Sialic acids as ligands in recognition phenomena

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ABSTRACT **The sialic acids are acidic monosaccharides typically found at the outermost ends of the sugar chains of animal glycoconjugates. They potentially can inhibit intermolecular and intercellular interactions by virtue of their negative charge. However, they can also act as critical components of ligands recognized by a variety of proteins of animal, plant, and microbial origin (sialic acid binding lectins). Recognition can be affected by specific structural variations and modifications of sialic acids, their linkage to the underlying sugar chain, the structure of these chains, and the nature of the glycoconjugate to which they are attached. Presented here is a summary of the various proteins that can recognize and bind to this family of monosaccharides, comparing and contrasting the structural requirements and mechanisms involved in binding. Particular attention is focused on the recently evolving information about sialic acid recognition by certain C-type lectins (the selectins), I-type lectins (e.g., CD22 and sialoadhesin), and a complement regulatory protein (the H protein). The last two instances are examples of the importance of the side chain of sialic acids and the effects of natural substitutions (e.g., 9-O-acetylation) of this part of the molecule.—Varki, A. Sialic acids as ligands in recognition phenomena. *FASEB J.* 248–255 (1997)**

Key Words: lectins · recognition · binding proteins

THE SIALIC ACIDS ARE 9-carbon monosaccharides usually found at the outermost position of the oligosaccharide chains that are attached to glycoproteins and glycolipids (1–6). All sialic acids have a carboxylate at the 1-carbon position that is typically ionized at physiological pH. Because of their terminal location and negative charge, these monosaccharides have the potential to inhibit many intermolecular and intercellular interactions. Such inhibition can be of major biological relevance, as in the case of polysialic acid chains on the neural cell adhesion molecule, which can regulate both homotypic and heterotypic interactions involving neuronal cells (7). In contrast to their role as inhibitors of interactions, sialic acids can also be critical components of ligands for various recognition phenomena involving carbohydrate binding proteins (lectins) (8–33). This review considers the natural occurrence, mode of recognition, and functions of these sialic acid binding lectins. It does not deal with other classes of sialic acid-recognizing proteins, such as the sialidase family of enzymes, or with antibodies that react with sialylated epitopes.

DIVERSITY IN THE SIALIC ACIDS

The term sialic acid is often equated with N-acetyl-neuraminic acid (Neu5Ac,[2] variously labeled in the past as NANA, NeuNAc, NeuAc, etc.). In fact, this "common" sialic acid is also the metabolic precursor of a family of more than forty 9-carbon acid sugars, in which structural diversity is generated by various substitutions at the 4, 5, 7, 8, and 9-carbon positions (see **Fig. 1** and refs 1–6). Nomenclature that permits easy abbreviation of the names of substituted sialic acids is now available (1–4) and has found general acceptance (the term Sia is used as a generic abbreviation for all family members). Further diversity in the presentation of these molecules is generated by several different linkages from the 2-carbon of sialic acids to a variety of underlying sugar chains (see **Fig. 2** for examples, and refs 1, 2, 4, 34). Combinations of the different substitutions and the variety of linkages afford many ways in which sialic acids can present themselves. Further complexity arises from the fact that O-acetyl esters can migrate along the side chain under physiological conditions (1, 2, 4). This diversity is found in a cell type-specific and developmentally regulated manner, implying important roles in intercellular recognition phenomena. In keeping with this, the structural diversity of sialic acids can determine or alter the specific recognition of sialylated sugar chains by a variety of lectins.

SIALIC ACID BINDING LECTINS

Table 1 lists many of the proteins that have been reported to be sialic acid binding lectins. It is evident that the sialic

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[2] Abbreviations: Sia, sialic acid, type unspecified; Neu5Ac, N-acetylneuraminic acid; CD, cluster of differentiation antigen; Sn, sialoadhesin; MAG, myelin-associated glycoprotein; SMP, Schwann cell myelin protein, Allo A-II, *Allomyrina Dichotoma* lectin II, LFA, Limax Flavus agglutinin, SNA, *Sambucus Nigra* agglutinin, TJA, *Tricosanthes japonicum* agglutinin, MAA, *Maackia Amurensis* agglutinin; InfA HA, influenza A hemagglutinin; InfC HE, influenza C hemagglutinin-esterase.

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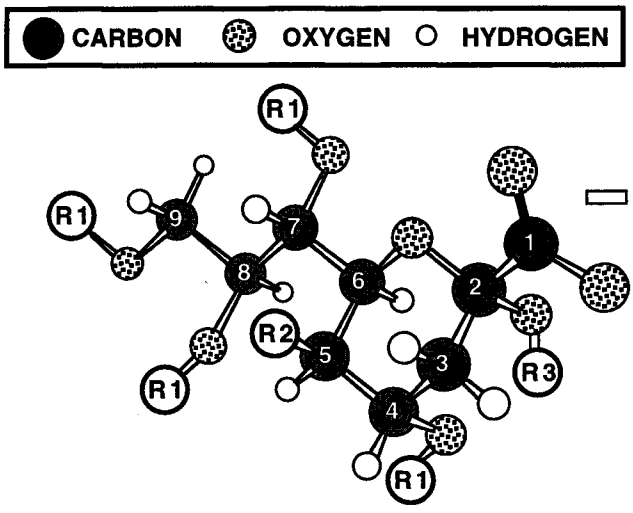

Figure 1. The sialic acids. The 9-carbon backbone common to all sialic acids is shown. Natural substitutions described to date at C_4, C_5, C_7, C_8, and C_9 are indicated. Additional diversity is generated by various types of glycosidic linkage via at C_2, by generation of lactones via C_1, by dehydro forms (eliminating the possibility for a linkage via C_2), and by anhydro forms. R1 = Acetyl(4,7,8,9), lactyl(9), methyl(8), sulfate(8,9), phosphate(9), anhydro(4,8 or 2,7), sialic acid(8,9), fucose(4), glucose(8), or galactose(4). R2 = N-acetyl, N-glycolyl, N-glycolyl-O-acetyl, amino, hydroxyl. R3 = Gal(3/4/6), GalNAc(6), GlcNAc(4/6), Sia(8,9), or 5-O-Neu5Gc (absent in 2,6 and 2,7 anhydro molecules).

acids can be recognized by a wide variety of lectins of animal, plant, and microbial origin, as well as by certain naturally occurring antibodies. It is impossible to do justice to the published information on all of these lectins in either the text or the bibliography of this brief review; for further information, the reader is referred to the reviews and representative references provided.

Some of these lectins were first discovered by their ability to agglutinate red blood cells in vitro, and the loss of this hemagglutination upon sialidase treatment of the cells. In other cases, the discovery of sialic acid binding occurred in the course of investigating various cell-cell interaction phenomena, e.g., the binding of various microbes to target cells was shown to be sensitive to sialidase treatments. In this regard, sialidase treatments generally tend to enhance cell-cell interactions, probably because negative charge repulsion is reduced. Thus, if sialidase treatments (with proper controls) consistently abolish a binding phenomenon, the likelihood of involvement of a sialic acid-specific lectin is high. In more recent examples, sialic acid binding lectins have been uncovered because of their sequence homology to other known lectins. In the case of water-soluble lectins, the next typical step in their investigation has been isolation by affinity chromatography on columns derivatized with sialylated molecules (for examples, see refs 35–43). For membrane-bound lectins, it has usually been necessary to isolate proteolytic fragments of their extracellular domains (9, 44–46) or to design recombinant soluble forms that can be secreted into the medium of cultured cells (27, 47–51). The isolated lectins can then be studied in more detail for their ability to recognize specific sialylated sugar chains, and for the effects of various substitutions and linkages on such recognition (16, 18, 19, 21, 22, 26, 28, 38–42, 51–60). The crystal structures of a few of these lectins have been elucidated, sometimes in a complex with a cognate-sialylated oligosaccharide (45, 61–63).

DETERMINANTS OF SIALIC ACID RECOGNITION BY LECTINS

It is reasonable to predict that the structural diversity in substitutions and linkages of sialic acids (Fig. 1 and Fig. 2) would affect recognition by these lectins. In fact, this matter has not been thoroughly investigated for most of the lectins. **Table 2** summarizes examples in which such information has been partly or completely worked out. It can be seen that the group at the 5-position (commonly N-acetyl or N-glycolyl) varies in its importance for binding (in the case of CD22, the human molecule recognizes both forms, whereas the murine homolog strongly prefers the N-glycolyl form) (33, 64). In this regard, the discoveries of natural sialic acids with either free amino groups (65) or hydroxyl groups (6) at the 5-position can also be expected to affect recognition by some lectins; this possibility has yet to be investigated. The role of the exocyclic polyhydroxylated side chain (C-7, 8, and 9 carbons) in recognition is also highly variable, ranging from being completely dispensable (e.g., for the selectins) to being an absolute requirement for binding (e.g., the influenza A hemagglutinin, the I-type lectins, and the H protein of the alternate complement pathway). In the latter instances, it is not surprising to find that natural substitutions of this side chain (the most common being 9-O-acetylation) can completely abrogate binding (66–69). On the other hand, natural substitutions can also be crucial for recognition, as in the case of influenza C and some coronaviruses, which will only bind to 9-O-acetylated sialic acids (14, 20, 66), and some lectins that strongly prefer this substitution (37, 39, 42).

One convenient way to explore the importance of the side chain has been the use of very mild periodate oxidation (see ref 67 for an example). This chemical treatment has proved to be remarkably selective for cleaving this side chain, even when applied to complex cell surfaces. It is of practical interest that 9-O-acetylation can block the action of periodate under these mild conditions and that this periodate oxidation reaction generates a reactive aldehyde on the side chain (at C-8 or C-7, depending on the extent of cleavage). Although this aldehyde is usually reduced to an alcohol before further studies (to avoid unwanted side reactions), it can sometimes be deliberately used to cross-link lysine residues adjacent to the binding site (e.g., in selectins) (70, 71). Also, some types of substitutions (e.g., O-acetylation) can render the sialic acid molecule partially or completely resistant to the action of sialidases (1–4). Finally, a recombinant soluble form of the 9-O-acetyl-specific esterase from influenza C is now available (67, 72). Thus, various combinations of treat-

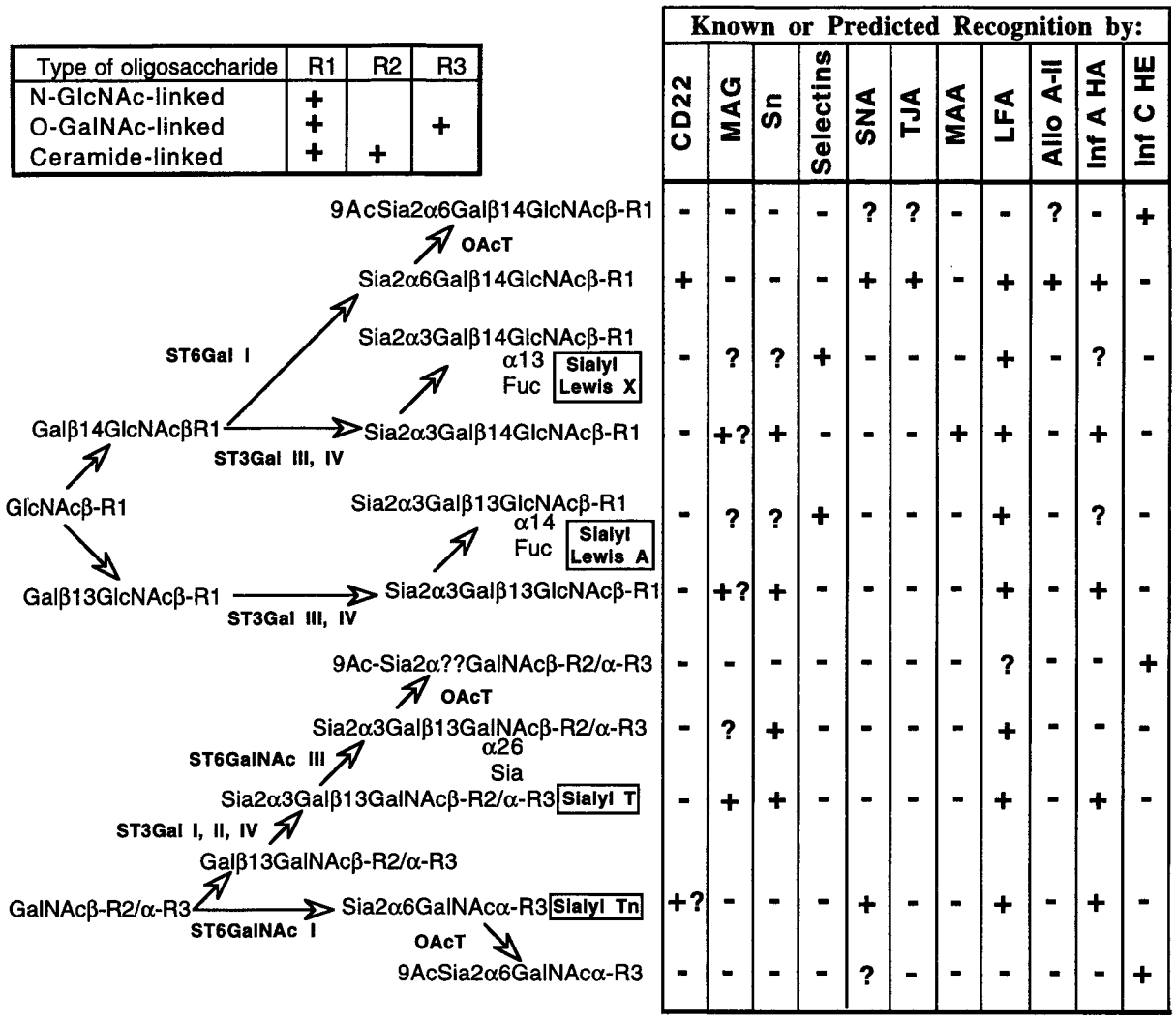

Figure 2. Terminal oligosaccharide sequences recognized by some sialic acid binding lectins, and the biosynthetic pathways generating them. GlcNAc or GalNAc residues on the oligosaccharides of glycoproteins and/or glycolipids can be extended by several biosynthetic pathways, some common examples of which are indicated by the arrows. The sialylated sequences shown as being recognized by some sialic acid binding lectins are based on published literature and/or reasonable predictions based on known specificities. The sequences shown are generally the minimal structural motifs necessary for binding; natural high-affinity ligands may be more complex. Also, recognition can be affected by modifications of Sia other than 9-O-acetylation (see Table 2 for examples, many modifications have not yet been tested). In the case of influenza A HA, relative preference for α2–3 and α-6 linkages can vary widely with different strains. Some of the key enzymes involved in the final biosynthetic steps are shown: ST3Gal I = core 1, α2–3sialyltransferase; ST3Gal II = Gal, α2–3Sialyltransferase; ST3Gal III = LacNAc, α2–3sialyltransferase; ST3Gal IV = Gal, α2–3sialyltransferase; ST6GalNAc I = core 1, α2–6sialyltransferase; ST6Gal I = LacNAc, α2–6sialyltransferase; and OAcT = sialate, O-acetyltransferase.

ments with sialidases, 9-O-acetylesterases, and mild periodate oxidation can be used to explore the importance of the side chain in recognition.

In most instances studied, the negatively charged carboxylate group at the C-1 position has proved to be critical for recognition. An interesting exception is that of wheat-germ agglutinin, where the specific binding of Neu5Ac is based on the similarity of the configuration of this sugar to that of N-acetylglucosamine at the 5-N-acetamido group and the C-3 hydroxyl group, and not on the carboxylate (61, 73). Also, the carboxylate group can sometimes be involved in the formation of intramolecular esters (lactones) under physiological conditions, which would eliminate the negative charge (1, 2). The significance of this modification for lectin recognition has not been well studied. The role of divalent cations in lectin binding varies from being absolutely required (e.g., in the selectins) to being nonessential (e.g., the I-type lectins). The role of the underlying oligosaccharide can vary from being nonessential (e.g., influenza C hemagglutinin-esterase) to being absolutely required (e.g., the *Mycoplasma pneumoniae* hemagglutinin recognizes an extended polylactosamine chain that is terminally sialylated) (74).

As with most lectins in nature, the affinity of a single binding site for the cognate-sialylated oligosaccharide may not be very good (e.g., the K_d of CD22 for binding the basic cognate sequence Siaα2–6Galβ1–4Glc is only ~30 μm) (75). Thus, in most instances it is probably the multiva-

TABLE 1. *Naturally occurring sialic acid binding lectins*

Vertebrate lectins

C-type lectins: E-selectin (endothelium), P-selectin (platelets, endothelium), L-selectin (leukocytes).

I-type lectins: CD22 (B lymphocytes), sialoadhesin (macrophages of spleen, marrow and lymph nodes), CD33 (myeloid cells and macrophages), myelin-associated glycoprotein (MAG, mammalian peripheral nerve myelin), Schwann cell myelin protein (SCMP, avian peripheral nerve myelin).

Unclassified lectins: Complement factor H (human plasma), ganglioside binding protein (rat brain), SAS agglutinins (rat endometrium), sialic acid lectin of frog eggs (*Rana catesbeiana*), calcyclin (bovine heart), human placental lectin (probably a natural antibody).

Arthropod lectins

Crab lectins: Limulin (*Limulus polyphemus*, American horseshoe crab), carcinoscorpin (*Carcinoscorpius rotunda*, Indian horseshoe crab), CA agglutinin (*Cancer antennarius*, Pacific crab), Asian horseshoe crab lectin (*Tachypleus tridentatus* and *Tachypleus gigas*), coconut crab lectin (*Birgus latro*), marine crab lectin (*Scylla serrata*).

Lobster and prawn lectins: L-agglutinin (lobster, *Homarus americanus*), monodin (black tiger prawn lectin, *Peneaus monodon*), freshwater prawn lectin (*Macrobrachium rosenbergii*).

Scorpion lectins: Whip scorpion lectin (*Masticoproctus giganteus*), wood scorpion lectin (*Vaejovis spinigerus*), Indian scorpion lectin (*Heterometrus granulomanus*), Saharan scorpion lectin (*Androctonus Australis*), Arizona lethal scorpion lectin (*Centruroides sculpturatus*), hairy scorpion lectin (*Hadrurus arizonensis*).

Other insect lectins: Allo A-II (beetle lectin, *Allomyrina Dichotoma*), Walker insect lectin (*Teleogryllus commodus*), caterpillar lectin (*Hyalophora cecropia*), American spider lectin (*Aphonopelma cepaehortensis*).

Mollusc lectins

Slug and snail lectins: Limax Flavus agglutinin (slug, *Limax flavus*), achatininH (land snail, *Achatina fulica*), garden snail lectin (*Capaea hortensis*), sea snail lectin (*Dolabella*), freshwater snail lectin (*Biomphalaria glabarata*), apple snail lectin (*Pila globosa*).

Mussel and oyster lectins: Mussel lectin (*Mytilus edulis*), Pacific oyster lectin (*Crassostrea gigas*).

Protozoal lectins

Parasite lectins: Merozoite erythrocyte binding antigen (*Plasmodium falciparum*), tritrichomonas lectin (*Tritrichomonas mobilensis*), sporozoite lectin (*Cryptosporidium parvum*).

Plant and fungal lectins

SN agglutinin (elderberry bark lectin, *Sambucus Nigra*), TJ agglutinin (*Tricosanthes japonicum*), MA agglutinin (*Maackia Amurensis*), wheat-germ agglutinin (*Triticum vulgaris*), prickly lettuce lectin (*Lactuca Scariole*), mistletoe lectin (*Viscum album*), mushroom lectin (*Hericium erinaceum*), mucin-specific elderberry lectin (*Sambucus sieboldiana*).

Bacterial lectins

Bacterial adhesins: S-adhesin (*Escherichia coli* K99), adhesins I and II (*Helicobacter pylori*), Sia-1 adhesin (*Neisseria subflava*), SfaS adhesins (*Escherichia coli, Streptococcus suis*), pseudomonas adhesin (*Pseudomonas aeruginosa*), streptococcal adhesin (*Streptococcus sanguis* and *Streptococcus mutans*).

Bacterial toxins: Cholera toxin (*Vibrio cholerae*), heat-labile enterotoxin (*Escherichia coli*), tetanus toxin (*Clostrodium tetani*), botulinum toxin (*Clostrodium botulinum*), pertussis toxin (*Bordetella Pertussis*), heat-stable toxin (*Vibrio parahemolyticus*), α-toxin (*Staphylococcus*) and δ-toxin (*Clostridium perfringens*).

Mycoplasma lectins: *Mycoplasma pneumoniae* hemagglutinin.

Viral lectins

Hemagglutinins: Influenza A and B viruses, primate polyoma viruses, rotaviruses, encephalomyocarditis virus, type 3 reovirus, enterovirus type 70.

Hemagglutinin neuraminidases: New Castle disease virus, Sendai virus, fowl plague virus, human parainfluenza type 3, porcine paramyxovirus LPM.

Hemagglutinin esterases: Influenza C viruses, human and bovine coronaviruses.

lency of interaction that generates the avidity necessary for functionally relevant binding. One possible exception may be the selectins, where high-affinity binding has been reported for recombinant soluble monovalent lectins (76–78). In this case, early studies indicated that the sialylated, fucosylated structure sialyl Lewis^x was the sole ligand for the selectins. It is now evident that this structure is necessary but not sufficient for recognition, and that there are even some selectin ligands that do not contain this motif (15, 18, 19, 21, 25, 27, 31, 32, 79).

FUNCTIONS OF SIALIC ACID BINDING LECTINS

Of all the sialic acid binding lectins in nature, most attention has recently focused on the selectins (15, 18, 19, 21, 25, 27, 31, 32, 78). This family of three vascular adhesion proteins recognizes a variety of sialylated ligands in a calcium-dependent manner, thereby mediating critical steps in many important vascular events, such as leucocyte diapedesis and thrombosis (25, 27). At least some animal studies have indicated that sialylated ligand mimics can have salutory effects on various pathological processes involving the selectins, such as reperfusion injury and excessive inflammatory responses (25, 27). A more recently recognized family of sialic acid binding proteins is the subset of I-type lectins related to CD22, which include other immunoglobulin superfamily members such as sialoadhesin, CD33, and myelin-associated glycoprotein (26, 33). The cell type-specific and regulated expression of these lectins and of the sialyltransferases that generate their cognate ligands has raised expectations that they are involved in highly specific biological roles. Indeed, CD22 may be involved in interactions with the tyrosine phosphatase CD45 (80), sialoadhesin may mediate macrophage

TABLE 2. *Structural requirements for recognition by some sialic acid binding lectins**

Lectin name†	Preferences/requirements for recognition						
	Sia linkage	Underlying saccharide	Carboxylate	5-Acyl group	Side chain	9-O-acetyl	4-O-acetyl
E-selectin	α2–3	Lewis^x or Lewis^a	Yes	No	No		
P-selectin	α2–3	Lewis^x or Lewis^a on O-linked	Yes	No	No		
L-selectin	α2–3	? 6′-sulfo-Lewis^x or Lewis^a	Yes	No	No		
CD22	α2–6	Galβ1-4Hex(NAc)	Yes	Ac, Gc	Yes	Blocks	
Sialoadhesin (Sn)	α2–3	Galβ1-(3)4HexNAc	Yes	Ac	Yes	Blocks	
Myelin-associated glycoprotein (MAG)	α2–3	Galβ1-(3)4HexNAc	Yes	Ac	Yes	Blocks	
CD33	α2–3	Galβ1-(3)4HexNAc	Yes		Yes		
Complement factor H	All?		Yes		Yes	Blocks	
Limulus polyphemus lectin (limulin)	All?			Ac = Gc	No		
Cancer antennarius lectin (CA agglutinin)						Prefers	Prefers
Scylla serrata (marine crab) lectin				Gc		Blocks	Blocks
Allomyrina Dichotoma lectin (Allo A-II)	α2–6	Galβ1-4GlcNAc					
Limax Flavus agglutinin (LFA)	All			Ac	Yes	Blocks	Accepts
Achatina fulica lectin (achatininH)	α2–6 > 3					Prefers	
Capaea hortensis (garden snail) lectin	α2–3, 6	O-linked chains	Yes	Ac	Yes		
Apple snail (*Pila globosa*) lectin			Yes	Gc	Yes	Blocks	
Plasmodium falciparum merozoite lectin	α2–3	Galβ1-4Glc(NAc)				Blocks	
Sambucus Nigra agglutinin (SNA)	α2–6	Gal or GalNAc			Yes		
Tricosanthes japonicum agglutinin (TJA)	α2–6	Galβ1-4GlcNAc					
Maackia Amurensis agglutinin (MAA)	α2–3	Galβ1-4Glc(NAc)		Gc/Ac	No		
E. coli K99 S-adhesin	α2–3	Galβ1-4Glc-Cer, G_{M3}		5Gc			
Helicobacter pylori adhesin I	α2–3	Gal					
Helicobacter pylori adhesin II	α2–3, 6	Polyglycosylceramide			Yes		
Vibrio cholerae toxin (B subunit)	α2–3	GA1 glycolipid core					
Influenza A hemagglutinin	Varies				Yes	Blocks	
Influenza C hemagglutinin esterase	All					Yes	
Mycoplasma pneumoniae hemagglutinin	α2–3	$(Galβ1-4GlcNAc)_n$					

* The data presented are based on published literature and some unpublished observations. In some cases, the data represent reasonable assumptions based on precedent in very similar situations. † See also Table 1.

interactions with developing myeloid precursors (81), and myelin-associated glycoprotein may interact with specific gangliosides on neuronal cells to maintain the integrity and function of myelin (51, 82, 83). Analysis of these functions is complicated by the fact that the cognate oligosaccharide sequences for some of these lectins are found in small numbers on a wide variety of glyconjugates. It appears that these lectins may function by specifically recognizing a few high-affinity ligands in the midst of a mileu of low-affinity inhibitors. Further confusion arises because some of these lectins can become functionally inactivated by binding in *cis* to sialylated ligands present on the same cell surface as the lectin itself (84). For these and other reasons, elucidating the functional roles of the I-type sialic acid binding lectins remains a challenge.

Another interesting case involves the binding of the complement regulatory factor H, a soluble factor in serum that binds to surfaces via the intact exocyclic (C_7-C_8-C_9) side-chain of sialic acids (85–89) and restricts alternative pathway activation. The biosynthetic addition of a 9-O-acetyl group to the side chain of cell-surface sialic acids (or the oxidation of the unsubstituted side chain with mild periodate) blocks the binding of factor H and abrogates its function as a negative regulator of the alternative pathway (69). This phenomenon has so far been demonstrated only with synthetic targets (87, 88) or in heterologous systems (e.g., with mouse erythrocytes and human complement) (69). Its potential importance in various pathological conditions involving complement activation needs to be explored.

Since the original discovery and characterization of sialic acids as ligands for the influenza viruses (9), many microbial-host interactions have been shown to depend on recognition of sialylated ligands (see Table 1, and refs 9, 10, 12, 24, 30). Some examples of medical relevance are the binding of *Helicobacter pylori* (the etiological agent of peptic ulcer disease) to gastric mucins via at least two different sialic acid-dependent mechanisms (90, 91), the binding of various pathogenic microbial toxins to mammalian cells (see Table 1, and ref

17), and the recognition of erythrocytes by *Plasmodium falciparum* merozoites (92, 93). In these cases, production of the sialylated ligands by the mammalian organism is obviously of detrimental value to the host. It is interesting that the interactions of some microbial lectins with sialic acids can be abolished by substitutions such as 9-O-acetylation, which can be found on mucosal surfaces. Indeed, it is possible that some of the extreme complexities of sialic acid diversification are the outcome of the ongoing evolutionary battle between animals and microbial pathogens. On the other hand, the modified sialic acids in some internal organs and tissues may be required for recognition by endogenous lectins that are yet to be discovered.

The widespread expression of large quantities of soluble multivalent sialic acid binding lectins in the body fluids of many lower organisms raises the possibility that they are meant to mediate host defense against microbes expressing sialic acids on their surfaces. In keeping with this, limulin, a pentraxin family member (29) found in the hemolyph of the American horseshoe crab *Limulus polyphemus*, has recently been shown to mediate foreign cell hemolysis (94).

THE NATURAL LIGANDS OF SOME SIALIC ACID BINDING LECTINS MAY NOT BE SIALIC ACIDS

One of the puzzles about sialic acid binding lectins is that they are so often found in organisms that do not themselves express sialic acids at easily detectable levels: e.g., plants and insects. Two possible explanations can be considered. First, their primary function may be in defense against exogenous sialylated pathogens that interact with these hosts. A second possibility is that their sialic acid binding properties are serendipitous, and that their real ligands are other anionic carbohydrates yet to be identified. It is interesting that the sialic acid binding lectin Carcinoscorpin from the Indian horseshoe crab can recognize both 2-keto-KDO (a component of the lipolysaccharide of gram-negative bacteria) and glycerol phosphate (a component of membrane techoic acids of Gram-positive bacteria) (35). Likewise, the lectin of the prawn *Macrobrachium rosenbergii* can recognize a variety of anionic bacterial sugars (95). Another suspicious finding is that upon cloning, some of these plant lectins turn out to have unexpected homologies with proteins known to bind other anionic carbohydrates; for example, the NeuAc(α-2,6)Gal/GalNAc-binding lectin from elderberry (*Sambucus nigra*) bark is most closely related to a type-2 ribosome-inactivating protein (96, 97). On the other hand, it is hard to completely reconcile this suggestion with the remarkable specificity of some plant and invertebrate lectins for specific aspects of the structure of sialic acids and/or the underlying sugar chain (see Table 2).

SIALIC ACID BINDING LECTINS AS TOOLS TO EXPLORE THE EXPRESSION AND BIOLOGY OF SIALIC ACIDS

Regardless of whether a particular lectin was originally evolved for the purpose of recognizing sialic acids, it could be used as a tool for exploring the expression and biology of these molecules. For example, WGA and Limax Flavus agglutinin (LFA) have been used as general tools to bind sialylated glycoconjugates, and combinations of *Tricosanthes japonicum* agglutinin (SNA), *Tricosanthes japonicum* agglutinin (TJA), and *Maackia Amurensis* agglutinin (MAA) are powerful tools to distinguish among different types of sialic acid linkages on terminal lactosamines (28, 46, 98). However, for the reasons mentioned earlier, additional controls should be used whenever possible to confirm the significance of binding results obtained with such lectins. An example would be the use of α2–3 linkage-specific sialidases (99) to eliminate the binding of some of these lectins. One is also on safer ground when using lectins of vertebrate and microbial origin, whose the natural ligands are definitely sialic acids. Thus, for example, recombinant soluble forms of CD22 and sialoadhesin are excellent tools to detect high-level expression of α2–6-linked and α2–3-linked sialic acids, respectively (98), and influenza C hemagglutinin-esterase can specifically probe for 9-O-acetylated sialic acids (67, 72, 100). Of course, in all instances in which a lectin is used as a detection device, the absence of binding does not necessarily imply the complete absence of the structure. First, the structure may be present in too low a density to achieve the multivalency required to generate detectable binding. Alternatively, the sialic acid or the underlying oligosaccharide could be modified in some way that prevents recognition. Also, in some cases (e.g., the selectins) high-affinity binding may be dependent on more complex structural motifs. For example, even though the sialyl Lewis^x structural motif is necessary for recognition of most selectin ligands, it may be insufficient for high-affinity binding without the addition of sulfate esters and/or proper clustering of multiple recognition determinants (19, 21, 27, 31, 78).

With these caveats in mind, the many lectins listed in Table 1 are productive ground for the generation of new tools with which to study the expression and biology of sialic acids. In particular, many microbial lectins have remarkably specific binding properties that have yet to be harnessed as tools. One can also safely predict that the microbial world contains many undiscovered lectins that will specifically recognize many other modifications of sialic acids (e.g., 4-O-acetylation, methylation, etc.) that have yet to be studied in any detail in vertebrate systems. One can also envision that some of these modified molecules will have intrinsic roles within the vertebrate organism synthesizing them, and hence will have endogenous lectins as recognition partners. The identification, characterization, and cloning of such

endogenous lectins deserves close attention in the near future. FJ

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