *et al.* 1993). These differences indicate that carbohydrate residues vary considerably according to the species and the site of the organ.

A rhabdovirus, vesicular stomatitis virus, infected the olfactory mucosa leaving the respiratory mucosa nearly intact, while a paramyxovirus, Sendai virus, preferably infected the respiratory mucosa (Lundh *et al.* 1987). Such a tropism can be the result of a difference in glycoconjugates on the cell surface. Different carbohydrate structures of sialomucin between respiratory and olfactory epithelium indicate that there might be a difference in the susceptibiliy to bacteria or virus and the viscoelasticity of two different kinds of mucus. The difference in viscoelasticity of nasal mucus indicates that mucociliary transport in respiratory mucosa is faster than that in olfactory mucosa. It also indicates that olfactory mucus is more viscous than respiratory mucus and that the olfactory nerve easily adapts to continuous stimuli. However, considering that lectin-binding patterns can be influenced by fixative and embedding material (Roth, 1980), lectin studies of human nasal mucosa need to be conducted using Lowicryl K4M, known to be the most sensitive material for lectin histochemistry.

REFERENCES

Bar-Shavit Z, Ofek I, Goldman R, Mirelman D, Sharon N: Mannose residues on phagocytes as receptors for the attachment of Eschrichia coli and Salmonella typhi. *Biochem Biophys Res Commun 78: 455-460, 1977*

Castells MT, Ballesta J, Pastor LM, Madrid JF, Marin JA: Histochemical characterization of glycoconjugates in the epithelium of the extrapulmonary airways of several vertebrates. *Histochem J 22: 24-35, 1990*

Cuatrecasas P: Interaction of Vibrio Cholerae enterotoxin with cell membrane. *Biochemistry 12: 3547-3558, 1973*

Ledeen RW, Mellanby J: *Gangliosides as receptors for bacterial toxins. In Bernheimer AW,* ed. Perspectives in toxinology. New York, John Wiley & Sons, Inc, *1977, 16-42*

Lee JG, Park HQ, Yoon JH, Park IY: Lectin-binding patterns of canine olfactory mucosa. *Korean J Otolaryngol 34: 718-731, 1991*

Lim DJ, Coticchia JM, Ueno K, Heiselman FA, Bakaletz LO: Glycoconjugates in the chinchilla tubotympanum. *Ann Otol Rhinol Laryngol 100: 933-943, 1991*

Lundh B, Brockstedt U, Kristensson K: Lectin-binding pattern of neuroepithelial and respiratory epithelial cells in the mouse nasal cavity. *Histochem J 21: 33-43, 1989*

Lundh B, Kristensson K, Norrby E: Selective infections of olfactory and respiratory epithelium by vesicular stomatitis and Sendai viruses. *Neuropathol Appl*

Neurobiol 13: 111-122, 1987

Markwell MAK, Paulson JC: Sendai virus utilizes specific sialoligosaccharides as host cell receptor determinants. *Proc Natl Acad Sci USA 77: 5693-5697, 1980*

Montreuil J: Primary structure of glycoprotein glycans: Basis for the molecular biology of glycoproteins. *Adv Carbohydr Chem Biochem 37: 157-223, 1980*

Roth J: Application of lectin-gold complexes for electron microscopic localization of glycoconjugates on thin sections. *J Histochem Cytochem 31: 987-999, 1983*

Sata T, Lackie PM, Taatjes, Peumans W, Roth J: Detection of the Neu5Ac(α2, 3) Gal(β1, 3) GlcNAc sequence with leukoagglutinin from Maackia amurensis: Light and electron microscopic demonstration of differential tissue expression of terminal sialic acid in α2, 3- and α2, 6-linkage. *J Histochem Cytochem 37: 1577-1588, 1989*

Schulte BA, Spicer SS: Histochemical methods for characterizing secretory and cell surface sialoglycoconjugates. *J Histochem Cytochem 33: 427-438, 1985*

Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ: Fractionation of sialylated oligosaccharides, glycopeptides, and glycoproteins on immobilized Elderberry(Sambucus nigra L.) Bark Lectin. *Arch Biochem Biophys 254: 1-8, 1987a*

Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ: The elderberry (Samnucus nigra L.) bark lectin recognizes the Neu5Ac(α 2, 6) Gal/GalNAc sequence. *J Biol Chem 262: 1596-1601, 1987b*

Suzuki Y, Nagao Y, Kato H, Suzuki T, Matsumoto M, Murayama J: The hemagglutinin of the human influenza viruses A and B recognize different receptor microdomains. *Biochim Biophys Acta 903: 417-424, 1987*

Taatjes DJ, Roth J, Peumans W, Goldstein IJ: Elderberry bark lectin-gold techniques for the detection of Neu5Ac(α 2, 6) Gal/GalNAc sequences: applications and limitations. *Histochem J 20: 478-490, 1988*

Ueno K, Hanamure Y, Ohyama M: Differences in terminal carbohydrate structures of sialomucin in the murine nasal cavity. *Eur Arch Otorhinolaryngol 251: 119-122, 1994*

Ueno K, Lim DJ: Heterogeneity of glycoconjugates in the secretory cells of the chinchilla middle ear and eustachian tubal epithelia: A lectin-gold cytochemical study. *J Histochem Cytochem 39: 71-80, 1991*

Ueno K, Ohyama M, Lim DJ: Expression of sialic acids in the developing murine tubotympanum. *Acta Otolaryngol (Stockh) 112: 824-830, 1992*

Wang WC, Cummings RD: The immobilized leukoagglutinin from the seeds of Maackia amurensis binds with high affinity to complex-type Asn-linked oligosaccharides containing terminal sialic acid-linked α -2, 3 to penultimate galactose residues. *J Biol Chem 263: 4576-4585, 1988*

Yoon J-H, Lee J-G, Hong SS, Park IY: Lectin binding patterns in the nasal epithelium of growing Guinea pigs. *Korean J Otolaryngol 36: 943-952, 1993*